Clinical Validation of Wideband Absorbance Tympanometry in Detecting Middle Ear Disorders

A Doctoral Thesis

Submitted to the University of Mysore

for the award of the degree

Doctor of Philosophy (Ph.D.) in Audiology

By

ARUNRAJ K.
(Reg. No.: DOR.9.1/Ph.D/AK/378/2014-15, dated 27.08.2015)

Prepared under the guidance of

Dr. ANIMESH BARMAN
Professor of Audiology
Department of Audiology
All India Institute of Speech and Hearing, Mysuru.

Research Centre
All India Institute of Speech and Hearing, Mysuru.

March 2021

CERTIFICATE

This is to certify that the thesis entitled **"Clinical Validation of Wideband Absorbance Tympanometry in Detecting Middle Ear Disorders"** submitted by Mr. Arunraj K. for the degree of Doctor of Philosophy (Audiology) to the University of Mysore, Mysuru was carried out at the All India Institute of Speech and Hearing, Mysuru.

Place: Mysuru
Date: 22.03.2021

Dr. (Prof.) M. Pushpavathi
Director
All India Institute of Speech and Hearing
Mysuru

CERTIFICATE

This is to certify that the thesis entitled **"Clinical Validation of Wideband Absorbance Tympanometry in Detecting Middle Ear Disorders"** submitted by Mr. Arunraj K. for the degree of Doctor of Philosophy (Audiology) to the University of Mysore, Mysuru was carried out at the All India Institute of Speech and Hearing, Mysuru, under my guidance. I further declare that the results of this work have not been previously submitted for any other degree.

Place: Mysuru
Date: 22.03.2021

Dr. Animesh Barman
Professor of Audiology
Department of Audiology
All India Institute of Speech and Hearing
Mysuru

DECLARATION

I declare that this thesis entitled **"Clinical Validation of Wideband Absorbance Tympanometry in Detecting Middle Ear Disorders"** submitted herewith for the award of the degree of Doctor of Philosophy (Audiology) to the University of Mysore, Mysuru is the result of work carried out by me at the All India Institute of Speech and Hearing, Mysuru, under the guidance of Dr. Animesh Barman, Professor of Audiology, All India Institute of Speech and Hearing, Mysuru. I further declare that the results of this work have not been previously submitted for any other degree.

Place: Mysuru

Date: 22.03.2021

Arunraj K.

Ph.D. Research Scholar

ACKNOWLEDGEMENTS

! by God's Grace with lots of blessings from Shri Navaladi Periyasamy, Mohanur !

I am extremely grateful to my parents, Karuppannan and Kulanthiammal, for their love, prayers, caring and sacrifices for educating and preparing me for my future. Also, I express my thanks to my brother Anandraj, Sumathi (sister-in-law) and their daughters Rithanya & Chellam for their support and valuable prayers. I am very much thankful to my wife and my son (Adheran) for their love, understanding, and continuing support to complete the doctoral research work. I also thank Dr. Kuppuraj's family, who became part of my life thinking of goodness and their lovely conversation.

At the outset, I would like to express my sincere appreciation to my beloved Professor Dr. Animesh Barman, for his advice during my doctoral research endeavour. As my supervisor, he has constantly forced me to remain focused on achieving my goal, though I took lots of time. His observations and comments helped me establish the overall direction of the research and move forward with the investigation in depth. Your advice on both research as well as on my career, has been invaluable. I thank him for all the support during my entire tenure of doctoral research.

I would like to thank Dr. Vijaya Kumar Narne, who helped me to start my doctoral research with lots of confidence. Though his presence was for a shorter period with me and flew worldwide for his career, but still the support and guidance till date is truly a blessing. He has played a major role in making me understand the concept and been a source of knowledge for my research! I also express my sincere thanks to Dr. Arivudai Nambi, who has always been in support and inspiration since my bachelor's degree. Special thanks to Dr. Kishan and Dr. Priya for their invaluable support.

Time is a necessitate factor for doctoral research and I am extremely grateful to Dr. Pushpavathi M., Director, who supported me in all ways and had provided me ample time to complete my research. Though it was tougher performing multitasking with lots of prime duties assigned during my doctoral research tenure, it was she who understood what exactly required for me and got me everything to complete my thesis. I am really thankful to her for believing me and having lots of hopes in me.

Dr. Sreedevi N., Head of Prevention of Communication Disorders dept.… My understanding was during my Icry research project with her being a project head and it was amazing to work for it with you. Since then, it's fortunate to work together. Always dedicated to work and loving and caring Professor who supported me in completing my doctoral research. Without her support and cooperation, it would not be possible to submit my thesis in time. Thanks a lot, and I vow a lot to you, Ma'am.

I would like to express my sincere thanks to my committee members, Dr. Manjula P., Dr. Sandeep M., Dr. Vasanthalakshmi M S., for serving as my committee members and for your thoughtful comments and suggestions, thanks to all. A special thanks to Dr. Vasanthalakshmi M S., Associate professor in Bio-Statistics and Mr. Srinivas, Lecturer in Bio-statistics, who had significantly contributed for their statistical support in my thesis.

I cannot forget two senior-most Professor in my early carrier. Dr. Manjula R., who brought me to this level, trained me in all aspects, including work ethics, writing and managing skills. It's you who made my name visible at AIISH till date. I vow a special place in my life and thank you for everything, ma'am. Other was Dr. Asha Yathiraj, who indirectly moulded me to face any problems in my carrier. Now I am proud that I had changed the way you think about me in later days.

I thank Mr. Joe Huijnen from Interacoustics, Germany for sharing lots of information and data on the WBT that enabled me to carry forward my doctoral research. Our chats on various concepts on WBT that continued for years had helped me a lot, and literally, we had good learning moments. You got me a lot to support my research, specifically the Research module and the Special probe unit with calibration couplers that too free-of-cost and sharing of data extraction MATLAB and Excel file is truly appreciable. A special thanks to M/s. Interacoustics who had agreed to support my research through you.

I thank all the Heads of Audiology and Prevention of Communication Disorders during my research tenure to provide excellent infrastructure to carry forward my research. Also, I thank all my colleagues and friends from both the departments, specifically Dr. Sharath, Dr. Jithin, Dr. Megha, Mr. Antony, Mr. Vikas, and Mr. Nagaraju for supporting me by sharing my clinical work and providing me enough time to focus on my doctoral research. Special thanks to Ms. Priyanka and Dr. Jithin for their lovely time in quarters with lots of snacks and tea on most of the days. Heartfelt thanks to Dr. Megha, Dr. Amulya, Darshan H S and Kalaiyarasan for their comments and for proofreading my thesis and research publications.

I greatly appreciate the Journals - 'Aurix, Nasus and Larynx' European Journal of Oto-Rhino-Laryngology' and 'Journal of Hearing Science' who believed my research and provided generous time in reviewing, editing and accepting it for publications. I also thank their Publishers- 'Springer' 'Elsevier' and 'Institute of Sensory organs' who shared my findings that influenced and boosted my research credentials.

Friends who are always the stress busters. I am lucky enough to have you all in my life… Navitha, Sruthy, Ramesh, Ismail, Sunil, Pragathi, Poornachandran, Naresh, Ridhima, Vivek, Shuchi, Amith Kishore and Sriram. Thank you for all the happiness and memories that you guys gave. Special thanks to Dr. Kuppuraj Sengottuvel, though you left us early, your achievements are many and are still in our memories. Zebu, Litty, Sabarish and Sanjitha... Miss you guys!! Such a loving and caring friends that I ever got and never let me down!! Niranjan, Sanjeev Anil Abbur and Teju, who would always give me a break from my continual research work to refresh my mind, thank you guys…. I am forever thankful to my junior friends and their families who are with me and always looked for the best in me - Rakshith, Ashwath, Shiva Belagi, Chaitra and Vikram... I cannot think of my life without you guys and such awesome soulmates... Love you guys!!! 'Aytalla sir, ennenu' from Vishwa (Shiva bro) for his dedicated research in everything and his easy-going in life…. Keerthi (Ashwath bro) 'mane ge banni sir' never miss asking me about my health and always been invited for special lunch in his home!! All the best bro's. Never last, my school friends, especially Praveen C N., my accident partner too, who care for me and support me in all ways without a second thought.

Special mentioning of Pramod and his family, fortunate to meet and am loving the way their concern and affection towards me. Thanks to Tejas, Somu Sumanth and AIISH Chetan for being with me and for your moral support. Thanks to Sumanth for giving his time to finish my doctoral research. Thanks to Ashwath friends -Bharath, Shive adam, Manu, Chetan and Shashank for their lovely time in their home.

Thanks to all the participants who were willingly volunteering to be part of my doctoral research

I thank the entire AIISH fraternity, the University of Mysore and their management for their support to carry forward and to complete my research work.

Thankful to everyone whom I have missed mentioning and who have contributed towards shaping this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure No. **Figure Title with description** **Page No.**

LIST OF ANNEXURES

LIST OF ABBREVIATIONS

WBT - Wideband Absorbance/Reflectance Tympanometry

WBA - Wideband Absorbance

WBT_{avg} - Wideband Average Tympanometry

TM - Tympanic Membrane

TmP - Tympanic Membrane Perforation

MEE - Middle Ear Effusion

ETD - Eustachian Tube Dysfunction

SNHL - Sensorineural Hearing Loss

TEOAE - Transient Evoked Oto-Acoustic Emission

ABG - Air-Bone Gap

SNR - Signal-to-Noise Ratio

ECV - Ear Canal Volume

TPP - Tympanometric Peak Pressure

ANOVA - Analysis of Variance

MANOVA - Multivariate Analysis of Variance

ICC - Inter-class Correlations Coefficient

HSD - Honestly Significant Difference

ROC - Receiver Operating Characteristics

AUROC - Area under Receiver Operating Characteristics

SD - Standard Deviation

CI - Confidence Interval

ABSTRACT

Objective: To obtain norms for wideband absorbance tympanometry in adults with normal hearing and normal middle ear, and to clinically validate the results with ear related disordered populations.

Methods: Three groups in the age range of 22 to 50 years were considered in the study. Group I consisted of normal middle ear functioning with normal hearing sensitivity (n=1127 ears), Group II consisted of middle ear disorders [Tympanic membrane perforation (n=109), Middle ear effusion without perforation (n=122 ears), Otosclerosis (n=140 ears), and Eustachian tube dysfunction (n=106 ears)], and Group III consisted of Sensorineural hearing loss (n=140 ears). All the participants have undergone WBA measurements across frequencies and wideband average tympanometry.

Results: The WBA measured in Group I across frequencies and average tympanogram showed a significant difference only for pressure conditions. Though the gender showed a significant effect on WBA at low frequencies, the effect size was small, whereas no significant difference was seen for ears. The WBA at peak pressure was higher compared to the ambient pressure conditions. The WBA was lowest at 250 Hz, increased steeply with increasing frequency to about 1250 Hz, rose to a second maximum at about 2000 Hz, and reduced to a minimum absorbance at 6000 Hz and above. Also, the analysis of reliability measures (test re-test and Inter-tester) had shown a moderate to good reliability. Further, the study had shown a reduction in WBA for all the middle ear pathological groups compared to the normal ear group, specifically at the lower frequencies below 2000 Hz. ROC also showed high sensitivity and specificity at the lower frequencies for the identification of middle ear disorders. However, there was no specific frequency absorbance value which can be used to differentiate different middle ear disorders from each other. However, a unique pattern for each pathological group was estimated in the study. While in ears with Sensorineural hearing loss, the WBA pattern is indistinguishable from normal ears having similar WBA values with lower sensitivity and specificity.

Summary and conclusions: The study has estimated the WBA norms at peak and ambient pressure. This study demonstrated the clinical utility of wideband absorbance tympanometry in detecting ear-related disorders.

Keywords: Wideband absorbance tympanometry, middle ear disorders, Peak pressure, and ambient pressure.

Chapter 1

INTRODUCTION

Sound transmission through the auditory system is a complex process that depends on the middle ear's acoustic transmission characteristics. The efficiency in transmitting sound from the environment to the inner ear may be altered and/or disrupted if there are any abnormalities in the middle ear structure. This results in greater impedance mismatch due to variations in the middle ear's mass and stiffness components (Shaver, 2010). Therefore, the precise functioning of the middle ear plays a major role in the efficient transmission of sound to the inner ear.

Many basic auditory tests are available, including pure tone audiometry, tympanometry, acoustic reflex measurements, and otoacoustic emission measurements, which provides information about the process of sound transmission to the auditory system (Baiduc et al., 2013). Immittance measurement is one of the key components of the test battery widely used to assess the middle ear system (Lantz et al., 2004). The earliest method of measuring the immittance of middle ear function was performed using ear canal pressure and plotted as a function of acoustic reactance and resistance with respect to frequency (Lilly & Margolis, 2013). Based on the reactance and resistance pattern, the differential diagnosis was made to identify conductive hearing loss (Lilly, 1984). Later, immittance measurements had undergone further modification, such as introducing changes in air pressure in the closed ear canal; single-component tympanogram with low-frequency probe tones; and multi-frequency Multi-component tympanometric measurements (Lilly & Margolis, 2013). In addition to these, wideband absorbance tympanometry measurements are the latest development to aural acoustic-immittance measurements to study the middle ear function.

To date, single-frequency tympanometry using a probe tone frequency of 226 Hz has been a widely applied diagnostic procedure for evaluating middle ear transmission function (Ibraheem, 2014). Despite the fact that tympanometry is the most commonly used diagnostic tool for the evaluation of the middle ear system (Hunter & Sanford, 2014; Margolis et al., 1999), a few research findings reported low accuracy in identifying middle ear pathologies (Hunter et al., 2017; Kaf, 2011; Shahnaz & Polka, 1997). This is because of the extent of change in the middle ear structures, i.e. ossicular mass, stiffness of tympanic membrane and supporting structures due to middle ear pathologies, resulting in frequency-specific attenuation or filtering (Norrix et al., 2013). Thus, there is a substantial overlap in clinical findings indicating false-positive results even in normal-hearing individuals (Keefe & Levi, 1996; Shahnaz & Polka, 1997). Research on test-retest reliability of low-frequency probe tone tympanometry have observed higher admittance on repeated testing (Gaihede & Ovesen, 1997; Karzon, 1991). Studies have shown elevation of middle ear admittance by an average of 14% between the ninth and the first trials (Gaihede, 1996).

An increasing number of studies have indicated that tympanometry with single probe tone frequency has poor sensitivity and specificity for identifying middle ear pathologies, especially in those who have slightly altered mechanical aspects of the tympanic membrane (Kaf, 2011) and pathologies affecting the ossicular chain such as otosclerosis (Browning et al., 1985; Shahnaz & Polka, 1997). In the literature, the lack of sensitivity and specificity has been attributed to the inability to detect subtle changes in the resonance frequency of the middle ear (Iacovou et al., 2013; Vanaja & Manjula, 2003), which is one of the key parameters used in the detection of middle ear pathologies (Shahnaz & Bork, 2008; Suat Terzi et al., 2017).

Multi-frequency tympanometry (MFT) is considered superior to 226 Hz probe tone tympanometry, which provides information from 226 Hz up to 2000 Hz (Keefe & Feeney, 2009). High impedance pathological conditions such as middle ear effusion, otosclerosis, and chronic otitis media are accurately detected with the inclusion of several probe tone frequencies (Keefe & Levi, 1996; Shahnaz, Bork, et al., 2009). Thus, MFT was found to be highly sensitive than single probe tone frequency tympanometry in detecting middle ear pathology (Shahnaz & Polka, 1997). However, the use of MFT in clinical practice has limitations. The presence of standing waves above 1500 Hz, which causes a large deviation in sound pressure, can affect and prevent valid measurements at high frequencies (Keefe & Feeney, 2009; Margolis et al., 1999). There is also no standard calibration for these high probe tone frequencies, which would provide inaccurate information about the middle ear, especially at higher frequencies (Keefe & Feeney, 2009). Thus, existing tympanometric measurements can lead to misdiagnosis, either false-positive or false-negative results. Therefore, an alternative measure that accurately detects middle ear pathology with greater sensitivity and specificity is needed, even for mild pathology.

Wideband absorbance or reflectance tympanometry (WBT) is a new technique that has been introduced recently to measure the transfer functions of the middle ear (Allen, 1986; Shahnaz & Bork, 2006). WBT provides clinical information regarding the impedance over a wide frequency range from 226 Hz up to 8000 Hz than the single probe tone tympanometry, which provides information regarding admittance at a specific frequency (Vander Werff et al., 2007). In recent years, much more information has emerged in the literature showing that WBT can identify pathologies of the middle ear, including subtle pathologies that affect the ossicular chain (Hunter et al., 2013;

Hunter, Bagger-Sjoback, et al., 2008; Keefe & Simmons, 2003; Shahnaz & Polka, 1997).

The researchers had found several benefits of WBT in evaluating the middle ear, over 226 Hz and multi-frequency tympanometry. WBT uses multiple frequencies simultaneously using transient stimuli so that no interference from myogenic noise is generated by patient movements (Prieve et al., 2013). WBT can also be recorded even without applying any pressure to the ear canal (Feeney et al., 2003; Huang et al., 2000; Keefe & Levi, 1996; Keefe & Simmons, 2003). In-addition, the positioning of the probe tip in the ear canal does not influence the WBT measurements (Keefe & Levi, 1996; Voss et al., 2008), while the tympanometric measurements are strongly influenced by the depth of the probe tip insertion (Margolis et al., 1999; Shanks & Lilly, 1981). Therefore, the WBT is emerging as a promising tool for identifying middle ear pathologies with high accuracy and possibly replacing the single probe tone and multi-frequency tympanometry (Sanford et al., 2013).

1.1 Need for the study

Keeping in view of all the reported advantages of WBT, it appears that WBT is a concept that has gained momentum in recent years for routine audiological evaluation. Researchers have been working in this direction to strengthen the application of WBT for middle ear assessment as part of the routine audiological evaluation. With lots of advantages using WBT over conventional single frequency and multi-frequency tympanometry, there is a need to establish the norms in the concerned area. This will facilitate differential diagnosis of middle ear conditions and precise identification of the pathology in the human middle ear.

1.1.1 Need for establishing normative data

1.1.1.1 Variation in WBT values across studies: Understanding that the WBT seems to be a lot more advantageous in detecting middle ear disorders than conventional single frequency tympanometry, there is a need to develop the norms for WBT. Normative reflectance/absorbance pattern was estimated in a few Western studies for different age groups (Margolis et al., 1999; Merchant et al., 2010; Werner et al., 2010). However, these normal reflectance/absorbance patterns were reported to vary across studies. Most published normative data for middle ear measures have not specified the subject's ethnicity related information.

Shahnaz and Bork (2006) estimated the normative reflectance across frequencies in Caucasians and Chinese populations and compared it with already existing data (Feeney & Sanford, 2004; Keefe et al., 1993; Mimosa Acoustics, 2002; Voss & Allen, 1994). It was observed that the mean reflectance value reported in Shahnaz and Bork (2006) study was relatively lower than what was reported in the already existing studies from 250 Hz to 3000 Hz. This clearly indicates that WBT does not have a unique pattern across frequencies (Feeney & Sanford, 2004; Keefe et al., 1993; Mimosa Acoustics, 2002; Voss & Allen, 1994). Hence, it is crucial to estimate WBA across frequencies for different population having healthy middle ear function.

1.1.1.2 Structural variation across regions/races: Research on structural and size differences of the outer and middle ear observed differences among the individuals differing in ethnicity, gender and age that could affect the middle ear transfer function (Shahnaz, 2010). The difference that exists among different ethnicities could be due to the differences in melanin level in the cochlea (Solano, 2014; Sun et al., 2014), genetics (Lin et al., 2011, 2012) and also anatomical and body-size differences (Chan & McPherson, 2001; Shahnaz & Davies, 2006). It has been shown that the average height and weight of the body are larger in the Caucasian than the Chinese group (Bell et al., 2002; Shahnaz & Bork, 2006). Several researchers observed that the increase in body size leads to increased middle ear air space compliance. At the same time, the frequency at which the reflectance minimum occurs decreases with body size (Huang et al., 2000).

1.1.1.3 Variation of immittance test results across regions/races: Middle ear measures have exhibited differences across racial groups. It has been indicated that Asians have lower admittance, smaller ear-canal volume, wider tympanometric width and higher middle ear resonant frequencies than Caucasians (Chan & McPherson, 2001; Shahnaz & Davies, 2006; Wan & Wong, 2002). It was also reported that American black infants and preschoolers had different mean admittance values than the respective Caucasian group (Robinson et al., 1988; Robinson & Allen, 1984; Wei De, 2020). Studies have also reported higher resonant frequencies for Asians than Caucasians (Shahnaz & Davies, 2006) and lower acoustic reflex thresholds for African Americans than Asians and Caucasians (Whitehead et al., 1993). Wan and Wong (2002) reported that 48% of Chinese children failed tympanometry screening when the same norms developed on Caucasians were used. Similarly, studies on WBT showed a significant difference between Caucasians and Chinese individuals (Shahnaz & Bork, 2006; Wang et al., 2019).

It is understood from the studies that even small variation in anatomy and physiology between ethnicities likely to underlie the differences in middle ear sound transmission. Thus, the studies implicate that body size differences could be a crucial factor in the variations noted in the middle ear transfer function between the different ethnic groups. Using ethnic-specific norms and comparing the individuals' results could assess the presence of an exact middle ear problem (Shahnaz et al., 2013). Studies had also reported that hit rates for detecting otosclerosis improved when ethnic-specific norms were applied (Shahnaz & Bork, 2006). This indicates that ethnic-specific norms are warranted for precise identification of middle ear abnormalities.

1.1.2 Need for estimating gender-specific WBT patterns

Among the factors that need attention are the gender-specific differences in reflectance patterns. Few researchers have found slightly lower peak admittance and broader tympanometric width in females when compared to males (Roup et al., 1998; Wiley et al., 1996), whereas others have reported no gender effect (Holte, 1996; Margolis & Goycoolea, 1993; Wan & Wong, 2002). In general, there exist a difference in length of the adult ear, and the cross-sectional area of the ear canal in male adults is larger than in female adults (Maroonroge et al., 2009; Rosowski et al., 2012). This difference could be contributing to variation in middle ear transfer function between males and females.

Studies on WBT and its effects on gender were reported to be uncertain. Rosowski et al. (2012) observed small differences in the power reflectance but not significant. Studies also reported that the female ear is stiffer than the male ear acoustically (Keefe et al., 2000; Margolis et al., 1999; Mazlan et al., 2015). There are also studies indicating no significant difference in gender on WBT measures in adults (Shahnaz & Bork, 2006; Sliwa et al., 2020) and children (Beers et al., 2010; Hunter, Tubaugh, et al., 2008).

Since the studies on the effects of gender variation are inconclusive, there is a need to estimate the reflectance/absorbance patterns across frequencies between the genders to see if there is any variation in WBA pattern between males and females in the Indian population.

1.1.3 Need to study the effect of pressure change on WBT

Some of the studies investigated the reflectance with the change in pressure mode and had observed changes of reflectance pattern significantly (Liu et al., 2008; Margolis et al., 1999; Robinson et al., 2016). Margolis et al. (1999) compared WBR across the broad range of discrete ear canal pressure (+300 to – 300 daPa) with that of ambient pressure (0 daPa). The study results showed increased reflectance from 250 Hz through 3000 Hz and decreased reflectance above 3000 Hz for the pressure change compared with the ambient pressure of 0 daPa. The authors have attributed this to the increase in stiffness of the tympanic membrane.

The effect of two pressure mode (ambient pressure, tympanometric peak pressure) was studied by Kenny (2011) in Caucasian and Chinese young adults. The results indicated that there exists significant interaction between ethnicity, frequency, and mode of measurement. During ambient pressure measurement, absorbance from 250 to 2500 Hz was less for both the groups, whereas higher absorbance was seen between 4000 to 5000 Hz only in the Caucasian group. Also, the 90% range of the dynamic measurement is notably higher than that of the static measurements in the low to mid-frequency region. These observations were consistent with the measurements reported by Liu et al. (2008).

Even though the literature in this area is very limited, the existing studies had indicated the change in reflectance patterns across the pressure mode. Thus, there is a need to conduct a comprehensive study of different pressure modes in estimating the reflectance/ absorbance patterns.

1.1.4 Need for validating WBT on different middle ear disorders

The widely used middle ear transfer function tests currently available can detect only the middle ear status. However, it does not provide appropriate information related to various middle ear disorders. For example, patients with otosclerosis often have conductive hearing loss and normal or low compliance tympanogram. Similar observations are also seen in other conditions such as middle ear effusion or a low-compliance eardrum that may confuse this profile (Browning et al., 1985; Browning & Gatehouse, 1992). Moreover, it shows poor diagnostic accuracy in detecting otosclerosis (Margolis et al., 1999; Muchnik et al., 1989). The sensitivity and specificity of 226 Hz in identifying otosclerotic ears were low (Shahnaz & Polka, 1997, 2002).

The development of objective methods with superior test performance, such as WBT, may enhance middle ear pathology identification. It was found to be more sensitive than traditional 226 Hz tympanometry and Multi-frequency tympanometry in diagnosing otosclerosis (Shahnaz, Longridge, et al., 2009). Hunter, Bagger-Sjoback, et al. (2008) found a higher sensitivity of WBT in detecting otitis media in infants and children with a cleft palate. Feeney et al. (2003) suggested a distinctive WBT pattern in different pathologies of the middle ear. However, the reflectance profiles of different middle-ear pathologies are not well-established. There is a need to establish the sensitivity and specificity of a variety of middle-ear disorders, including otosclerosis and tympanic membrane perforations that can be detected using WBT. Thus, there is a need for population-specific norms to improve any test’s sensitivity and specificity (Shahnaz et al., 2013).

Similarly, the tympanometry accuracy in identifying different middle ear pathologies can be increased compared to 226 Hz or 1000 Hz tympanometry, with the inclusion of additional frequencies. The test's sensitivity would significantly improve as the results would greatly differ between normal and pathological ears (Sanford et al., 2009). Sanford et al. (2009) indicated that the mid-frequencies from 800 Hz to 2000 Hz had the largest difference in absorbance values between normal and pathological ears and probably should be considered to detect middle ear problems. Thus, the wideband average tympanometry that averages the absorbance values across frequencies between 375 Hz to 2000 Hz (Interacoustics, 2020) might provide distinct values that might help identify different middle ear pathologies. Further, it provides reliable results even in noisy conditions, as it averages over 30 tympanogram to obtain a single curve in a shorter time that cancels out the noise and artefacts (Martin, 2019). However, there are very limited studies (Karuppannan & Barman, 2020, 2021; Kaya et al., 2019; Kim et al., 2019; Sliwa et al., 2020; Terzi et al., 2015) which has used wideband absorbance average to identify different middle ear pathologies. Thus, there is also a need to study the wideband average tympanometry and to establish normative data that could become a potential tool to detect middle ear disorders.

The above summary suggests that there could be a lot of variation in reflectance patterns reported and observed in the literature. The studies' sample sizes have generally been small, and only sparse data are available on clinically feasible wideband reflectance criteria and procedures in disordered individuals. To date, there are also no such studies in the Indian population on WBT. Considering all the above and the importance of WBT in the clinical decision-making process over single frequency and Multi-frequency tympanometry, there is a need to establish specific normative data with outsized samples that could lead to precise diagnosis and identification of middle ear disorders. There is also a need to clinically validate the absorbance results in various middle ear conditions, facilitating WBT that can fit into the routine clinical protocol for assessing middle-ear function in adults.

1.2 Aim of the study

The present study aimed to obtain norms for wideband absorbance tympanometry in adults with normal hearing and normal middle ear and clinically validate the results with ear-related disordered populations.

1.3 Objectives of the study

1. To estimate the wideband absorbance across frequencies in normal (healthy) ears.
2. To compare the wideband absorbance in normal (healthy) ears between males and females.
3. To compare the wideband absorbance measured at peak pressure to that of the ambient pressure in normal (healthy) ears.
4. To compare the wideband absorbance between middle ear disorders (Tympanic membrane perforation, Middle ear effusion without perforation, Otosclerosis, and Eustachian tube dysfunction) and normal (healthy) ears. Also, to estimate sensitivity and specificity of wideband absorbance in identifying different middle ear disorders.
5. To compare the wideband absorbance between the ears with sensorineural hearing loss and normal (healthy) ears and to estimate sensitivity and specificity of wideband absorbance in identifying sensorineural hearing loss.
6. To compare wideband absorbance across different pathological conditions.

1.4 Hypotheses of the study

1. There is no significant difference in wideband absorbance in normal (healthy) ears between males and females.
2. There is no significant difference in wideband absorbance measured at peak pressure to that of the ambient pressure in normal (healthy) ears.
3. There is no significant difference in wideband absorbance between middle ear disorders (Tympanic membrane perforation, Middle ear effusion without perforation, Otosclerosis, and Eustachian tube dysfunction) and normal (healthy) ears.
4. There is no significant difference in wideband absorbance between the ears with sensorineural hearing loss and normal (healthy) ears.
5. There is no significant difference in wideband absorbance across different pathological conditions.

Chapter 2

REVIEW OF LITERATURE

The auditory system is dependent on the mechanical transmission of sound transmitted through the tympanic membrane to the inner ear. The sound waves vibrate the tympanic membrane and the middle ear's ossicles, which set the fluid motion in the cochlea. The cochlea's fluid motion causes neurochemical changes that help transmit the sound to the brain (Martin & Clark, 2019). Any structural or physiological changes in the middle ear can affect the sound transduction to the inner ear (Norrix et al., 2013). This change may be due to a variety of factors, such as the presence of middle ear pathology or anatomical differences observed by gender, ethnicity, and maturation, which can attenuate frequency-specific sound transmission (Beers et al., 2010; Mazlan et al., 2015; Shahnaz & Davies, 2006).

The change in sound transmission through the middle ear is usually assessed using a middle ear analyser, which uses a single or multiple frequencies tones and measures transmission of sound energy across the pressure (Keefe et al., 1993; Margolis et al., 1999; Shahnaz & Polka, 2002; Voss & Allen, 1994). However, the introduction of WBA tympanometry in recent years has the potential to assess the middle ear function at a wide range of frequencies and can also be measured even without any change in pressure (Keefe et al., 1993; Liu et al., 2008). The recent addition of WBA tympanometry showed good sensitivity in identifying middle ear disorders (Aithal, Aithal, Kei, Anderson, et al., 2019; Lilly & Margolis, 2013; Margolis et al., 1999; Nakajima et al., 2013; Shahnaz & Polka, 2002). Thus, the literature review focuses on the principles of WBA tympanometry and several sources of variability that influence the WBA measurements.

2.1 Wideband tympanometry (WBT)

WBT is the proportion of acoustic energy absorbed or reflected by the middle ear, i.e. when a calibrated transient broadband stimulus is directed towards the tympanic membrane in a sealed ear canal, the amount of energy that is absorbed or reflected at the tympanic membrane is calculated across frequencies (Hunter & Shanaz, 2014; Liu et al., 2008; Margolis et al., 1999). The parameters measured in WBT measurements include reflectance or absorbance, generally called Wideband absorbance (WBA) or Wideband reflectance (WBR). Other terms used in the literature are acoustic reflectance/acoustic absorbance (Margolis et al., 1999); Power reflectance/Power absorbance (Nakajima et al., 2013) and Energy reflectance (Jaffer, 2016; Wang et al., 2019)/ Energy absorbance (Burdiek & Sun, 2014; Hougaard et al., 2020; Liu et al., 2008). However, “Wideband acoustic immittance (WAI)” is an alternative term of WBT, which includes both WBA and WBR measurements (Feeney, 2013).

WBA is represented as a percentage equal to the ratio of acoustic energy absorbed by the middle ear to the acoustic energy of a stimulus presented in the ear canal (Liu et al., 2008). Conversely, WBR is the inverse ratio of WBA or the acoustic energy reflected, to the total acoustic energy delivered to the ear canal, i.e. WBR equals 100% minus WBA (Keefe & Feeney, 2009; Neely et al., 2013; Sanford et al., 2013). Both WBA and WBR are optimal measures of the middle ear's mechanical properties (Liu et al., 2008). The present study uses the term wideband absorbance (WBA) to quantify the amount of sound energy entering the middle ear. The WBA measurements are quantified in a real number that ranged between ‘0’ and ‘1’ and are expressed only in magnitude without dimension and phase (Feeney et al., 2013; Liu et al., 2008; Stinson, 1990). The value ‘one’ in WBA indicates that the middle ear absorbed all the sound energy, while ‘zero’ indicates that all sound energy is reflected to the ear canal (Liu et al., 2008; Stinson, 1990).

WBA measurements are currently available in three equipment, namely Otostat and HearID systems from Mimosa Acoustics, USA and Titan from Interacoustics, Denmark. Mimosa Acoustics use the chirp and pure-tone stimuli to measure power reflection and power absorbance (Mimosa Acoustics, 2002; Shahnaz & Bork, 2006). The difference in stimuli (Chirp, Pure-tone) on power reflectance/absorbance measurements in infants with a normal middle ear showed similar results, indicating that both stimuli were equivalent (Santos et al., 2015). Although there exists no significant difference between the stimulus, the pure-tone stimuli provide reliable results in noisy situations, while the chirp stimulus provides good frequency resolution (Mimosa Acoustics, 2014). The Titan system from Interacoustics, Denmark, uses the click stimulus and measures only the WBA (Hein et al., 2017; Interacoustics, 2020).

As similar to the 226 Hz tympanogram, the WBT data was presented in a two-dimensional graph representing frequency (226 Hz to 8000 Hz) in the x-axis and absorbance (0 to 1) in the y-axis, called as ‘Wideband tympanogram’. In addition, the Titan system provides a three-dimensional graph, called as 3-D tympanogram with pressure (y-axis), frequency (x-axis), and absorbance (z-axis) to represent WBT data (Hein et al., 2017; Interacoustics, 2020).

Although the WBA is a new middle ear analyser to assess the middle ear's status, studies have shown more advantages than traditional tympanometry and multi-frequency tympanometry (Keefe et al., 1993). The main advantage of the WBA is the use of a wide range of frequency to assess the condition of the middle ear (Hein et al., 2017; Shahnaz et al., 2013). It supports evidence for comparison with other hearing tests such as pure tone audiometry, otoacoustic emissions, and auditory brainstem response that uses a wide range of frequencies important for speech and language comprehension (Hunter & Shanaz, 2014). In addition, the WBA can be measured without applying pressure in the ear canal, i.e. at ambient pressure, which would be beneficial for infants, post-operative conditions and even in cases where the ear canal seal is difficult to maintain. Another advantage is that the WBA measurement is not affected by the depth of the probe insertion. Therefore, the probe's position is not a decisive factor than traditional tympanometric measurements (Allen et al., 2005; Margolis et al., 1999; Voss & Allen, 1994). Studies have also shown that the WBA is sensitive in detecting middle ear disorders over multi-frequency and single-frequency tympanometry (Kim et al., 2019; Nakajima et al., 2013; Shahnaz, Longridge, et al., 2009).

However, several factors such as age, gender, ethnic difference, and pressure variations can influence the WBA measures, affecting its sensitivity (Feeney et al., 2014; Voss et al., 2008, 2013). The sources of variability in wideband absorbance measurements are described further in the review section.

2.2 Effect of gender on WBA measures

Earlier studies on WBA had shown inconclusive results, with few indicating a significant effect on gender and a few other studies showing no effects. Feeney and Sanford (2004) investigated the effect of gender on WBA at ambient pressure in 40 young adults (mean age - 21.4 years) and 30 older adults (mean age - 71.6 years). The results showed a significant reduction in absorbance for female than young male adults in the low frequencies at 794 and 1000 Hz, but higher absorbance at 5040 Hz. A similar WBA pattern trend as that of young adults was also reported in elderly individuals but did not show any significance. Feeney et al. (2014) demonstrated a reduction in mean absorbance at frequencies below 3000 Hz and a higher mean absorbance above 3000

Hz for females than in male participants aged 23.9 – 51.4 years. Mazlan et al. (2015) also have noted lower WBA in frequencies ranging from 2830 to 4490 Hz in males compared to females and higher WBA in males at frequencies below 1000 Hz.

Males had higher WBA values at all frequencies except for the range of 3150-5000 Hz. Certain studies showed a significant difference in increased absorbance values only at higher frequencies around 3000 Hz to 6000 Hz in females than young male adults (Jaffer, 2016; Kenny, 2011; Polat et al., 2015; Rosowski et al., 2012; Shahnaz et al., 2013). Whereas, Riddler (2017) had shown higher WBA values at all frequencies in males except for the range of 3150-5000 Hz. Kenny (2011) had reported a significant interaction between gender, ethnicity and frequency at ambient pressure, showing higher absorbance between 4000 and 5000 Hz for Chinese females than males, while the Caucasian group did not show any difference. Whereas at peak pressure, both the Chinese and Caucasian group showed a significant difference, with females having higher absorbance than males at 5000 Hz. Jaffer (2016) studied the gender difference on WBT_{avg} measurements and had shown higher averaged absorbance values in males than females at both the peak and ambient pressure.

Several authors had attributed this gender difference to the difference in sizes of the body, middle ear cavity, and ear canal (Brucker et al., 2003; Jaffer, 2016). Males found to have a larger body size than females that could probably have larger ear canal volume and middle ear cavity, leading to an increase in the middle ear’s mass component. Studies have also shown that males had less stiffness dominated tympanic membrane and greater middle ear resistance for frequencies below 1000 Hz but had less resistance between 2000 Hz and 4000 Hz (Margolis et al., 1999; Mazlan et al., 2015). Females had higher reactance magnitudes and resistance magnitudes at low frequencies below 600 Hz than males (Werner et al., 2010). This contributes to the large impedance and leads to decreased absorbance at low-frequencies and increased absorbance at high frequencies (Allen et al., 2005; Beers et al., 2010; Feeney et al., 2014; Shahnaz & Bork, 2006). Keefe et al. (2000) found lower WBA values below 2000 Hz in males than female neonates, and the authors had related to the middle ear stiffness where female ears were stiffer than male ears.

However, few other studies have shown no significant differences between males and females in adults (Shahnaz & Bork, 2006; Sliwa et al., 2020; Werner et al., 2010), children (Beers et al., 2010; Hunter, Tubaugh, et al., 2008) and in neonates (Aithal et al., 2013; Werner et al., 2010) at ambient pressure conditions. Though most of the studies had shown a significant gender effect, especially in adults, there is no uniformity and showed variable results in WBA across frequencies.

2.3 Effect of ear on WBA measures

The effects of the ear on WBA measurements have not been studied extensively. Researches on the effect of the ear on WBA showed variable results, with the majority of the studies showing no significant difference between right and left ears (Burdiek & Sun, 2014; Feeney & Sanford, 2004; Jaffer, 2016; Kenny, 2011; Liu et al., 2008; Riddler, 2017; Shahnaz & Bork, 2006; Shaw, 2009; Sliwa et al., 2020). Few studies have found higher WBA for the left ear than the right ear (Feeney & Sanford, 2004; Rosowski et al., 2012; Werner et al., 2010). In contrast, Feeney et al. (2014) showed higher absorbance in the right ear than in the left. This difference of higher absorbance could be due to reduced stiffness in the left ear than the right ear (Feeney & Sanford, 2004; Rosowski et al., 2012; Werner et al., 2010).

Though the studies have shown a significant difference in WBA between the ears, the difference is relatively small (Feeney & Sanford, 2004; Werner et al., 2010) and may have little or no impact on WBA norms.

2.4 Effect of maturation and age on WBA measures

Several changes in the human auditory system can occur during their developmental and ageing progress. It was reported that the middle ear is not matured until six years and undergoes lots of changes during their infancy and continues across the life span (Hunter et al., 2016; Mishra et al., 2017). Studies have investigated the effect of maturational changes on WBA and have shown higher absorbance at low and mid-frequencies compared to adults (Aithal et al., 2014; Hunter et al., 2008, 2016; Keefe et al., 1993; Keefe & Levi, 1996; Sanford & Feeney, 2008; Werner et al., 2010)

Keefe et al. (1993) compared the WBA obtained from 78 infants aged 1 to 24 months with ten adults and found changes in absorbance with increasing age. The study showed a significant decrease in absorbance from birth to six-month for frequencies below 1000 Hz, attributed to the flaccid ear canal movement. Similar results of higher absorbance at low and mid-frequencies was reported in the literature during the infancy period (Sanford & Feeney, 2008; Werner et al., 2010). The WBA decreases as the age progress with lower mean absorbance in young adults than neonates and infants up to 6 months (Feeney & Sanford, 2004; Hunter et al., 2016).

Hunter et al. (2016) observed that the WBA pattern was flat at birth and changes to a peaked pattern similar to adult by six months of age. Further, the maximum absorbance was obtained slightly below 2000 Hz in infants compared to adults with a broad maximum around 2000 to 4000 Hz. Shahnaz et al. (2014) study on infants from birth to six months showed decreased absorbance at low frequencies and increased absorbance at high frequencies as a function of age with little change around 600 to 1600 Hz. Few studies had reported a slight difference in absorbance across frequencies in infants from birth to six months but not significant except at 2000 Hz (Merchant et al., 2010) and 6000 Hz (Hunter, Tubaugh, et al., 2008). The authors attributed the differences to the methods, equipment and probe tips used (Hunter, Tubaugh, et al., 2008; Merchant et al., 2010).

WBA values also vary between paediatric and adult populations. Shahnaz (2008) compared the WBA obtained from 26 NICU neonates infants with normal hearing adults. Results showed higher absorbance for NICU babies below 727 Hz compared to Normal hearing adults. Beers et al. (2010) compared the energy reflectance of 78 school-aged children to adult data from Shahnaz and Bork (2006). Lower energy reflectance (high absorbance) was reported between 2500 and 5000 Hz in children than adults. Mazlan et al. (2015) indicated higher mean absorbance values in the high-frequency region (2000 to 5000 Hz) for young adults than middle-aged and older adults.

Feeney et al. (2014) did a longitudinal study and found that the absorbance values were significantly higher at low-frequency in middle-aged adults (30 to 39 years) compared to older (40 to 49 years) and younger adults (20 to 29 years). In contrast, the younger and older age group did not show any significant difference in WBA. Feeney and Sanford (2004) study on young (18-28 years) and older adults (60- 85 years) showed a reduction in energy reflectance (increase in absorbance) from 800 to 2000 Hz in young adults while an increase in reflectance (decreased absorbance) around 4000 Hz in older adults. The study suggested that this could be due to a reduction in stiffness in older adults compared to younger adults. Similar results of decrease in energy reflectance (increased absorbance) from 1007 to 5039 Hz in young adults (18–25 years) than older adults (50–65 years) were reported by Carpenter et al. (2012). The authors attributed the change in energy reflectance to the difference in hormones between the age groups. In contrast, Rosowski et al. (2012) did not report any difference in energy reflectance as a function of age except at 1000 Hz.

Further, studies have shown the difference in WBA pattern among the age groups. Feeney and Sanford (2004) showed two reflectance minima (maximum absorbance) around 1000 to 2000 Hz and between 4000 and 5000 Hz in the majority of the older adults (63%), while only 15% of the young adults had a similar pattern. They attributed this to the reduction in stiffness in older adults than younger adults. Similarly, Margolis et al. (1999) reported two maxima around 1200 Hz and 3500 Hz in 20 adults aged between 20 and 53 years, while other studies had shown a single broad maximum absorbance around 1000 to 4000 Hz (Keefe et al., 1993; Sanford & Feeney, 2008; Voss & Allen, 1994).

It is understood that there is a change in absorbance from infancy to older adults indicating the developmental change to WBA, and these changes are not consistent. This variation in WBA from infancy through older adults could be the difference in body size and also change in stiffness characteristics of the middle ear, i.e. increased stiffness for adults while decreased stiffness for paediatrics and older adults was reported (Howarth & Shone, 2006; Ruah et al., 1991; Shahnaz et al., 2013). Thus, age-specific normative WBA data separately for paediatric and adults is essential, as Shahnaz et al. (2013) suggested.

2.5 Effect of Ethnicity on WBA measures

Reports from previous studies have indicated the variation in WBA on different ethnic populations due to the difference in the middle ear's acoustical properties (Shahnaz, 2010; Shahnaz & Bork, 2006; Shaw, 2009). Shahnaz and Bork (2006) studied ethnicity's effect on energy reflectance in 126 normal-hearing adults in the age of 18 to 32 years. The Chinese individuals had a higher mean energy reflectance (lower mean WBA) in the lower frequencies from 469 Hz to 1500 Hz and lower mean reflectance (higher mean WBA) from 3891 Hz to 6000 Hz than the Caucasian individuals. Also, the energy reflectance pattern of Chinese individuals had a single minimum near 3141 Hz, while the Caucasian individuals had two minima, around 1617 Hz and 3164 Hz. These findings are in consensus with other studies, which also found interactions between ethnicity and frequency for mean WBA in adult subjects (Jaffer, 2016; Kenny, 2011; Shaw, 2009).

Likewise, two maxima in the 1200 Hz and 4000 Hz region were reported in Singaporean adults (Tan & Martin, n.d.), similar to the Caucasian individuals reported in Shahnaz and Bork (2006)'s study. Similarly, there are reports of increased energy reflectance in the lower frequency regions, with the maximum absorbance seen around 4000 to 5000 Hz in Danish Caucasians adults (Hougaard et al., 2020). Although exact reasons for the difference among the ethnic groups are unknown, body size variations between ethnic groups are considered as a contributing factor according to several scholars (Jaffer, 2016; Kenny, 2011; Shahnaz & Bork, 2006; Shaw, 2009). Generally, the average height and weight are larger in Caucasian than in Chinese individuals (Rosowski et al., 2013; Shahnaz & Bork, 2006). Large body size may have larger ear canal and middle ear volume that could alter the middle ear stiffness, thereby increasing low-frequency energy for Caucasian than Chinese individuals (Hunter & Shanaz, 2014; Shahnaz, 2010). Also, an increase in body size tends to have increased size of the middle ear structures that could increase the middle ear mass component (Jayesh et al., 2014; Werner et al., 1998; Werner & Igić, 2002). This could reduce absorbance at higher frequencies in the Caucasian group than the Chinese group (Allen et al., 2005; Shahnaz & Bork, 2006).

These differences are also noted in paediatric subjects of varying ethnicities (Abbott, 2018; Aithal et al., 2014; Beers et al., 2010). Beers et al. (2010) on 78 school going children had reported lower WBA values over the mid-frequency range in

Caucasian children compared to Chinese children. Similarly, Aithal et al. (2017) reported a significant difference in WBA obtained at ambient pressure between aboriginal and non-aboriginal neonates. Aboriginal neonates who passed the screening test showed reduced WBA from 400 Hz to 2000 Hz than non-aboriginal babies. Likewise, those aboriginal neonates who failed the screening tests also showed lower absorbance values between 1500 Hz and 3000 Hz than non-aboriginal neonates. Such variation in WBA was attributed to the difference in birth weight, with non-aboriginal neonates have higher birth weight than Australian aboriginal.

In contrast, few studies showed no significant difference among the ethnic groups considered in their study (Aithal et al., 2019; Wali & Mazlan, 2018). Wali and Mazlan (2018) studied the difference in WBA measured at 0 daPa in well nursery neonates of three ethnic groups, i.e. Malays, Chinese, and Indians. The study did not report any significant difference among the ethnic groups. However, the lower absorbance values were observed in Chinese babies between 1250 Hz and 5000 Hz, while identical WBA response across frequencies was seen in Malays and Indians neonates. This could be because of the similarity in the average height and weight between Chinese, Malays and Indians (Wali & Mazlan, 2018). Similarly, Aithal et al. (2019) compared WBA measurements in Caucasian and Australian Aboriginal children at peak and ambient pressure (0 daPa). The study showed a significant effect of ethnicity with a small effect size on WBA measured at peak pressure, with Caucasian children showing higher absorbance values than Aboriginal children at 3000, 4000 and 8000 Hz.

Thus, irrespective of the age group, studies showed a significant difference in WBA among different ethnicities. It shows the importance of measuring ethic specific norms to validate with the clinical population.

2.6 Effect of instrument on WBA measures

Widely available wideband reflectance/absorbance instruments reported in the literature are Mimosa Acoustics HearID, Otostat Mimosa Acoustics, Titan Interacoustics and Reflwin Interacoustics. Several researchers have proven that the absorbance varies among the instrument used to measure WBA (Kenny, 2011; Shahnaz et al., 2013; Shaw, 2009).

Shaw (2009) had reported higher absorbance at ambient pressure for frequencies below 2000 Hz measured using the Reflwin Intercoustics system compared to those obtained with the Mimosa Acoustics HearID system. In comparison, Kenny (2011) found a higher estimate of absorbance only at 5000 Hz in Caucasian individuals measured using Reflwin Interacoustics than those measured using Mimosa Acoustics HearID system, while Chinese individuals did not show any significant difference. Jaffer (2016) studied the difference in WBA at ambient pressure obtained using HearID and Otostat from Mimosa Acoustics; and Titan and ReflWin from Interacoustics. The study showed a significant difference in mean absorbance across frequencies between the instruments. Interestingly, the difference was also observed significantly between the devices of the same company, i.e. Titan and Reflwin of Interacoustic devices (400 Hz to 2000 Hz and 3150 to 5000 Hz); and HearID and Otostat from Mimosa acoustics (6300 Hz).

The authors above had attributed this variation in WBA to the difference in calibration procedures used among the devices, the estimation of ear canal area and the variation in the ear tip used to achieve a seal. However, the observed difference was found to be small between these systems compared to the difference in absorbance obtained among the different middle ear pathologies that are reported in the literature

(Feeney et al., 2003; Shahnaz et al., 2013; Shahnaz, Bork, et al., 2009; Shahnaz & Bork, 2006).

2.7 Effect of pressurization method on WBA measures

WBA measurements can be performed at ambient pressure, i.e. static pressure present in the ear canal (Shahnaz, Longridge, et al., 2009) and at tympanic peak pressure (Hunter & Shanaz, 2014; Keefe & Levi, 1996). During the pressurized conditions, the tympanic membrane and ossicular chain are stiffened at extreme positive and negative pressures leading to reduced WBA values. While at tympanic peak pressure, the ear canal's pressure approximates pressure in the middle ear cavity and has the highest WBA because of the tympanic membrane's maximum mobility (Hunter & Shanaz, 2014).

Studies have shown the difference in WBA measured between ambient and peak pressure (Aithal, Aithal, Kei, & Manuel, 2019; Feeney et al., 2017; Keefe et al., 2015; Liu et al., 2008; Sun, 2016). The WBA at ambient pressure was reported to be lower at low frequencies, increasing to a maximum around 1000 Hz to 4000 Hz and decreasing at high frequencies in ears with normal middle ear (Kenny, 2011; Shahnaz & Bork, 2006; Shaver, 2010). While at peak pressure, it was reported an increase in absorbance at low frequencies and decreases at high-frequency just above the resonance frequency (Margolis et al., 1999). Also, the maximum absorbance at peak pressure was slightly towards higher frequencies compared to the ambient pressure. The increase in the middle ear's stiffness due to pressurisation could explain the difference in absorbance between pressure conditions (Margolis et al., 1999).

Liu et al. (2008) compared energy absorbance obtained at peak and ambient pressure. The study found higher energy absorbance at peak pressure than ambient pressure at low frequencies and slightly lower at high frequencies. The author attributed this variation to the positive residual pressure present during the probe insertion for ambient-pressure measurement. Also, the study measured energy absorbance at different pressure-sweep speed (75, 100, 200, and 400 daPa⁄s) and direction (ascending and descending directions) at peak pressure. The results showed similar energy absorbance for the pressure speed of 75, 100, and 200 daPa⁄s while there is a difference in pressure sweep direction.

Aithal et al. (2019) compared WBA obtained between peak and ambient pressure on 171 ears from 171 Caucasian children and 87 ears from 87 Aboriginal children. The study reported differences in WBA obtained between peak and ambient pressure conditions at frequencies ranging from 250 Hz to 1500 Hz for Caucasian children and up to 1250 Hz for aboriginal children. Similarly, a large difference of WBA up to 2000 Hz has been observed in young adults (Feeney et al., 2017; Liu et al., 2008; Sun, 2016). Sun (2016) on 84 healthy young adults showed a significant difference in energy absorbance between the pressure conditions with lower absorbance seen at ambient pressure than peak pressure. Shaver (2010) reported higher power absorbance measured at peak pressure than ambient pressure for Caucasians and Chinese groups. Similar results of higher absorbance at peak pressure than ambient pressure in the low and mid-frequencies up to 2000 Hz were reported in the Indian population aged 22 to 50 years (Karuppannan & Barman, 2021b). In another study on the Indian population by Karuppannan and Barman (2021a), WBA at peak pressure was higher than ambient pressure for low and mid-frequencies but significant only at 250 and 500 Hz. While Karuppannan and Barman (2020) observed similar results with higher WBA at peak pressure than ambient pressure in the normal hearing individuals but did report any significant difference between the two pressure conditions. This could be because of the limited sample size in their study and the negligible pressure difference between peak and ambient pressure.

Several studies had examined WBA by varying middle ear pressure towards negative and compared with normal middle ear pressure (Karuppannan & Barman, 2021b; Margolis et al., 1999; Shaver & Sun, 2013; Sun & Shaver, 2009). A large difference with the decrease in WBA at low and mid-frequencies was reported for negative middle ear pressure compared to normal middle ear pressure. This difference was greatest at ambient pressure conditions, while no difference was observed at peak pressure (Karuppannan & Barman, 2021b; Margolis et al., 1999; Shaver & Sun, 2013).

There is a dearth of literature on Wideband average absorbance tympanometry and its comparison between peak and ambient pressure. Jaffer (2016) showed slightly higher WBT_{avg} at peak than the ambient pressure for both the Chinese and Caucasians. The author had attributed this difference to the increase in stiffness of the eardrum due to pressurization. While no significant difference in WBT_{avg} between peak and ambient pressure was reported on the Indian population having a normal middle ear (Karuppannan & Barman, 2020, 2021a).

On the whole, there is a difference in WBA between the pressure conditions mostly reported in low and mid-frequencies and limited research on WBT_{avg}. A separate normative WBA data at both ambient and peak pressure is recommended.

2.8 Effect of WBA measures on middle ear disorders:

Generally, the presence of middle ear disorders affects the transmission of sound to the inner ear. This may be due to changes in the middle ear's mass and/or stiffness properties (Kim & Koo, 2015). Studies have shown that conditions related to stiffness, such as otosclerosis, negative middle ear pressure, or middle ear effusion, reduce low-frequency sound transmission. In contrast, the reduction of high-frequency sound energy is related to the increased mass of the middle ear bones, usually seen in the tympanic membrane perforation or ossicular chain discontinuity (Allen et al., 2005; Kim & Koo, 2015). Thus, the use of WBA at all frequencies provides valuable information of the middle ear's stimulus characteristics reaching the inner ear. There are a few reports on reflectance or absorbance measures on specific middle ear disorders that are reported to alter the WBA pattern across frequencies and are given below:

2.8.1 Effect of Tympanic membrane perforation (TmP) on WBA measures

The tympanic membrane plays a key element of the tympano-ossicular system for sound transmission. Few studies have reported the effect of tympanic membrane perforation on WBA measurements (Allen et al., 2005; Feeney et al., 2003; Nakajima et al., 2013; Voss et al., 2001c, 2001b, 2001a, 2012). The effect of different sized tympanic membrane perforation on energy reflectance was extensively studied in cadaveric specimens by Voss et al. (2012). The study demonstrated a reduction in energy reflectance, i.e. increase in WBA at low frequencies up to 2000 Hz. Further, the effect of perforation size showed a large variation, with the smallest perforation showing the largest effect. The authors attributed this large effect to the low-frequency mass-generated due to air accumulation in the perforation region. This reduces the impedance, thereby allowing the low-frequency sound transmission into the middle ear (Jeng et al., 2008). As the tympanic membrane perforation size increases, the resonance moves relatively towards the high-frequency region.

Similarly, Ibraheem (2014) measured energy reflectance in tympanic membrane perforation individuals who had ‘B’ type tympanogram with high ear canal volume and compared with the control adult group's results having ‘A’ type tympanogram. The tympanic membrane perforation group showed reduced energy reflectance (higher absorbance) at low frequencies and higher energy reflectance (lower absorbance) at higher frequencies above 1000 Hz. A similar pattern was reported in the literature with reduced energy reflectance (increased WBA) up to 800 Hz and increased reflectance (decreased WBA) at higher frequencies (Feeney et al., 2003), while Allen et al. (2005) described a lower than normal energy reflectance (higher WBA) below 1500 Hz. On the contrary, Kim et al. (2019)'s study on human subjects demonstrated lower absorbance values (higher energy reflectance) at ambient pressure condition in the low and mid-frequency region, especially below 1000 Hz.

Similarly, there is a dearth of research on WBT_{avg} in ears with TmP. To date, there is only one study in the literature that had reported an average absorbance measured at ambient pressure (Kim et al., 2019). It was observed that ears with TmP had a flat absorbance curve without any measurable peaks, i.e. 'B' type tympanogram. The study also reported measurable peak pressure in a few of the individuals. However, WBT_{avg} measurements at those peak pressure were not reported.

On the whole, there is no uniformity in the frequencies with highly variable energy reflectance/absorbance pattern reported on TmP individuals. In addition, these studies were conducted with limited sample size. Further, there is a lack of research on WBT_{avg} measurements in ears with TmP, and no studies have estimated the accuracy of identifying TmP from normal ears.

2.8.2 Effect of middle ear effusion (MEE) on WBA measures

The middle ear is an air-filled cavity that allows the tympanic membrane and ossicles to vibrate efficiently for sound transmission to the inner ear. Loss of aeration results in the secretion of fluid in the middle ear leading to effusion (AAP/AAFP Guidelines, 2004). This reduces the middle ear space, thereby increase in cavity's impedance, and affects the transmission of sound to the inner ear (Nakajima et al., 2013). Research has focused on the effects of middle ear effusion on WBA measurements primarily in children (Allen et al., 2005; Beers et al., 2010; Ellison et al., 2012; Keefe et al., 2012; Piskorski et al., 1999; Terzi et al., 2015) and limited studies on adults (Feeney et al., 2003). Studies had shown increased reflectance, i.e. decreased absorbance at all frequencies and further, absorbance decrease with increased fluid in the middle ear (Dai et al., 2008; Voss et al., 2012).

The effect of fluids on wideband reflectance measurements was performed on cadaver specimens by Voss et al. (2012). In their experiments, the change in reflectance pattern was minimal when the middle ear was filled with fluid lesser than 50%. When the middle ear's fluid exceeded 50%, there was an increase in reflectance across frequencies. In addition, a drastic reduction in absorbance was observed at lower frequencies (200 to 500 Hz), where the middle cavity was almost filled with fluid. Similar experiments have been conducted to study the umbo velocity in the fluid-filled middle ear cavity (Gan et al., 2006; Ravicz et al., 2004). The results showed a decrease in umbo velocity across frequencies. This difference at low frequencies was attributed to the decrease in the middle ear volume and at high frequencies due to increased fluid in the middle ear and tissue load on the tympanic membrane.

Studies on human ears have also shown increased reflectance, i.e. decreased absorbance (Allen et al., 2005; Beers et al., 2010; Ellison et al., 2012; Piskorski et al., 1999) as similar to the experimental studies, except the sharp minima at lower frequencies. However, Feeney et al. (2003) showed increased energy reflectance below 4000 Hz in adults, while there was a notch at high frequencies nearing the normal value. Further, the increment in reflectance value is constant over all the frequencies, especially below 4000 Hz (Allen et al., 2005; Jeng et al., 2008).

Few studies have compared the WBA obtained at the peak and ambient pressure in ears with middle ear effusion (Keefe et al., 2012) and the WBT_{avg} measurements

(Terzi et al., 2015). As similar to the existing studies on MEE, Keefe et al. (2012) showed a reduction in absorbance in children with MEE for both pressure conditions. However, the study did not show any significant difference in WBA measured between peak and ambient pressure in ears with MEE. Terzi et al. (2015) had measured WBT_{avg} (375 Hz to 2000 Hz) at ambient pressure in children. They reported lower average absorbance in the otitis media with effusion group than the otitis media and healthy ear groups.

The precision of WBA measurements in MEE has previously been studied in infants and children (Beers et al., 2010; Ellison et al., 2012; Terzi et al., 2015). Beers et al. (2010) found a high test performance in identifying MEE from normal ears for frequencies above 800 Hz with the largest AUROC at 1250 Hz with a sensitivity of 96% and specificity of 95%. While Terzi et al. (2015) had reported a high diagnostic value for frequencies at 1000 Hz and 1500 Hz, as well as for wideband average absorbance tympanometry in identifying MEE. Likewise, Ellison et al. (2012) had determined the accuracy of wideband acoustic transfer functions measured at ambient pressure in children with MEE. The authors have reported that the mid-frequencies of 800 Hz to 2000 Hz are most sensitive in identifying MEE. Overall, there is a reduction in absorbance across frequencies in ears with MEE than a normal ear and are evident at mid frequencies. However, most of the studies are performed in children with a limited sample size. Further, there is dearth of literature on adults with MEE, and further, no studies have used WBT_{avg} measurements in ears with MEE. In addition, no studies have reported the accuracy of WBA in identifying MEE from normal ears in the adult population.

2.8.3 Effect of Otosclerosis on WBA measures

Otosclerosis is generally seen in the adult, due to the fixation of the stapes footplate in the oval window causing conductive hearing loss (Nakajima et al., 2012; Shahnaz & Polka, 1997). This fixation increases the tympanic membrane's stiffness, thereby decreasing the sound transmission to the middle ear, especially at the low-frequencies (Møller, 2014). Recent studies on WBA measurements have shown to differentiate otosclerosis from normal ears (Allen et al., 2005; Feeney et al., 2003; Karuppannan & Barman, 2020, 2021; Keefe et al., 2017; Kelava et al., 2020; Shahnaz, Bork, et al., 2009; Wang et al., 2019).

Shahnaz, Bork, et al. (2009) had considered surgically confirmed otosclerotic ears to evaluate the wideband reflectance measurements across frequencies in adults. The results showed a statistically significant increase in energy reflectance (decrease absorbance) below 1000 Hz compared to the normal ears. Similar results of higher energy reflectance (decrease absorbance) below 1000 Hz were reported by Feeney et al. (2003) in two otosclerotic adult patients. Energy reflectance at high frequencies was similar to the normal ears. Whereas Allen et al. (2012) reported an increase in energy reflectance (decrease absorbance) below 800 Hz in bilateral otosclerosis patients. Few other studies had shown a reduction of energy absorbance (increase in reflectance) beyond 1000 Hz, and this could be due to the severity of the stapes fixation at the oval window (Karuppannan & Barman, 2020, 2021a; Neto et al., 2014; Shahnaz, Bork, et al., 2009).

According to the literature, the reduction of absorbance (increase in reflectance) at lower frequencies has been related to the increased tympanic membrane and annular ligament stiffness. This creates a high impedance, thus reflecting most of the low-frequency energy into the ear canal (Allen et al., 2005; Feeney & Keefe, 1999). While at high frequencies, the studies had reported no difference in wideband reflectance/absorbance compared to normal ears (Feeney et al., 2003; Pickles, 2012). In contrast, the study reported higher energy reflectance for frequencies below 4000 Hz in Chinese otosclerotic patients compared to the normal ear group (Wang et al., 2019). Ibraheem (2014) also showed similar results of higher reflectance at frequencies up to 4000 Hz but did not find a significant difference.

There are limited studies that have noted the difference in WBA obtained between peak and ambient pressure conditions in otosclerosis (Karuppannan & Barman, 2020, 2021a). Studies on WBA at peak pressure in Indian adults have shown a significantly higher absorbance up to 1500 Hz than ambient pressure conditions (Karuppannan & Barman, 2021a). Another study also found differences between peak and ambient pressure conditions in the otosclerosis group but did not reach significance (Karuppannan & Barman, 2020). This could be because of the negligible pressure difference between peak and ambient pressure in the Otosclerosis group.

The findings of the wideband average absorbance tympanometry (375 Hz to 2000 Hz) showed significantly lower average absorbance values in the otosclerosis group than the normal ear group (Karuppannan & Barman, 2021; Kim et al., 2019). Likewise, Neto et al. (2014) showed a high reflectance rate, i.e. lower absorbance in ears with Otosclerosis, averaged between 250 Hz and 6000 Hz. In contrast, Niemczyk et al. (2019) had reported a similar average absorbance (250 Hz and 4000 Hz) in the Otosclerosis group with that of a normal ear. Thus, there exists no consistency in the average absorbance measurements that are reported in the literature.

Only limited studies have demonstrated ROC analysis and reported the sensitivity and specificity in-ears with otosclerosis (Karuppannan & Barman, 2021a; Nakajima et al., 2012; Shahnaz, Longridge, et al., 2009). Most studies have shown a decrease in absorbance till 1000 Hz in otosclerosis. Also, the frequencies of 315 Hz and 500 Hz have shown to be a better indicator in identifying otosclerosis with a sensitivity of 82% at 500 Hz (Shahnaz, Longridge, et al., 2009). Whereas, Karuppannan and Barman (2021) reported high sensitivity and specificity for all the frequencies from 250 Hz to 1000 Hz, with the highest accuracy (>90%) seen at 1000 Hz (Karuppannan & Barman, 2021a). Another WBA study that averaged over 600 Hz and 1000 Hz at peak pressure showed 86%sensitivity and 100% specificity to differentiate otosclerosis in combination with an air-bone space at an average of 1 to 4 kHz (Nakajima et al., 2012). Regarding WBT_{avg} measurements, there was one study by Karuppannan and Barman (2020) that had reported high accuracy in detecting otosclerosis from normal ears only at ambient pressure conditions.

To conclude, the WBA for otosclerosis have demonstrated reduced low-frequency WBA values, but studies report that individual WBA results for otosclerotic ears overlap with the normal range. Also, there is variation in WBA values among the studies reported in the literature.

2.8.4 Effect of Eustachian tube dysfunction/negative middle ear pressure on WBA measures

One of the most common middle ear conditions is the negative middle ear pressure caused by Eustachian tube dysfunction (Shaver, 2010). It affects the transmission of sound to the inner ear due to abnormal negative middle ear pressure caused due to ETD (Shaver & Sun, 2013). Only a few studies have investigated WBA in ears with ETD in adults, and most of these studies were based on the stimulated conditions in healthy individuals/cadaveric ears (Ibraheem, 2014; Margolis et al., 1999, 2001).

Ibraheem (2014) measured energy reflectance at ambient pressure in three adult patients with ETD. The study results showed a high reflectance (reduced absorbance) ratio at low and mid frequencies. Further, as the middle ear pressure decreases towards negative, the energy reflectance increases to the maximum (decrease in absorbance), especially at the low and mid-frequencies up to 4000 Hz. These results were consistent with the outcome reported by Feeney et al. (2003). The mid-frequencies showed a large gap in admittance between normal and negative middle ear pressure conditions compared to the lower frequencies (Shaver & Sun, 2013). Karuppannan and Barman (2021b) had studied WBA at peak and ambient pressure in adults with normal middle ear pressure, positive middle ear pressure and negative middle ear pressure. The study indicated a significant difference between the groups with a greater reduction in WBA seen till 3000 Hz for positive and negative middle ear pressure conditions than the normal middle ear pressure in peak and ambient pressure. Even in children, the change in WBA is similar to that of adults and are evidently seen around 400 Hz to 2000 Hz in children with negative middle ear pressure (Aithal, Aithal, Kei, Anderson, et al., 2019; Beers et al., 2010; Hunter, Tubaugh, et al., 2008; Sanford & Brockett, 2014).

In a simulated study by Robinson et al. (2016), the evaluation of WBA in eight ears having negative middle ear pressure had shown a reduction in WBA across frequencies with the largest reduction seen between 800 Hz and 2000 Hz compared to WBA measured at ambient pressure and peak pressure. Similarly, Voss et al. (2012) studied the effect of negative middle ear pressure on WBA in cadaveric ears. In their experiment, the absorbance values decreased with a corresponding decrease in middle ear pressure for frequencies less than 2000 Hz. This was due to an increase in middle ear stiffness caused by negative middle ear pressure, which produces high impedance at the tympanic membrane level, reflecting most of the energy to the ear canal

(Robinson et al., 2016). While at high frequencies, there exhibited an increase in WBA (Robinson et al., 2016; Voss et al., 2012). Further, the change in WBA with the change in middle ear pressure is supported by the umbo-displacement measurements. In the negative middle ear pressure conditions, reduced umbo movements for frequencies below about 2000 Hz were reported. Whereas the umbo displacement at high frequencies showed an asymmetry above 2000 Hz with either increase or no change in umbo velocity (Dai et al., 2008; Gan et al., 2006; Murakami et al., 1997).

Although the studies had reported of reduction in WBA at ambient pressure in ears with negative middle ear pressure, while the WBA at peak pressure showed similar absorbance values to that of the normal ear (Aithal, Aithal, Kei, Anderson, et al., 2019; Karuppannan & Barman, 2021b; Margolis et al., 1999; Robinson et al., 2016; Shaver & Sun, 2013; Voss et al., 2012). Margolis et al. (1999) had measured WBA in a 10-year-old having recurrent otitis media and negative middle ear pressure. The WBA measurement at ambient pressure seen to have reduced, while a normal WBA pattern was observed at peak pressure. Aithal et al. (2019) had investigated the effect of WBA in children with ETD. They had demonstrated a differential absorbance pattern with significant lower WBA only at ambient pressure compared to the normal ears.

Similarly, the difference between peak and ambient pressure was studied in ears with positive, negative and normal middle ear pressure. The negative middle ear pressure group had the largest difference in WBA between peak and ambient pressure, followed by the positive middle ear pressure group. The normal middle ear pressure group had the smallest difference, and this difference in WBA was mostly seen between 600 Hz and 1000 Hz.

Thus, applying pressure in the ear canal compensates for the negative middle ear pressure, i.e. the pressure across the tympanic membrane is the same, making it more effective in the transmission of sound to the middle ear as similar to that of the normal ears (Onusko, 2004; Robinson et al., 2016; Schlagintweit, 2018). Hence, it is suggested to measure WBA at peak pressure and ambient pressure in ears with negative middle ear pressure (Margolis et al., 1999; Robinson et al., 2016).

On the whole, the effect of middle ear pressure had shown a significant reduction in WBA at ambient pressure. While similar absorbance compared to that of the normal ear was reported at peak pressure. However, none of the studies has been performed on adults who are diagnosed with ETD. Further, WBT_{avg} measurements and the accuracy of WBA in detecting ETD are not studied till date.

2.8.5 Effect of Ossicular chain discontinuity on WBA measures

Feeney et al. (2009) used five cadaver temporal bones to examine the wideband energy reflectance in ossicular chain discontinuity. In their experiment, the energy reflectance was measured in the baseline condition, disarticulated condition, and after ossicular repair. The energy reflectance for the discontinuity condition showed a notch, i.e. decreased reflectance (increased absorbance) in the low-frequency region between 561 Hz and 841 Hz, while an increase in energy reflectance (decreased absorbance) was seen at high frequencies below 2000 Hz. The low-frequency notch was disappeared after ossicular repair and was similar to that of the baseline conditions. Voss et al. (2012) performed a similar experiment in cadaver ears showed decreased reflectance with a distinctive minimum below 1000 Hz.

Likewise, in-ears with ossicular chain discontinuity showed a prominent notch around 400 Hz to 800 Hz (Feeney et al., 2003; Karuppannan & Barman, 2020; Nakajima et al., 2012). This has been attributed to the increase in the middle ear mass component because of the discontinuity of the ossicles (Karuppannan & Barman, 2020). Thus, this limits the transfer of acoustic energy at high frequencies and allows maximum energy at low frequencies (Kim & Koo, 2015).

Karuppannan and Barman (2020) compared WBA measured at peak and ambient pressure conditions in ears with ossicular chain discontinuity. The study found no difference between the two across the frequencies. Similarly, the wideband average absorbance tympanometry (375 Hz to 2000 Hz) showed similar average absorbance values to that of the normal ears, with no significant difference in pressure conditions. Nakajima et al. (2012) measured average WBA accuracy (600 Hz to 1000 Hz) in identifying the ossicular chain discontinuity in combination with air-bone gap measurements. The results showed high sensitivity and specificity of 83% and 96%, respectively.

2.8.6 Effect of hypermobile tympanic membrane on WBA measures

Studies on the hypermobile tympanic membrane using wideband energy reflectance demonstrated a low-frequency notch below 1000 Hz, like that of ossicular chain discontinuity (Feeney et al., 2003; Ibraheem, 2014). There are variations in the reflectance pattern observed among the studies. Ibraheem (2014) reported reduced reflectance in the low-frequency region around 2800 Hz, with a significant reduction seen below 1000 Hz. Also, few studies had indicated a deep notch around 500 to 600 Hz and an increase to normal range beyond that (Feeney et al., 2003; Nakajima et al., 2012). However, no significant difference at higher frequencies beyond 1000 Hz was reported (Feeney et al., 2003; Ibraheem, 2014; Nakajima et al., 2012). The variation in reflectance pattern could be due to the difference in the scared part of the tympanic membrane and the absence of a mass component of the tympanic membrane.

Further, the energy reflectance pattern is found to be similar to ossicular discontinuity and are indistinguishable. In such conditions, Feeney et al. (2003) had suggested using the other audiological test findings to differentiate between the two. However, more research is warranted to study the wideband reflectance pattern in ears with hypermobile tympanic membrane and differentiate it from other middle ear disorders.

2.8.7 WBA measures in children with Down syndrome

Handful studies have examined the effect of WBA on individuals with Down syndrome, as they are prone to have middle ear disorders (Durante et al., 2019; Hunter et al., 2017; Kaf, 2011; Soares et al., 2016). Kaf (2011) measured wideband energy reflectance in children with Down syndrome, having 'A' type tympanogram on 226 Hz tympanometry. The energy reflectance of Down syndrome children was similar to that of the normal ear with a smaller difference below 4000 Hz. In contrast, abnormally lower reflectance (increased absorbance) was reported at high frequencies. This abnormal reflectance pattern could suggest associated congenital middle ear anomalies in them (Kaf, 2011). While Soares et al. (2016) reported a similar reflectance pattern across frequencies for both the control group (children with normal hearing) and Downs syndrome having intact middle ear with normal tympanometry curves.

Whereas Durante et al. (2019) observed lower energy absorbance in children with Down syndrome than the control group, centred at 2520 Hz for 'A' type tympanogram and 226-4000 Hz with type 'B' tympanogram for both peak and ambient pressure. Hunter et al. (2017) demonstrated significantly lower wideband absorbance at 1 to 4 kHz in-ears with conductive hearing loss than normal hearing in the Downs syndrome group. Thus, it clearly indicates that children with Downs syndrome having intact middle ear had similar absorbance pattern to that of normal hearing, while with conductive hearing loss showed reduced absorbance. Thus, lower absorbance, even in the presence of a normal tympanogram, may indicate that children with downs syndrome might have middle ear abnormalities (Durante et al., 2019).

2.8.8 Effect of cochlear hearing loss and other inner ear abnormalities on WBA measures

Very few researchers had measured WBA in ears with sensorineural hearing loss (Feeney et al., 2003) and other inner ear abnormalities such as third window lesions including superior semicircular canal dehiscence (Demir et al., 2019; Nakajima et al., 2012, 2013), large vestibular aqueduct syndrome (Olszewski et al., 2017); inner ear malformations (Kaya et al., 2019); and Meniere's disease (Cakir Cetin et al., 2019; Demir et al., 2020; Li et al., 2019; Tanno et al., 2020).

2.8.8.1 Cochlear hearing loss: One study in the literature evaluated WBA pattern in the adult with bilateral sensorineural hearing loss without any other associated conditions (Feeney et al., 2003). The energy absorbance measured across frequencies in the sensorineural hearing loss was similar to that of the normal ear and are indistinguishable. The cochlea's damage does not impact the WBA measurements, as the lesion occurs within the cochlear structure while the middle ear remains intact (Davis, 2015).

2.8.8.2 Inner ear malformations: Another study by Kaya et al. (2019) evaluated the WBA in ears with cochlear hypoplasia, incomplete partition I, incomplete partition II, cochlear aplasia and complete labyrinthine aplasia having sensorineural hearing loss and compared the results with the WBA measured in normal-hearing individuals. The authors reported distinctive effects of inner ear malformations on WBA measurements at peak and ambient pressure. Although the WBA was lower for all the malformations group than the normal hearing group, the complete labyrinthine aplasia showed a large difference. A significant difference was seen for frequencies from 226 Hz to 1000 Hz between complete labyrinthine aplasia and other groups. At high frequencies between 4237 Hz and 6535 Hz, a significant difference was reported between normal hearing and other malformation groups, while no difference was seen between the malformation groups. In addition, reduced wideband average absorbance was reported for the malformation groups with the largest reduction in absorbance reported for the complete labyrinthine aplasia group. The authors attributed this difference to the change in stiffness of the middle ear especially for the complete labyrinthine aplasia group. Thus, the authors suggested that the presence of low absorbance values, flat average wideband absorbance and high resonance frequency in ears with profound SNHL could indicate the presence of complete labyrinthine aplasia.

2.8.8.3 Third window lesions: WBA was measured in a patient with a bilateral sensorineural hearing loss having enlarged vestibular aqueduct syndrome and cochlear abnormalities (Olszewski et al., 2017). The WBA values reported being slightly abnormal with a large reduction near 1200 Hz in the right, while WBA in the left ear was within normative range with unusual peaks near 500 Hz and 1500 Hz. The difference in the WBA pattern obtained in the study was attributed to some abnormalities that might be present in the cochlea, as middle ear is intact (Olszewski et al., 2017). This difference in WBA pattern was almost similar to the values obtained in the superior semicircular canal dehiscence disorder (Nakajima et al., 2013).

Studies on semicircular canal dehiscence showed a notch in energy reflectance, i.e. maximum absorbance at around 750 Hz to 1000 Hz than other frequencies (Nakajima et al., 2012). A similar WBA pattern of increase in absorbance was also reported in ears with Ossicular chain discontinuity, but at a lower level around 400 to 800 Hz (Feeney et al., 2003; Karuppannan & Barman, 2020; Nakajima et al., 2012).

However, the increase absorbance was higher for OCD with a broader peak compared to Superior semicircular canal dehiscence (Nakajima et al., 2013). Further, the accuracy of average absorbance over 600 Hz to 1000 Hz in combination with the air-bone gap averaged over 1000 to 4000 Hz showed 100% sensitivity and 95% specificity in identifying the semicircular canal dehiscence disorder.

Likewise, other measurements such as resonance frequency, maximum absorbance frequency and maximum absorbance ratio were measured in ears with semicircular canal dehiscence and compared with normal hearing ears (Demir et al., 2019). A significant reduction of resonance frequency and maximum absorbance frequency was reported in the semicircular canal dehiscence group. In contrast, a high maximum absorbance ratio was reported compared to the control group. Further, ROC analysis showed 81% sensitivity and 77% specificity for maximum absorbance ratio of above 86%; 86% sensitivity and 81% specificity for resonance frequency below 728 Hz; and 79% sensitivity and 67% specificity for maximum absorbance frequency below 1835 Hz.

2.8.8.4 Meniere's disease/ Endolymphatic hydrops: Generally, an increase on the endolymphatic fluid in the inner ear due overproduction or inadequate reabsorption is considered as Meniere's disease/Endolymphatic hydrops (Mirza & Gokhale, 2017). In ears with Meniere's disease, there is an increase in perilymphatic pressure that pushes the stapes footplate towards the middle ear, thereby restricting the movement of the ossicles and reducing the middle's compliance ear (Bianchedi et al., 1996; Darrouzet et al., 2007). In recent years, multi-frequency tympanometry was used to diagnose the ears with Meniere's disease. However, it is reported to have limited diagnostics accuracy (Franco-Vidal et al., 2005; Sugasawa et al., 2013). However, studies on WBA measurements on endolymphatic hydrops indicated information about the inner ear's status when the middle ear is intact (Darrouzet et al., 2007).

A cross-sectional study of WBA on ears with symptomatic and asymptomatic Meniere's disease was performed at two pressure conditions (ambient, peak) and compared with the normal hearing ears (Tanno et al., 2020). Reduced absorbance was reported at lower frequencies up to 1000 Hz for symptomatic cases, while it is extended to 1260 Hz for asymptomatic cases, with the lowest absorbance being symptomatic individuals. In contrast, increased absorbance was observed in higher frequencies, i.e. at 2520 Hz, 3175 Hz and 4000 Hz, with a maximum change in absorbance was reported for symptomatic cases. In normal-hearing ears, the maximum absorbance was reported at mid-frequencies at 1000 Hz, 1260 Hz, and 1587 Hz. The symptomatic and asymptomatic groups had maximum absorbance at higher frequencies between 2520 Hz and 3175 Hz. This reduction could be due to the increased pressure in the inner ear that increases the stiffness of the cochlear windows and influences the ossicles' stiffness. This would reduce the transmission of low-frequency sounds to the inner ear and allow maximum sound energy at frequencies above 2000 Hz.

Similar results of low-frequency difference with reduced absorbance up to 1000 Hz was reported in unilateral Meniere's Disease, while no difference was observed at higher frequencies (Demir et al., 2020). Also, the accuracy in detecting Meniere's disease from normal ears using ROC analysis was reported to be high at 500 Hz and 1000 Hz. Other study had measured the integral area of the WBA absorbance curve, i.e. area formed by the absorbance curve on peak pressure and frequency in adult patients with unilateral Meniere's disease (Li et al., 2019). The integral area calculated in the absorbance graph was large in the affected side, showing high sensitivity but poor specificity in detecting Meniere's disease from the normal ears.

Li et al. (2019) showed no significant difference in WBA across frequencies and also average absorbance at low and high frequencies in an acute episode of Meniere's Disease with low-frequency sensorineural hearing loss, compared to the contralateral ear without sensorineural hearing loss/Normal hearing ears (Cakir Cetin et al., 2019).

Based on the study's review, it was observed that there is a greater difference in WBA measurement for different middle ear disorders. At the same time, it is least affected in the sensorineural hearing loss. However, the difference in WBA values depends on the extent of middle ear conditions and structural defects in the inner ear.

2.9 Reliability on WBA measures

Studies evaluating WBA reliability measures in adults have reported good reliability (Feeney et al., 2014; Rosowski et al., 2012; Vander Werff et al., 2007; Voss et al., 2013; Werner et al., 2010). Vander Werff et al. (2007) evaluated reliability measurements on WBA in ten adults. The study compared WBA values obtained from baseline measurements to the second measurement without change in probe placement and with re-insertion. The results indicated excellent reliability measures for all the conditions, i.e. with and without re-insertion having a mean difference of less than 0.05 across the frequencies.

Werner et al., 2010 studied test-retest reliability measures in 183 young adults (18 - 30 years). Absolute test-retest correlation measured after two weeks showed a high correlation in low and mid-frequencies with a small difference in mean absorbance than at high frequencies, indicating greater stability of the WBA measurements. Feeney et al. (2014) compared WBA measured annually for 05 years on 112 normal-hearing adults (27.7 - 45.9 years). The results showed the test re-test variance of 0.1 for frequencies at 1000 Hz, 2000 Hz and 4000Hz. A similar result was also reported by Rosowski et al. (2012), where WBA was measured for a period of four weeks.

Burdiek and Sun (2014) investigated the effects of consecutive trials on WBA measurements on 29 normal-hearing young adults. Increased absorbance at low frequencies (<1500 Hz) and decreased absorbance at high frequencies around 2000 and 6000 Hz were reported over the WBA trails. The change in absorbance was slightly greater in the ambient pressure than at peak pressure measurements. Similar results were also reported by Sun (2016), indicating the slight variation in WBA and WBT_{avg} measured on seven consecutive trials. However, such differences in WBA across frequencies was reported to be very minimal and hence can be used as a reliable tool.

In summary, the earlier studies had indicated variation in WBA across frequencies in terms of age, gender, ear, ethnicity, instrumentation and pressure conditions. Due to its large variation and no uniformity on WBA results across studies, most researchers recommend a separate set of normative data for different populations (Keefe et al., 2000; Shahnaz & Bork, 2006). Also, studies had indicated the difference in WBA among middle ear disorders. Shahnaz and Bork (2006) also suggested using composite norms to improve test accuracy in detecting middle ear disorders. However, there are limited normative studies in the literature focused on the western population, while there are no studies on WBA in the Indian population. Most of the studies have measured WBA at one pressure condition and have not compared across the pressure conditions. Also, wideband average tympanometry measurements were reported only in a few studies with a limited sample size. Thus, more research is needed to obtain ethnic-dependent norms and determine the WBA pattern in various middle ear disorders.

Chapter 3

METHODS

This study attempted to investigate the effect of gender and pressure conditions on WBA and determine the norms in a healthy adult population with normal hearing and normal middle ear function. The study also determined the effect of middle ear disorders and sensorineural hearing loss (SNHL) on WBA and compared the same with the WBA obtained in normal healthy individuals. The study is a cross-sectional research study design with standard group comparison (Orlikoff, Schiavetti, & Metz, 2015) performed in an academic institution. The participants were recruited through a purposive non-random sampling method (Pope & Mays, 1995), wherein all eligible and interested participants are enrolled for the study.

3.1 Participants

Three groups of participants in the age range of 22 to 50 years were recruited. The sample size for the current study was determined by G*Power analyses (Faul et al., 2007). G*Power suggests a minimum total sample size of 232 participants for normal ear group to detect an effect of η_p^2= 0.04 as suggested by Richard et al. (2003) with 95% power and alpha *p*=0.05 for repeated measures ANOVA, within-between groups interactions (02 groups). For the pathological group, G*Power suggests a minimal sample size of 50 participants in each of the group (n= 245) to detect an effect of η_p^2=0.04 (Richard et al., 2003) with 95% power and alpha, *p*=0.05 for within and between-groups repeated measures ANOVA (05 groups). However, the present study's objective was to develop WBA norms and clinically validate the results with the pathological groups. Also, there are no previous studies on WBA in the Indian population, and hence, the larger sample size has been considered in the present study.

Thus, Group I consisted of 802 healthy adults (1127 ears) with normal middle ear functioning with normal hearing sensitivity. Normal hearing with the normal middle ear is defined as healthy ears, and hereinafter, Group I will be referred to as the Normal ear group. Among those, 325 healthy individuals had a normal hearing with normal middle ear functioning in both ears. Group II consisted of 378 participants (477 ears) with different middle ear disorders that included Tympanic membrane perforation (TmP), Middle ear effusion (MEE) without perforation, Otosclerosis, and Eustachian tube dysfunction (ETD) without effusion. Group III consisted of 90 adults (140 ears) diagnosed as having SNHL. Details of the number of participants/ears under each group and the different pathological conditions are provided in Table 3.1.

Table 3.1

Details of the number of participants/ears in each group along with the mean age and Standard deviation

Groups	**Diagnosis**	**No. of Participants (No. of ears)**			**Mean age ± SD (in years)**
		Males (Right/Left)	**Females (Right/Left)**	**Total (Ears)**	
Group I	Normal hearing	455 (322/310)	347 (266/229)	802 (1127)	34.41 ± 8.64
Group II	Tympanic membrane Perforation (TmP)	49 (28/28)	47 (27/26)	96 (109)	34.22 ± 9.04
	Middle ear Effusion (MEE)	52 (31/31)	50 (30/30)	102 (122)	37.53 ± 8.91
	Otosclerosis	52 (37/33)	50 (29/41)	102 (140)	35.79 ± 8.71
	Eustachian tube dysfunction (ETD)	38 (20/30)	40 (36/20)	78 (106)	37.44 ± 9.63
Group III	Sensorineural hearing loss (SNHL)	44 (34/36)	46 (35/35)	90 (140)	34.04 ± 8.45

Note. Right - Total no. of Right ears; Left - Total no. of Left ears; SD – Standard deviation

3.2 Inclusion/Exclusion criteria

3.2.1 Group I

Group I consisted of healthy individuals having normal hearing with the normal middle ear, and subsequently, Group I will be mentioned as a Normal ear group. The participants in Group I had air conduction hearing thresholds within 15 dB HL at octave frequencies between 250 Hz and 8000 Hz with an air-bone gap of less than 10 dB HL from 250 Hz and 4000 Hz (ANSI, 2019). The speech identification score was greater than 90% having more than 100 dB HL of uncomfortable level for speech. All the participants had a normal middle ear and cochlear function confirmed by the immittance measurements [Type 'A' tympanogram with the presence of ipsilateral and contralateral reflexes (Feldman, 1977)] and Transient evoked otoacoustic emission (TEOAE) responses [>3 dB SNR with reproducibility of >70% and stimulus reproducibility of >75% (McPherson et al., 2006)]. Based on a structured interview and otoscopic examination, it was ascertained that none of the participants had any history of middle ear pathology, including ear discharge and or eardrum abnormalities. Also, the ear canal was devoid of cerumen which the experienced Otologist confirmed.

3.2.2 Group II

Group II consisted of participants with middle ear disorders, which was further divided into four subgroups, i.e., Tympanic membrane perforation (TmP) group, Middle ear effusion (MEE) group, Otosclerosis group, and Eustachian tube dysfunction (ETD) group. All the Group II participants had conductive hearing loss that ranged from mild to moderately severe degree based on the four frequency (500, 1000, 2000 and 4000 Hz) pure tone average threshold with abnormal immittance findings and absence of TEOAE responses. An experienced otologist made the diagnosis of those specific middle ear disorders mentioned in the subgroups. Clinically, a qualified audiologist also diagnosed the middle ear disorders based on the following tests and criteria.

3.2.2.1 Tympanic membrane perforation group (TmP):

The inclusion of participants in the TmP group was based on the otoscopic examination, immittance measurements and confirmation from the experienced

Otologist. The participants considered in this group had a perforated eardrum, large ear canal volume (>2.0 ml), 'B' type tympanogram using 226 Hz probe tone tympanometry and absent ipsi/contralateral acoustic reflexes (AAP/AAFP Guidelines, 2004). Those participants who had middle ear effusion with perforation, cholesteatoma, disorders of ossicles and/or any other associated middle ear disorders/infections were excluded.

3.2.2.2 Middle ear effusion group (MEE):

The participants' diagnosis in the MEE group was based on the clinical practice guidelines on Otitis media with effusion (Rosenfeld et al., 2016). The MEE group participants had air-fluid filled middle ear cavity or bubbles with retracted/bulging of tympanic membrane visualized through otoscopic examination. In addition, the 226 Hz probe tone frequency showed 'B' type tympanogram and absence of ipsi/contralateral acoustic reflexes with the ear canal volume of 0.3 to 2.0 ml. Exclusion criteria were the presence of otitis media with perforation, injury to ossicles or the presence of any other associated middle ear disorders/infections. Experienced Otologist confirmed the presence of MEE without any other associated middle ear disorders.

3.2.2.3 Otosclerosis group:

The clinically accepted criteria suggested by Danesh et al. (2018) was considered for diagnosing the Otosclerosis, i.e., participants who had a history of progressive hearing loss, low-frequency conductive hearing loss (raising pattern), 2 kHz Carhart notch in bone conduction threshold measured using pure tone audiometry; speech identification scores >90%; Presence of 'A_s' type tympanogram with absent/atypical (biphasic) acoustic reflex patterns measured using 226 Hz probe tone frequency measurements; and intact tympanic membrane with no other middle ear pathologies visualized using an otoscope were considered in this group (Danesh et al., 2018). Also, participants who had a positive family history based on the structured interview were considered cofactors to confirm the disorder. Participants with tympanic membrane abnormalities, middle ear fluid, cholesteatoma, ETD, and other associated mass related pathologies were excluded. Along with the clinical findings, Otosclerosis cases with no associated middle ear disorders were confirmed by the Otologist.

3.2.2.4 Eustachian tube dysfunction group (ETD):

The ETD group consisted of participants diagnosed with ETD without effusion and no other middle ear disorders, confirmed by the Otologist. All the ETD group participants had either normal/abnormal but intact tympanic membrane with a type 'C' tympanogram having a middle ear pressure of < -100 daPa determined by 226 Hz probe tone tympanometry and an air-bone gap of ≥ 15 dB HL (Browning & Gatehouse, 1992). The participants also had either or all of the clinical signs and symptoms of 'aural fullness' or 'popping' or discomfort/pain, tinnitus, and muffled hearing. Participants with other associated middle ear disorders such as otitis media with effusion, tympanic membrane perforation, and cholesteatoma were excluded.

3.2.3 Group III

The participants in Group III were diagnosed as having SNHL with air conduction pure-tone audiometric thresholds of not less than 30 dB HL at octave frequencies (250 Hz to 8000 Hz) and an air-bone gap of ≤ 10 dB HL (250 Hz to 4000 Hz) with appropriate speech identification scores corresponding to their degree of hearing loss. An experienced Otologist ruled out the presence of external or middle ear pathology. Further, it was confirmed with immittance evaluation showing an A/A_s-type tympanogram with absence or elevated ipsi/contralateral reflex thresholds. In-addition, they had absent TEOAE responses revealing cochlear hearing loss.

3.3 Ethical consent

The study was approved by the institutional Ethical Committee for Bio-Behavioral Research Involving Human Subjects at the All India Institute of Speech and Hearing, Mysuru, India (WOF-0404/2014-15, dated 12.11.2015, see Annexure 3.1). Prior to testing, all the participants were explained about the test procedures as well as the potential risks and benefits. Written consent from each of the participants was obtained for their voluntary participation. A copy of the informed consent document, approved by the Institutional review board and signed by the participant, is given in Annexure 3.2.

3.4 Instrumentation

The data was collected using the following equipment:

1. Horus HD digital video scope system (Medimaging Integrated Solution Inc., Taiwan) examines the ear canal and tympanic membrane status.
2. Ear Check™ Middle ear monitor (Innovia Medical, Lenexa) to detect fluid in the middle ear.
3. GSI AudioStar Pro™ dual-channel clinical audiometer (Grason-Stadler Inc., Eden Prairie, USA) for estimating pure tone thresholds and speech identification scores.
4. GSI TympStar Version 2 (Grason-Stadler Inc., Eden Prairie, USA) clinical middle ear analyzer for measuring tympanogram and acoustic reflex thresholds using 226 Hz probe tone.
5. Echoport 292 II with ILOv6 ver. 6.40.0.0 (Otodynamics Ltd, Hatfield, United Kingdom) otoacoustic emission analyzer software for measuring TEOAE.

6. Titan Suite IMP440/WBT440 advanced research module (Interacoustics A/S, Middelfart, Denmark) connected to a laptop for measuring wideband absorbance tympanometry.

All the tests were carried out in a sound-treated audiometric room with the background permissible noise levels as per ANSI/ASA S3.1-1999 (R2018) standards (ANSI, 2018). The audiological equipment was calibrated once in three months as per the recommendations of ANSI/ASA S3.6-2004 (R2010) standards (ANSI, 2010) and ANSI/ASA S3.39-1987 (R2012) standards (ANSI, 2012) during the period of the data collection.

3.5 Test Procedure:

All the participants had undergone the following audiological test procedure to confirm the eligibility criterion prior to the experimental test procedure.

3.5.1 Preliminary evaluations

3.5.1.1 Structured Case history: A structured case history interview was conducted with all the participants before testing. The detailed information regarding the status of the ear and hearing, along with clinical signs and symptoms, were collected. Also, the participants' medical records indicating any confirmed middle ear pathologies or SNHL diagnosed by the Otologist were recorded.

3.5.1.2 Otoscopic Examination: All the participants were subjected to visual inspection using an otoscope to examine the ear canal's status and tympanic membrane. Each participant was seated comfortably on a chair. The physical inspection of the external ear was carried out to ensure no abnormalities in the ear canal and the tympanic membrane and the absence of excessive cerumen. The presence of middle ear effusion was estimated by observing the tympanic membrane's fluid level using an Ear-check middle ear monitor.

3.5.1.3 Pure tone and speech audiometry: After confirming the ear's status, participants were subjected to pure-tone audiometry and speech audiometry testing. Air conduction (0.25, 0.5, 1, 2, 4, and 8 kHz) and bone conduction (0.25, 0.5, 1, 2, and 4 kHz) thresholds were estimated using a modified version of Hughson and Westlake procedure (Carhart & Jerger, 1959). Speech identification scores were measured at the most comfortable level using a standardized phonemically balanced word list (Yathiraj & Vijayalakshmi, 2005). The degree of hearing loss was based on Clark's classification (Clark, 1981) by averaging four frequency's hearing thresholds (0.5, 1, 2 & 4 kHz). The diagnosis was made for each of the participants based on the audiometric results.

3.5.1.4 Immittance Audiometry: Tympanogram at 226 Hz probe-tone and acoustic reflex thresholds (Ipsi/contralateral at 0.5, 1, 2 and 4 kHz) were measured to determine the middle ear status of each participant. They were made to sit comfortably and instructed to be quiet while the test was performed. Ear canal volume, static admittance, and tympanometric peak pressure were recorded from 226 Hz probe-tone tympanometry. Feldman (1977) classification system was used to determine the type of pathology. Acoustic reflex pattern (Jerger & Jerger, 1977) based on the presence or absence of ipsilateral and contralateral reflexes was also used to identify the disorders.

3.5.1.5 Transient evoked otoacoustic emissions (TEOAE): TEOAEs were measured using non-linear click trains presented at 80 dB peSPL to confirm normal hearing and cochlear hearing loss. The signal-to-noise ratio of ≥03 dB SPL with >70% reproducibility and stimulus strength of >75% (McPherson et al., 2006) indicated the presence of TEOAE. Participants with the presence of TEOAE without any neural pathology was considered normal hearing, while the absence of TEOAE without any middle ear pathology or any obstruction in the ear canal was considered as SNHL.

Prior to participating in the study, all the participants had undergone preliminary evaluations for inclusion and exclusion criterion. Table 3.2 shows a summary of the clinical criterion that was used for the diagnosis of each participant. Based on the clinical findings, diagnosis and medical report of the Otologist, participants were considered for each of the designed groups and were enrolled for performing wideband absorbance tympanometry.

Table 3.2

Summary of the clinical criterion used for grouping the participants

Groups	**Audiometric findings**	**Immittance findings**	**TEOAE findings**
Group I	**Normal**	**Normal**	**Normal**
Normal ear group	- <15 dB HL across all octaves - ABG ≤10 dB HL	- ‘A’ type tympanogram - Normal Ipsi/Contra acoustic reflexes - No Middle ear fluid	- SNR: >3 dB - Reproducibility: >75 - Stimulus Stability:>70
Group II	**Conductive hearing loss**	**Abnormal**	**Absent**
Tympanic membrane perforation (subgroup)	- >26 dB HL and < 70 dB HL across all octaves for air conduction thresholds - <10 dB HL across all octaves for bone conduction thresholds - ABG >10 dB HL	- ‘B’ type tympanogram - ECV: >2 ml - Absent Ipsi/Contra acoustic reflexes - No Middle ear fluid	- SNR: <3 dB - Reproducibility: <75 - Stimulus Stability:<70
Group II	**Conductive hearing loss**	**Abnormal**	**Absent**
Middle ear effusion (subgroup)	- >26 dB HL and < 70 dB HL across all octaves for air conduction thresholds - <10 dB HL across all octaves for bone conduction thresholds - ABG >10 dB HL	- ‘B’ type tympanogram - ECV: 0.3 to 2.0 ml - Absent Ipsi/Contra acoustic reflexes - Moderate to high risk of Middle ear fluid	- SNR: <3 dB - Reproducibility: <75 - Stimulus Stability:<70
Group II	**Conductive hearing loss**	**Abnormal**	**Absent**

Otosclerosis (subgroup)	- >26 dB HL and < 70 dB HL across all octaves for air conduction thresholds - <10 dB HL across all octaves for bone conduction thresholds - ABG >10 dB HL	- 'As' type tympanogram - ECV: 0.5 to 2.0 ml - Absent ipsi/Contra acoustic reflexes - No Middle ear fluid	- SNR: <3 dB - Reproducibility: <75 - Stimulus Stability:<70
Group II	**Conductive hearing loss**	**Abnormal**	**Absent**
Eustachian tube dysfunction (subgroup)	- > 26 dB HL and < 70 dB HL across all octaves for air conduction thresholds - <10 dB HL across all octaves for bone conduction thresholds - ABG >10 dB HL	- 'C' type tympanogram - Absent ipsi/Contra acoustic reflexes - No Middle ear fluid	- SNR: <3 dB - Reproducibility: <75 - Stimulus Stability:<70
Group III	**Sensorineural hearing loss**	**Normal**	**Absent**
Sensorineural hearing loss	- > 26 dB HL across all octaves for air conduction and bone conduction thresholds - ABG ≤10 dB HL	- 'A/As' type tympanogram - Elevated/Absent ipsi/Contra acoustic reflexes - No Middle ear fluid	- SNR: <3 dB - Reproducibility: <75 - Stimulus Stability:<70

3.5.2 Experimental test procedures - Wideband absorbance tympanometry

Before initiating WBA measurements, calibration of the Interacoustics Titan Suite IMP440/WBT440 was performed daily by placing the probe assembly in each of four metal calibration units of 0.2, 0.5, 2 and 5 cc volumes. The source reflectance and incident pressure were determined for carrying out the WBA measurements. It was ensured that the reflectance value remains below 15% up to 2 kHz and below 30% thereafter, as recommended by the manufacturer (Interacoustics, 2016; Liu et al., 2008).

All the participants who met the inclusion criteria were subjected to experimental test procedures. The participants were seated comfortably and asked to relax during testing without any active involvement. A brief testing procedure, including the insertion of a probe tip into the ear canal to achieve an airtight seal, the building of pressure and presentation of click stimuli, were informed to each of the participants.

The participants were also instructed not to swallow, cough, talk and avoid unnecessary body or head movements while the test is being carried out.

The probe tip with a suitable size was firmly placed in the participant's ear canal. Wideband click stimulus of 100 dBpeSPL was presented at a fixed rate of 21.5 Hz (Interacoustics, 2017). The pressure was automatically swept between +200 daPa and – 600 daPa at the medium level pump speed corresponded to 200 daPa/sec. The WBA values were measured automatically at 1/24th octave frequencies between 226 Hz and 8000 Hz (121 frequencies) by averaging the click stimuli response with 32 sweeps. The WBA values were displayed in a 3-dimensional graph with frequency (in Hz from 226 Hz to 8000 Hz) on the x-axis; pressure (in daPa from +200 to -600 daPa) on the y-axis; and absorbance values (in percentage from 0 to 100%) on the z-axis (as shown in Figure 3.1).

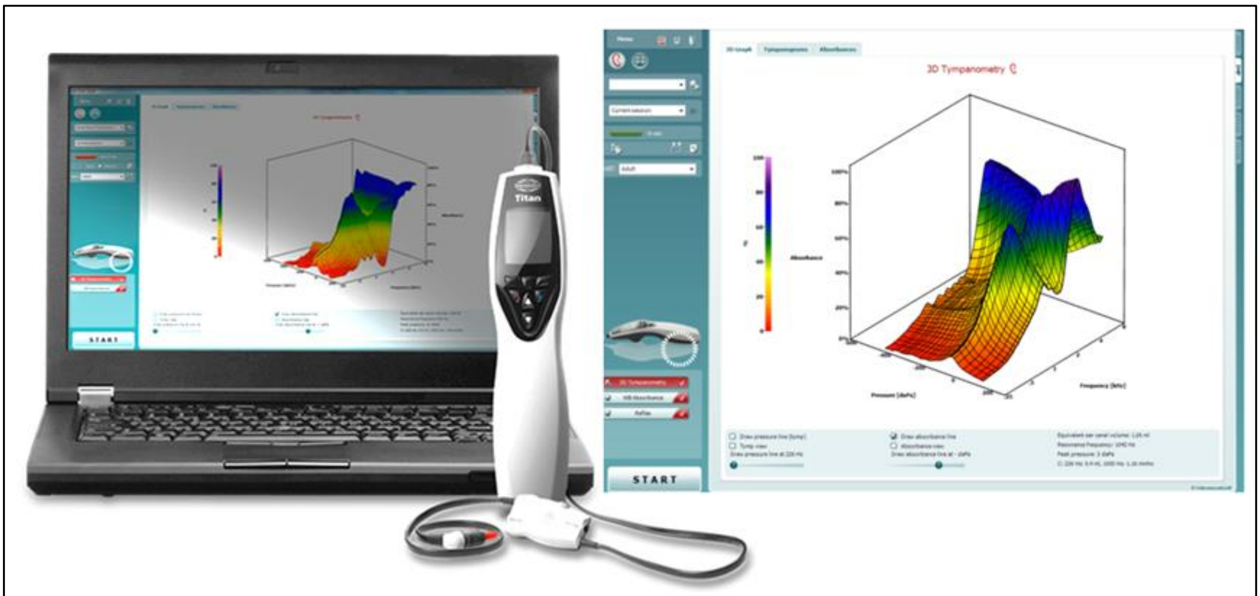


Figure 3.1. Interacoustics Titan IMP/WBT440 equipment used in this study and a three-dimensional WBA graph displayed in Titan Suite module.

Generally, WBA values range between 0.0 and 1.0, where '1' indicates that the middle ear absorbs all the sound energy while '0' indicates that all the sound energy is reflected from the middle ear (Stinson, 1990). The Titan IMP/WBT440 module provides WBA measurements at two pressure conditions, i.e., Tympanometric peak pressure and ambient pressure (corresponding to 0 daPa, within the pressure sweep) across the frequencies. Further, frequency averaged tympanogram was also measured that includes Ear canal volume (ECV), Tympanometric peak pressure (TPP), Wideband averaged absorbance (WBT_{avg}) at TPP and ambient pressure and also the resonance frequency.

Wideband averaged absorbance (WBT_{avg}) tympanometry value is an averaged absorbance value obtained from 375 to 2000 Hz by sweeping pressure from positive to negative (Hunter & Shanaz, 2014). The tympanometric peak pressure was measured as a point at which the maximum peak, i.e., maximum absorbance was detected by the equipment (Interacoustics, 2016), which is considered for eliciting WBA across the frequencies. Similarly, the ECV is calculated at 226 Hz tympanometry using the susceptance value calculated from the positive tail of the pressure sweep (Interacoustics, 2016). The resonance frequency was calculated by obtaining the lowest frequency at which the magnitude of the mass susceptance is equal to the stiffness, resulting in zero susceptance (Interacoustics, 2016). All the measurements were performed automatically by the Titan Suite and displayed in the 3-D graph. WBA measurements were performed thrice for each participant to check for consistency in WBA pattern/results and eliminate the abnormal WBA pattern, which could be an artifact elicited due to involuntary movements or swallowing, etc. If all the three recordings showed a similar pattern, then the second recording value was considered for the analysis.

The study extracted WBA at tympanometric peak pressure and ambient pressure at 1/24th-octave bands (121 frequency data points), which were averaged into 1/3rd octave bands (16 frequency data points) to reduce the inflated type I errors for the current analysis. Apart from WBA at each frequency, wideband average tympanogram (WBT_{avg}) at peak and ambient pressure were also extracted. The extracted data were exported into an excel sheet using MATLAB software version 9.7 (MATLAB R2019b, Math Works, Inc., Natick, US) and then transferred into IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA) for statistical analysis.

3.6 Reliability measures

Reliability measures were performed for WBA measurements, i.e. absorbance across 1/3rd octave frequencies and wideband average tympanogram (WBT_{avg}) at peak and ambient pressure. A total of 120 ears (minimum 10% of the total participants) was selected randomly from Group I (Normal ears) for investigating the test re-test reliability and inter-tester reliability. The ear was chosen alternatively, i.e. if the right ear was chosen for test re-test reliability, then the left ear was considered for inter- tester reliability for the same participants during the re-test measurement and vice versa for the other participants.

Testing was repeated immediately by removing and re-inserting the probe to the ear canal after the first measurements for measuring the test-retest reliability. Similarly, another qualified audiologist had repeated the WBA measurements to the same participants' opposite ear to investigate the inter-tester reliability. The audiologist was not revealed about the first measurements' results and the probe tip selection for testing. The data obtained at the initial and repeated measurements from each of the selected participants were extracted for further analysis to study WBA measures' consistency and inter-tester reliability.

Thus for analysis, the WBA data across 1/3rd Octave frequencies from 226 Hz to 8000 Hz (16 frequency) and the average absorbance (WBT_{avg}) were obtained for two ears (right, left), two pressure conditions (Peak, ambient pressure), and gender (Male, Female) from all the groups. These variables were considered to study the effect of gender and pressure conditions on WBA from Group I participants and establish the WBA norms. Further, the WBA across the frequencies and WBT_{avg} measurements obtained from Group II and Group III were compared with the Group I data to study the effect of various middle conditions and SNHL on WBA measurements.

3.7 Statistical analysis

The distribution of WBA measurements obtained across the frequencies and wideband averaged (WBT_{avg}) absorbance tympanogram in both pressure conditions was assessed for normality using the Shapiro-Wilks test. The results showed that the data were normally distributed for frequencies from 250 Hz to 800 Hz and 4000 Hz ($p>0.05$), as shown in Table 3.3. Similar results were also obtained using the Kolmogorov-Smirnov Test for Normality.

Table 3.3

Shapiro-Wilks test statistics for normality of WBA obtained at peak and ambient pressure conditions

Frequency (Hz)	**Peak pressure**		**Ambient pressure**	
	Statistic *(df=1127)*	***p-value***	**Statistic** *(df=1127)*	***p-value***
250	0.994	0.30*	0.987	0.13*
300	0.994	0.33*	0.982	0.08*
400	0.986	0.07*	0.992	0.09*
500	0.993	0.15*	0.992	0.09*
600	0.989	0.06*	0.992	0.09*
800	0.994	0.24*	0.997	0.89*
1000	0.990	0.03	0.984	<0.01
1250	0.967	<0.01	0.958	<0.01
1500	0.965	<0.01	0.956	<0.01
2000	0.951	<0.01	0.937	<0.01
2500	0.959	<0.01	0.954	<0.01
3000	0.985	<0.01	0.985	<0.01
4000	0.994	0.26*	0.991	0.06*
5000	0.981	<0.01	0.976	<0.01
6000	0.954	<0.01	0.954	<0.01
8000	0.861	<0.01	0.859	<0.01
WBT_{avg}	0.968	<0.01	0.988	0.01

Note. *Significant different at $p>0.05$.

Though the study sample did not follow the normal distribution ($p<0.05$) for most variables, parametric inferential statistics were still considered for statistical analysis. This is because the study has a large sample size with multiple within- and between-subject factors that include pressure conditions (02 levels), ears (02 levels), gender (02 levels) and frequencies (16 levels) to determine the significant difference of WBA (Altman & Bland, 1995; Ghasemi & Zahediasl, 2012). Hence, to investigate the main effect and their interactions, the parametric test was used. However, an equivalent non-parametric test was also performed for all the analysis to confirm the statistical test results obtained from the parametric test.

A mixed model analysis of variance (Mixed ANOVA) was used to analyze the normal and pathological groups. The WBA data collected were fitted with mixed

ANOVA statistics to study the main effect and its interactions. Initially, four-way mixed ANOVA was done with pressure conditions (peak, ambient) and ear (right, left) as within-subject factors; gender (male, female) as between-subject factors; and frequency (16 center frequencies of 1/3rd octave bands from 250 Hz to 8000 Hz) as a repeated measure. The initial analysis was done to study the ear's effect as within-subject factors along with other factors (pressure conditions, gender), as the participants who had a normal hearing with the normal middle ear in both ears were 325 individuals. Thus, both ear's (right, left) WBA data obtained from each of the participants, i.e. from 325 individuals were considered for initial analysis, among the total participants of 802 (1127 ears) from Group I. As the test results of mixed ANOVA did not show a significant effect ($p<0.05$) only for the ear across the frequencies and WBT_{avg} (details are given in results section), the data obtained from both ears were merged, and further analysis was done for a total of 1127 ears. This 1127 ears data has been considered to establish normative on WBA across the frequencies and WBT_{avg} for both the pressure conditions.

Further, three-way mixed ANOVA was performed with pressure conditions (peak, ambient) as within-subject factors; gender (male, female) as between-subject factors; and frequency (250 Hz to 8000 Hz) as a repeated measure to study the main effect and its interactions. Multivariate ANOVA (MANOVA) was performed to those groups where statistically significant differences were observed between the subject factors for the repeated measures without any Bonferroni corrections (Feise, 2002; Perneger, 1998). Differences in within-subject factors of pressure conditions (peak, ambient) were evaluated in each of the frequencies using a paired T-test with Bonferroni corrections. Equivalent non-parametric Mann-Whitney U test for between-subject factors (Gender) and Wilcoxon signed-rank test for within-subject factors (pressure conditions) was also performed to confirm the significant difference obtained from the parametric test. Descriptive statistics that include mean, standard deviation, median (50^{th} percentile), range, 95% confidence interval (CI), and the percentile (10^{th}, 25^{th}, 75^{th} and 90^{th}) were measured to provide the norms for WBA for each of the pressure conditions. The normative range for absorbance was defined as the region between the 10^{th} and 90^{th} percentiles (Aithal et al., 2013; Hunter et al., 2010; Liu et al., 2008).

For comparison of the pathological groups (TmP, MEE, Otosclerosis, ETD and SNHL) with the normal ear group (Group I), two-way mixed ANOVA was performed with pressure conditions (peak, ambient) as within-subject factors; pathological group (TmP, MEE, Otosclerosis, ETD and SNHL) as between-subject factors; and frequency (250 Hz to 8000 Hz) as a repeated measure. However, the study group comparison has an unequal sample size of a having large gap between the Normal ear group [n=1127] and the pathological group [TmP - 109 ears; MEE – 122 ears; Otosclerosis – 140 ears; ETD – 106 ears and SNHL – 140 ears]. Thus, 150 ears from the Group I (Normal ear group) were randomly selected (from the total sample size of 1127 ears) using an online random number generator (Stattrek.com) to compare with the pathological group. This is performed to improve the statistical analysis's power and avoid the heterogeneity of variance with the reduction of type I error rates (Rusticus & Lovato, 2014). MANOVA and Paired T-test with Bonferroni corrections were performed to determine the frequency range over which the significant difference existed between- and within-groups factors. An equivalent non-parametric Mann-Whitney U test for between-group factors and Wilcoxon signed-rank test for within-subject factors were also used. Independent Sample-T test was performed for WBT_{avg}, as the average absorbance values are obtained only at ambient pressure for all the groups and not at peak pressure.

For comparison between the pathological group, MANOVA and the equivalent non-parametric Kruskal-Wallis Test were performed to study the significant difference of WBA obtained across the frequencies among the pathological group. For WBT_{avg}, an Independent Sample T-test was performed for peak pressure conditions, as the average absorbance was elicited for only two pathological groups (Otosclerosis and SNHL). At the same time, Univariate ANOVA was performed for WBT_{avg} at ambient pressure. A post hoc Tukey's Honestly Significant Difference (HSD) test was administered to those groups where statistically significant differences were observed in WBA across the frequencies and WBT_{avg} at ambient pressure conditions. This post-hoc analysis was selected because it is considered an acceptable and conservative method of controlling for multiple comparisons, where sample sizes are unequal (Bender & Lange, 2001). An equivalent non-parametric Mann-Whitney test was administered for multiple comparisons of the pathological group.

A Greenhouse-Geisser Correction (Greenhouse & Geisser, 1959) was applied to all outcome measures to accommodate the violation of the sphericity assumption characteristic of this experimental design and avoid inflated type I errors. The Bonferroni alpha correction for between-subject factors was not required for multiple outcomes (Feise, 2002). The statistical significance level was set at $p<0.05$ for all the statistical analyses along with the measured effect size using partial Eta square (η_p^2)/ Correlation coefficient (r). However, Bonferroni corrections with an adjusted *p*-value of 0.003 (α altered =0.05/16 pairs of analysis) were considered significant for studying the multiple comparisons for within-subject factors and avoiding type I error.

Effect size criteria considered in this study to determine the true significance were small effect, $\eta_p^2= 0.01$ [<0.06]; medium effect, $\eta_p^2= 0.06$ [≥0.06 to <0.14]; and large effect, $\eta_p^2=0.14$ [≥0.14] (Cohen, 2013). Similarly, the effect size for a non-parametric test, i.e. Correlation coefficient (r), was calculated as Z value divided by the square root of the sample size. The interpretation values for r used in this study were: 0.1 to <0.3 as a small effect, 0.3 to < 0.5 as a moderate effect and ≥ 0.5 as a large effect (Cohen, 1992).

Intra-class Correlation Coefficients (ICC) of 2-way mixed-effects model (Koo & Li, 2016) were used to determine the test-retest and inter-tester reliability of WBA in the normal group (10% of the total participants from Group I) for each of the 16 test frequencies and WBT_{avg} in both the pressure conditions. ICC statistic was chosen, as the analysis consists of repeated measures, but the observations' pairs do not have an obvious order (Shrout & Fleiss, 1979). The reliability values of less than 0.5 indicate poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability and values greater than 0.90 indicate excellent reliability (Dunn et al., 2014; Koo & Li, 2016). The 95% confidence interval and a critical alpha level of the *p*-value of 0.05 were computed for every ICC to determine statistical significance (Koo & Li, 2016).

A Receiver Operating Characteristic (ROC) analysis was used to determine the clinical utility of WBA for the identification of each pathological group along with 95% CI as a function of WBA (Salkind, 2012). The variables were selected at the tests frequencies, which showed a significant difference in absorbance values between the groups of both pressure conditions. The ROC curve interpretation was also based on calculating the area under the ROC (AUROC) value (Kumar & Indrayan, 2011). All ROC analyses and AUROC were automatically calculated using MedCalc for Windows, trail version 19.4.1 (MedCalc Software, Ostend, Belgium). The AUROC ranges between 0.5 and 1.0, where '0.5' indicates that WBA at that frequency for distinguishing healthy middle ear from the pathological group is at a chance level and

‘1.0’ indicates perfect test performance in distinguishing between the two (Kumar & Indrayan, 2011). A significant *p*-value of <0.05 was considered to confirm the testability to differentiate between the groups. The sensitivity and specificity of each WBA univariate predictor were extracted based on the Youden Index criterion [max(sensitivity + specificity − 1)] of 50% (Youden, 1950), as the disease prevalence is unknown. Also, the Youden index reflects the intension to maximize the correct classification rate (Kumar & Indrayan, 2011; Perkins & Schisterman, 2006). Table 3.4 shows the summary of the test statistics used in the study to determine the test significance.

Table 3.4

Statistical analyses used to achieve the objectives of the study

Study effects	Parametric Test	Equivalent Non-parametric test
	Objectives 1, 2, & 3	
Main effect across the subjects factors *(Gender/Pressure conditions of Group I with frequency as a repeated measure)*	Mixed ANOVA	--
Between the subject factors across frequencies *(Gender)*	MANOVA	Mann-Whitney U test
Within-subject factors across frequencies *(Pressure conditions)*	Paired T-test with Bonferroni corrections	Wilcoxon signed-rank test with Bonferroni corrections
Establishment of WBA norms *(from Group I)*	Descriptive statistics [Mean, Median (50^{th} percentile), SD, Range, 95% CI, and Percentile (10^{th}, 25^{th}, 75^{th} and 90^{th})]	
Test-retest and Inter-tester reliability measures	Intra-class correlation coefficients (2-way mixed-effects model)	
	Objectives 4 & 5	
Main effect across the Groups *(Normal vs Pathological Groups)*	Mixed ANOVA	--
Between the Normal ear group and Pathological Group (TmP, MEE, Otosclerosis, ETD and SNHL)	MANOVA	Mann-Whitney U test
Within-subject factors for each of the pathological Group *(Pressure conditions)*	Paired T-test with Bonferroni corrections	Wilcoxon signed-rank test with Bonferroni corrections
Sensitivity and Specificity measures	Receiver Operating Characteristic (ROC) analysis and Area under ROC (AUROC)	
	Objective 6	
Comparison of between the pathological group	- MANOVA (Across the frequencies) - Univariate ANOVA (WBT_{avg} at ambient pressure) - Independent T-test (WBT_{avg} at peak pressure)	- Kruskal-Wallis Test - Kruskal-Wallis Test - Mann-Whitney U test
Post-hoc analysis between the pathological group comparison	Tukey's HSD	Mann-Whitney U test
Effect size measures (For all the analysis)	Partial Eta square (η_p^2)	Correlation coefficient (r)

Chapter 4

RESULTS

The present study aimed to obtain the norms for wideband absorbance in adults having normal middle ear with normal hearing sensitivity and clinically validate the results with the ear-related disordered population. To address the objectives of the present study, the data were analyzed both quantitatively and qualitatively and are given under the following headings:

1. Comparison of WBA between the gender in Normal ears.
2. Comparison of WBA between the pressure conditions in Normal ears.
3. Establishment of norms for WBA with reliability measures.
4. Comparison of WBA obtained in Normal ears with different ears with middle ear pathologies.
   a) Tympanic membrane perforation (TmP)
   b) Middle ear effusion (MEE)
   c) Otosclerosis
   d) Eustachian tube dysfunction (ETD)
5. Comparison of WBA obtained in Normal ears with Sensorineural hearing loss.
6. Comparison of WBA obtained between the pathological group.

4.1 Effect of ear, pressure and gender on WBA in Normal ears

Prior to establishing the normative, effect of ears, pressure conditions, and gender on WBA across the frequencies were done using mixed ANOVA. This was done to know whether there is any significant effect of the ear, gender, and pressure conditions on WBA pattern and whether normative needed to be established for each ear, gender, or pressure condition. If no significant effect is found for any one of these variables, the data could be combined for further analysis to establish the normative. To do so, a total of 325 (650 ears) out of 802 participants who had normal hearing with normal middle ear functioning in both the ears (right, left) were considered as the WBA could be obtained from both the ears. WBA was measured from both ears in 325 participants, and the rest of 477 participants' data were obtained only from one ear.

A Mixed ANOVA was fitted to the data with the ear (right, left) and pressure conditions (peak, ambient) as within-subject factors; gender (male, female) as between-subject factors; and frequency as a repeated measure-factor. Results showed a significant main effect of gender [F (1, 323) = 17.21, $p<0.05$, η_p^2 =0.05], pressure conditions [F (1, 323) = 66.94, $p<0.05$, η_p^2 =0.02] and frequency [F (3.08, 995.99) = 3271.24, $p<0.05$, η_p^2 =0.91]. However, the study did not show a significant main effect of ear on WBA [F (1, 323) = 3.19, $p>0.05$, η_p^2 =0.00] and all its interaction effect, i.e. Ear*Gender [F (1, 323) = 3.03, $p>0.05$, η_p^2 =0.01]; Ear* Pressure [F (1, 323) = 3.60, $p>0.05$, η_p^2 =0.01]; Ear*Frequency [F (4.035, 1303.45) = 10.81, $p<0.05$, η_p^2 =0.01]; Ear*Pressure* Gender [F (1, 323) = 2.05, $p>0.05$, η_p^2 =0.00]; Ear*Frequency*Gender [F (4.035, 1303.45) = 1.61, $p>0.05$, η_p^2 =0.00]; Ear*Frequency*Pressure [F (4.209, 1359.56) = 1.57, $p>0.05$, η_p^2 =0.00]; and Pressure * Frequencies * Ear * Gender [F (4.209, 1359.56) = 0.68, $p>0.05$, η_p^2 =0.00]. The mean WBA across the frequencies were comparable for both right and left ears, as shown in Figure 4.1. The mean WBA for the left ear was marginally lower than the right ear for frequencies from 250 Hz to 1500 Hz; no difference at 2000, 2500 and 3000 Hz, and increased for frequencies above 4000 Hz.

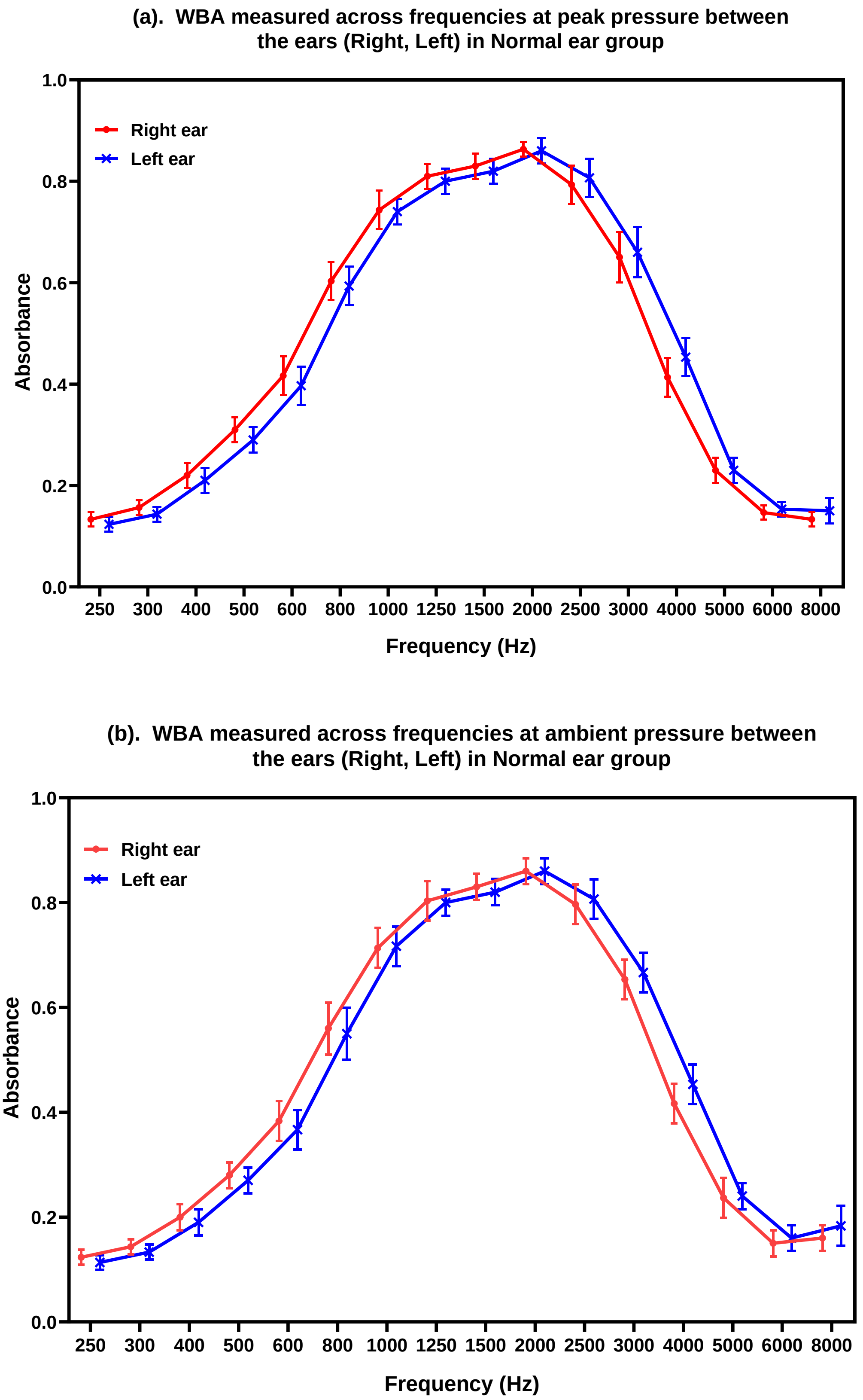


Figure 4.1 Graphical representation of mean WBA [vertical lines denoting 95% CI] measured between the ears at (a) peak pressure and (b) ambient pressure across frequencies in the Normal ear group.

Similarly, Wideband average tympanogram (WBT_{avg}) measurements were analyzed using mixed ANOVA to determine the effect of ear, pressure conditions and gender on average absorbance values. The WBT_{avg} data were fitted with pressure conditions (peak, ambient) and ear (right, left) as within-subject factors and gender (male, female) as between-subject factors. Results showed a significant main effect of gender [F (1, 323) = 11.31, p<0.05, η_p^2 =0.03], and pressure [F (1, 323) = 13.02, p<0.05, η_p^2 =0.04]. However, the study did not show any significant main effect of ear on WBT_{avg} [F (1,323, 0.001) = 2.54, p>0.05, η_p^2 =0.01]. The mean WBT_{avg} absorbance values between the right and left ear were identical for peak and ambient pressure conditions.

As there is no significant main effect of ears on WBA across the frequencies and WBTavg, the non-parametric Wilcoxon Signed Rank test with a Bonferroni correction was also performed between the ears to confirm the results of the parametric test. The results showed no significant difference in WBA between the ears (p>0.003) or the significant difference (p<0.003) with negligible effect size (r<0.3), and the statistical results are shown in Annexure 4.1. Thus, both ears data were combined with the remaining data of 447 normal-hearing ears for further analysis to see the effect of gender and pressure conditions on large data from 1127 ears and subsequently establish the normative.

A total of 1127 ears from 802 participants in Group I (Normal ear group) was considered for establishing WBA norms. All the ears in Group I had ‘A’ type tympanogram with a mean tympanometric peak pressure of -9.28 daPa (SD=16.60; Range = +50 to -97 daPa) and the mean static admittance of 1.07 mmho (SD=0.15; Range=0.50 to 1.55 mmho) measured at 226 Hz probe tone frequency. The average ear canal volume obtained was 1.16 cc (SD = 0.27; Range = 0.55 to 2.25 cc) and the mean resonance frequency was 903.33 Hz (SD =78.33; Range = 800 Hz to 1192 Hz). Also, the mean TPP obtained from WBT measurements that were used to record the WBA across the frequencies was -7.45 daPa (SD=16.15; Range = +50 to -92 daPa). The mean, SD, median and range for the WBA obtained across the frequencies at two different pressure conditions (peak, ambient) and gender (male, female) are given in Table 4.1.

Between the genders, irrespective of pressure conditions (peak, ambient), the mean WBA was the least at 250 Hz, increased steeply with increasing frequency reaching a maximum at 1250 Hz and increased further at 2000 Hz; and thereafter, the absorbance decreased moderately to a minimum at 6000 Hz and beyond. Thus, the mid-frequencies between 1000 Hz and 2500 Hz showed a maximum absorbance, whereas the lower absorbance was seen at low and high frequencies. In addition, the distributions of mean and median WBA of both the genders were comparable and superimposed to each other. The mean WBT_{avg} values obtained at peak pressure and ambient pressure were also identical for both the genders, as shown in Table 4.1.

Table 4.1

Descriptive statistics of WBA obtained in two pressure conditions [peak, ambient] and genders [male, female] in the Normal ear group

Pressure conditions	**Frequency (Hz)**	**Male (n=632)**				**Female (n=495)**			
		Mean	**SD**	**Median**	**Range**	**Mean**	**SD**	**Median**	**Range**
Peak pressure	250	0.14	0.05	0.14	0.02 - 0.28	0.12	0.04	0.12	0.02 - 0.23
	300	0.17	0.06	0.16	0.03 - 0.33	0.14	0.05	0.14	0.02 - 0.28
	400	0.24	0.08	0.23	0.04 - 0.45	0.21	0.08	0.20	0.03 - 0.41
	500	0.34	0.12	0.33	0.07 - 0.65	0.29	0.11	0.28	0.05 - 0.59
	600	0.45	0.15	0.44	0.12 - 0.80	0.40	0.14	0.39	0.09 - 0.79
	800	0.63	0.15	0.65	0.24 - 0.96	0.59	0.15	0.60	0.22 - 0.94
	1000	0.75	0.11	0.77	0.44 - 0.99	0.74	0.12	0.75	0.43 - 0.99
	1250	0.81	0.09	0.82	0.55 - 0.99	0.81	0.09	0.81	0.57 - 0.99
	1500	0.83	0.09	0.84	0.58 - 0.99	0.83	0.08	0.83	0.60 - 0.99
	2000	0.86	0.09	0.87	0.58 - 0.99	0.87	0.08	0.88	0.63 - 0.99
	2500	0.77	0.13	0.79	0.41 - 0.99	0.81	0.13	0.83	0.42 - 0.99
	3000	0.63	0.16	0.62	0.29 - 0.99	0.67	0.16	0.67	0.27 - 0.98
	4000	0.39	0.15	0.38	0.00 - 0.80	0.43	0.15	0.42	0.08 - 0.84
	5000	0.23	0.10	0.22	0.00 - 0.59	0.22	0.09	0.21	0.04 - 0.53
	6000	0.16	0.07	0.14	0.00 - 0.42	0.14	0.06	0.12	0.02 - 0.40
	8000	0.17	0.11	0.13	0.01 - 0.54	0.12	0.08	0.10	0.02 - 0.52
	WBT_{avg}	0.60	0.10	0.62	0.33-0.82	0.60	0.10	0.62	0.4--0.82
Ambient pressure	250	0.13	0.05	0.12	0.00 - 0.28	0.11	0.04	0.11	0.01 - 0.26
	300	0.15	0.06	0.14	0.02 - 0.33	0.13	0.05	0.13	0.02 - 0.31
	400	0.22	0.08	0.21	0.04 - 0.45	0.19	0.08	0.18	0.02 - 0.44
	500	0.30	0.11	0.29	0.05 - 0.59	0.27	0.11	0.25	0.03 - 0.60
	600	0.41	0.14	0.40	0.08 - 0.80	0.37	0.13	0.35	0.11 - 0.80
	800	0.59	0.15	0.60	0.17 - 0.94	0.56	0.15	0.56	0.21 - 0.98
	1000	0.73	0.12	0.74	0.36 - 0.99	0.72	0.13	0.73	0.40 - 0.98
	1250	0.81	0.10	0.82	0.54 - 0.99	0.80	0.09	0.82	0.53 - 0.99
	1500	0.83	0.09	0.85	0.58 - 0.99	0.83	0.09	0.84	0.58 - 0.99
	2000	0.86	0.09	0.88	0.51 - 0.99	0.87	0.09	0.89	0.60 - 0.99
	2500	0.78	0.13	0.80	0.40 - 0.99	0.81	0.13	0.84	0.41 - 0.99
	3000	0.63	0.15	0.63	0.29 - 0.98	0.67	0.16	0.68	0.28 - 0.99
	4000	0.40	0.15	0.39	0.00 - 0.78	0.43	0.15	0.42	0.08 - 0.80
	5000	0.23	0.10	0.22	0.00 - 0.59	0.22	0.09	0.21	0.04 - 0.53
	6000	0.16	0.07	0.14	0.00 - 0.41	0.14	0.06	0.12	0.02 - 0.37
	8000	0.18	0.11	0.14	0.01 - 0.57	0.12	0.08	0.10	0.02 - 0.50
	WBT_{avg}	0.60	0.09	0.61	0.34 - 0.86	0.59	0.09	0.59	0.33 - 0.82

To study the effect of gender (male, female) and pressure conditions (peak, ambient) on WBA across the frequencies, mixed ANOVA was applied with pressure conditions as within-subject factors, gender as between-subject factors, and frequency as a repeated measure-factor. The summary of the repeated measure ANOVA on WBA is tabulated in Table 4.2. The results indicated a significant main effect of gender, pressure conditions and frequency. Further, all the interactions between gender, pressure and frequency were significant. However, the partial eta-squared value that reflects the main and the interaction effect's strength had shown good strength of significance for pressure conditions and frequency only. In contrast, a small effect size was seen for gender and its interactions.

Table 4.2

Mixed ANOVA results on WBA obtained from the independent variables (genders, pressure conditions) and dependent repeated-measure variable (frequency) in the Normal ear group

Variables	**df, Error**	**F**	***p***	**η_p^2**
Gender	1, 1125	13.03	<0.01*	0.01
Pressure conditions	1, 1125	314.59	<0.01*	0.22
Pressure conditions * Gender	1, 1125	10.10	<0.01*	0.01
Frequency	3.37, 3792.70	9199.33	<0.01*	0.89
Frequency * Gender	3.37, 3792.70	20.73	<0.01*	0.02
Pressure conditions * Frequency	3.55, 3992.04	182.08	<0.01*	0.14
Pressure conditions * Frequency * Gender	3.55, 3992.04	10.03	<0.01*	0.01

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and Partial Eta-squared (η_p^2)—Effect size. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

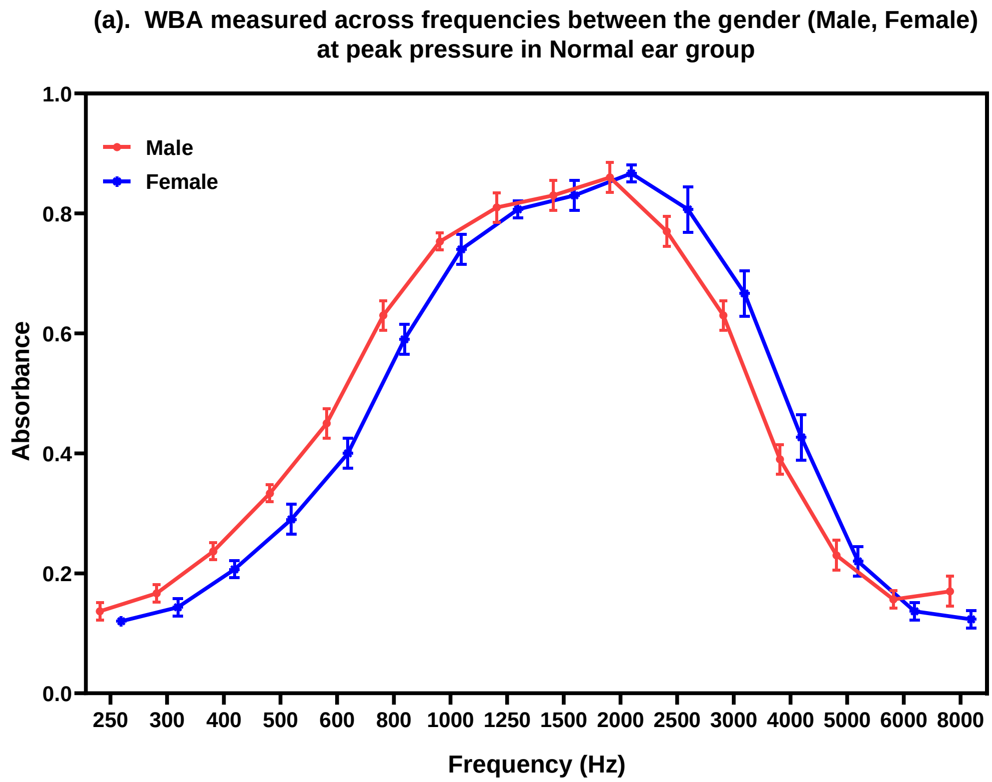
Similarly, Mixed ANOVA on WBT_{avg} was performed with pressure conditions (peak, ambient) as within-subject factors and gender (male, female) as between-subject factors. The results showed a significant main effect of pressure [F (1, 1125) = 3.957, p<0.05, η_p^2 = 0.00] and gender [F (1, 1125) = 4.332, p<0.05, η_p^2 = 0.00] with negligible effect size for both the variables. Also, the interaction between pressure and gender [F

(1, 1125) = 5.772, p<0.05, η_p^2 = 0.00] was significant with negligible effect size. The results of the main effect and the interaction effect of WBA across the frequencies and WBT_{avg} are discussed under each of the variables.

4.2 Comparison of WBA between the gender in Normal ears

The mean, standard deviation, median and range of WBA values on genders across frequencies in both the pressure conditions are given in Table 4.1. Irrespective of the pressure conditions (peak, ambient), the mean WBA for males was marginally higher than females from 250 to 1000 Hz and identical at mid-frequencies from 1250 to 2000 Hz. Further, WBA was lesser for high frequencies up to 4000 Hz for males and increased beyond, as shown in Figure 4.2.

Results of mixed ANOVA on WBA across the frequencies showed a significant main effect of gender [F (1, 1125) = 13.03, p<0.05, η_p^2 =0.01]. Further, its interaction with pressure and frequency were also significant, as indicated in Table 4.2. Though it showed a significant effect, it can be seen that the effect size on the main effect and its interaction on frequency and pressure were small (η_p^2 <0.06). Thus, gender has a minimal or negligible effect on WBA measurements.



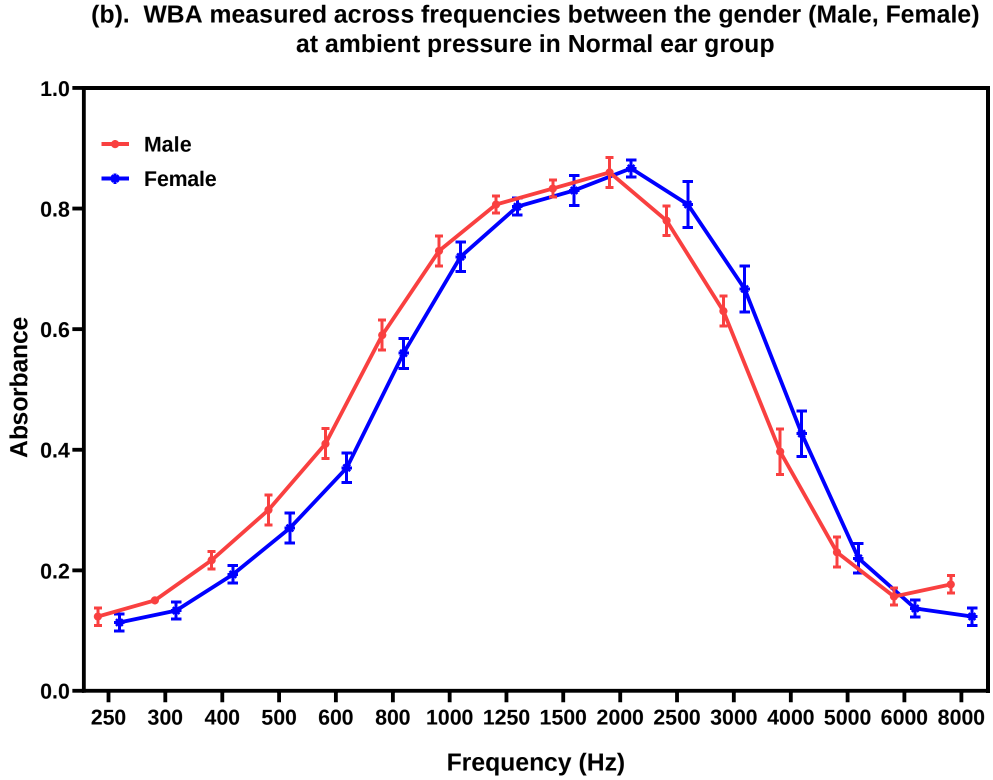


Figure 4.2 Graphical representation of mean WBA [vertical lines denoting 95% CI] measured at (a) peak pressure and (b) ambient pressure across frequencies for both the genders in the Normal ear group.

MANOVA was performed further to explore the significant effect of gender on WBA at different frequencies, and the statistical results are shown in Table 4.3. The mean WBA differed significantly between male and female participants for all the frequencies except at 1250 Hz, 1500 Hz, 2000 Hz and 5000 Hz (p>0.05) for both peak pressure and ambient pressure. However, the difference in mean WBA between males and females were meagre, i.e. less than 0.05 for both the pressure conditions. The effect size also showed small (η_p^2 <0.06) for those significant frequencies, indicating negligible differences between genders. Interestingly, the maximum absorbance occurred at those mid-frequencies (1250 Hz to 2000 Hz) where no gender difference exists. Similar results were also obtained with the non-parametric Mann-Whitney U test, and the statistical significance value across the frequencies at peak and ambient pressure are shown in Annexure 4.2.

Table 4.3

Summary of MANOVA results of WBA obtained between genders (male, female) in the Normal ear group

Frequency (Hz)	**Peak Pressure**			**Ambient pressure**		
	F *(df, Error = 1, 1125)*	***p***	**η_p^2**	**F** *(df, Error = 1, 1125)*	***p***	**η_p^2**
250	43.723	<0.01*	0.04	17.154	<0.01*	0.02
300	43.552	<0.01*	0.04	22.187	<0.01*	0.02
400	39.647	<0.01*	0.03	22.807	<0.01*	0.02
500	42.533	<0.01*	0.04	26.774	<0.01*	0.02
600	35.429	<0.01*	0.03	19.574	<0.01*	0.02
800	22.926	<0.01*	0.02	9.328	<0.01*	0.01
1000	6.560	<0.01*	0.01	2.290	0.13	0.00
1250	0.528	0.47	0.00	0.331	0.57	0.00
1500	0.006	0.94	0.00	0.500	0.48	0.00
2000	2.617	0.11	0.00	0.988	0.32	0.00
2500	16.464	<0.01*	0.01	13.169	<0.01*	0.01
3000	17.267	<0.01*	0.02	15.446	<0.01*	0.01
4000	13.200	<0.01*	0.01	13.267	<0.01*	0.01
5000	3.583	0.06	0.00	3.790	0.05	0.00
6000	28.506	<0.01*	0.03	30.015	<0.01*	0.03
8000	64.482	<0.01*	0.05	74.644	<0.01*	0.05
WBT_{avg}	10.605	<0.01*	0.00	0.979	<0.01*	0.00

Note. df—Degrees of freedom, F—F-test statistic, *p*—Significant level, and η_p^2—Effect size (Partial Eta-squared). *The actual *p*-value was not depicted as the initial three values after decimals were zero.

With respect to the WBT_{avg}, the mean average WBA obtained at peak pressure for both males (*Mean* = 0.60 ± 0.01 *SD*) and females (*Mean* = 0.60 ± 0.01 *SD*) were identical. At ambient pressure condition, the mean WBT_{avg} for males was marginally higher (*Mean* = 0.60 ± 0.09 *SD*) compared to females (*Mean* = 0.59 ± 0.09 *SD*) with a mean difference of 0.01. Results of mixed ANOVA on WBT_{avg} showed a significant main effect on gender [F (1, 1125) = 4.332, $p<0.05$, η_p^2 =0.00] with negligible effect size, indicating the difference has no practically significant. An equivalent non-parametric Mann-Whitney U test also showed no significant difference between males and females at peak pressure [U=156155.00, $p>0.05$, r=0.0] whereas, a significant difference (p<0.05) with negligible effect size [U=138294.50, p<0.05, r=0.1] was observed at ambient pressure.

4.3 Comparison of WBA between the pressure conditions in Normal ears

Descriptive statistics (Mean, Median, SD and Range) for WBA across frequencies measured at peak pressure and ambient pressure was given in Table 4.1. The mean WBA measured at peak pressure was slightly higher than the ambient pressure condition in the lower frequencies from 250 Hz to 1000 Hz, whereas, at the higher frequencies above 1000 Hz, the difference was negligible for both genders.

The results of mixed ANOVA showed a significant main effect of pressure conditions [F (1, 1125) = 314.59, p<0.05, η_p^2 =0.22] having a large effect size (η_p^2 ≥0.14). Further, the interaction of pressure conditions with frequency [F (3.55, 3992.04) =182.08, p<0.05, η_p^2 = 0.14] also showed a significant effect, indicating that mean WBA values varied significantly between the two pressure conditions across the frequencies. Though there was a significant interaction with gender [F (1,1125) =10.10, p<0.05, η_p^2 =0.01], there exists a small effect size. Paired T-test with Bonferroni corrections was performed to determine the frequencies at which the WBA differed between the pressure conditions. For this, both the genders' data were combined for analysis, as the negligible effect size of gender has been noticed.

The Paired T-test results showed a significant difference in absorbance values for frequencies from 250 Hz to 1500 Hz, 3000 Hz, and 4000 Hz (p<0.003) as shown in Table 4.4. The WBA at peak pressure had slightly higher absorbance values than the ambient pressure, as shown in Figure 4.3. However, the effect size using partial eta square showed a large effect size (η_p^2≥0.14) for the frequencies from 250 Hz to 1000 Hz and a small effect (η_p^2<0.06) for other frequencies above 1000 Hz. Similarly, an equivalent non-parametric Wilcoxon signed-rank test with Bonferroni corrections was performed to determine the significant difference in WBA between the pressure conditions in each of the frequencies. The Wilcoxon signed-rank test results showed identical results as that of the parametric (Paired-T) test and are shown in Annexure 4.3.

Table 4.4

Summary of Paired T-test result and its significant level of WBA obtained between pressure conditions (peak, ambient) in the Normal ear group

Frequency (Hz)	**t** *(df=1126)*	***p***	η_p^2
250	16.557	<0.001*	0.2
300	17.321	<0.001*	0.2
400	17.384	<0.001*	0.2
500	18.928	<0.001*	0.2
600	18.034	<0.001*	0.2
800	18.616	<0.001*	0.2
1000	14.382	<0.001*	0.2
1250	2.129	0.033	0.0
1500	-4.57	<0.001*	0.0
2000	-1.677	0.094	0.0
2500	-1.182	0.237	0.0
3000	-3.539	<0.001*	0.0
4000	-3.275	<0.001*	0.0
5000	-0.368	0.713	0.0
6000	1.018	0.309	0.0
8000	-2.883	0.004	0.0
WBT_{avg}	-1.706	<0.001*	0.0

Note. t- t-test statistics value, η_p^2 - Effect size of Partial Eta square values, and *p* - Significance level. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for $WBT_{avg,}$ and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Similarly, mixed ANOVA for WBT_{avg} (Gender, Pressure) showed a significant main effect of pressure [F (1, 1125) = 3.957, $p<0.05$, $\eta_p^2=0.00$] with negligible effect size. Further, paired T-test was performed to determine any difference between the pressure conditions. The results showed no significant difference [$t(1126) = -1.706$, $p>0.05$, $\eta_p^2=0.00$] between the pressure conditions with the mean average WBA of 0.61

± 0.10 SD at peak pressure and 0.60 ± 0.06 SD at ambient pressure. The non-parametric Wilcoxon signed-rank test also showed no significant difference in WBT_{avg} between the pressure conditions [Z= -1.747, p<0.05, r=0.1].

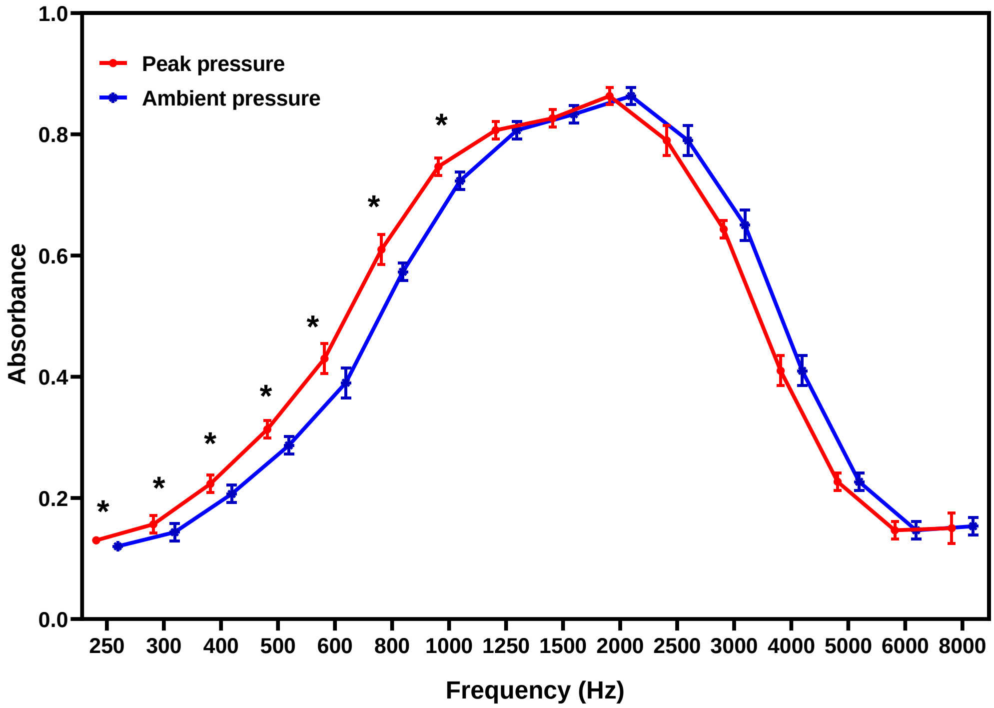
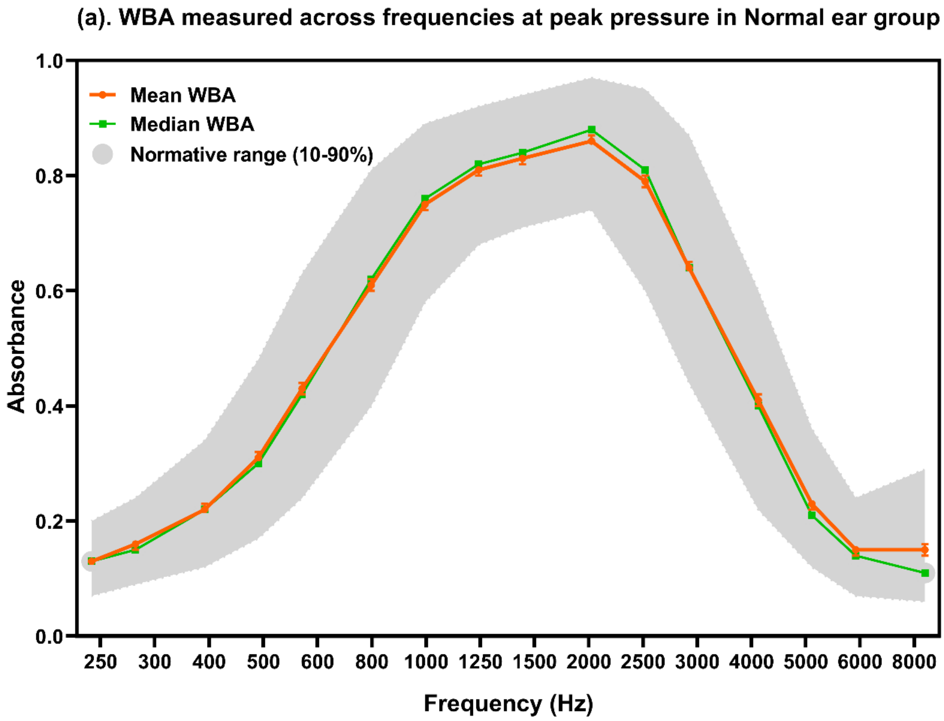


Figure 4.3 Graphical representation of mean WBA [vertical lines denoting 95% CI] for both pressure conditions (peak, ambient) across the frequencies in the Normal ear group. *indicates corrected significant difference, p<0.003 with medium to large effect size.

4.4 Establishment of norms for WBA with reliability measures

The current study results showed no significant effect on ears, whereas significant gender and pressure effect was seen. Though there is a significant effect of gender, the effect size is very small and can be considered negligible. Hence, the data obtained across the gender and ear were pooled together to provide WBA norms. However, a significant difference in WBA was exhibited between the pressure conditions showing large effect size in low and mid frequencies up to 1000 Hz. Therefore, WBA across the frequencies was analyzed independently for each pressure conditions to establish the clinical norms.

Figure 4.4 shows the trend of mean, median and normative range (percentile of absorbance between 10th and 90th percentile) across the frequencies for both peak and ambient pressure. Irrespective of the pressure mode, the mean WBA was lowest at 250 Hz, increased steeply with increasing frequency to about 1250 Hz, rose to a second maximum at about 2000 Hz, and reduced to a minimum absorbance at 6000 Hz and above.



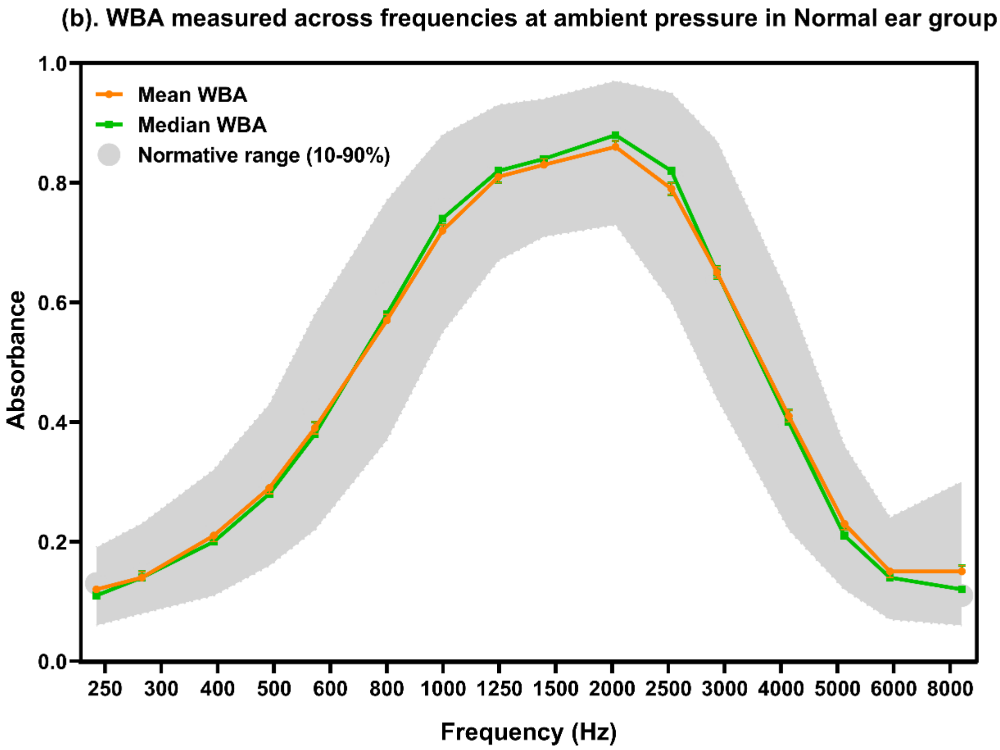


Figure 4.4 Graphical representation of WBA (Mean, Median) and Normative range (10th and 90th percentile) at (a) peak and (b) ambient pressure across the frequencies in the Normal ear group.

Table 4.5 show the WBA and WBT_{avg} norms developed in the current study at peak and ambient pressure conditions, including the mean, standard deviation, 95% CI, percentiles (10th, 25th, 50th, 75th, and 90th) along with interquartile range (25th and 75th percentiles) and normative range (10th and 90th percentiles). The inter-quartile (25th - 75th percentile) and normative ranges (10th - 90th percentile) of the WBA are slightly narrower at mid-frequencies from 1000 to 2000 Hz compared to other frequencies. The 95% CI was small for all the frequencies that fall around mean/median values. The established WBA values in each of the 1/3rd octave frequencies and WBT_{avg} at peak and ambient pressure can be used in clinics to determine an adult's middle ear status.

Table 4.5

Descriptive statistics [Mean, SD, Range, and 95% CI], percentiles (10th, 25th, 50th, 75th, and 90th), Interquartile range and Normative range of WBA measured at peak and ambient pressure in the Normal ear [n=1127] group

Pressure conditions	**Frequency (Hz)**	**Mean**	**SD**	**Range**	**95% CI**	**Percentiles**					**Inter-quartile range**	**Normative range**
						10	**25**	**50 (Median)**	**75**	**90**		
Peak pressure	250	0.13	0.05	0.02 - 0.28	0.13 , 0.13	0.07	0.09	0.13	0.16	0.20	0.07	0.13
	300	0.16	0.06	0.02 - 0.33	0.15 , 0.16	0.09	0.11	0.15	0.20	0.24	0.09	0.16
	400	0.22	0.08	0.03 - 0.45	0.22 , 0.23	0.12	0.16	0.22	0.28	0.34	0.12	0.22
	500	0.31	0.12	0.05 - 0.65	0.31 , 0.32	0.17	0.22	0.30	0.39	0.48	0.17	0.32
	600	0.43	0.14	0.09 - 0.80	0.42 , 0.44	0.24	0.32	0.42	0.53	0.63	0.21	0.39
	800	0.61	0.15	0.22 - 0.96	0.60 , 0.62	0.40	0.49	0.62	0.73	0.81	0.23	0.41
	1000	0.75	0.12	0.43 - 0.99	0.74 , 0.75	0.58	0.66	0.76	0.83	0.89	0.17	0.31
	1250	0.81	0.09	0.55 - 0.99	0.80 , 0.81	0.68	0.75	0.82	0.88	0.92	0.13	0.24
	1500	0.83	0.09	0.58 - 0.99	0.82 , 0.83	0.71	0.76	0.84	0.90	0.94	0.13	0.24
	2000	0.86	0.09	0.58 - 0.99	0.86 , 0.87	0.74	0.80	0.88	0.94	0.97	0.13	0.23
	2500	0.79	0.13	0.41 - 0.99	0.78 , 0.80	0.60	0.69	0.81	0.90	0.95	0.20	0.35
	3000	0.64	0.16	0.27 - 0.99	0.64 , 0.65	0.44	0.53	0.64	0.77	0.87	0.24	0.43
	4000	0.41	0.15	0.00 - 0.84	0.40 , 0.42	0.22	0.31	0.40	0.51	0.60	0.20	0.38
	5000	0.23	0.10	0.00 - 0.59	0.22 , 0.23	0.12	0.16	0.21	0.29	0.36	0.13	0.25
	6000	0.15	0.07	0.00 - 0.42	0.14 , 0.15	0.07	0.10	0.14	0.18	0.24	0.09	0.17
	8000	0.15	0.10	0.01 - 0.54	0.14 , 0.16	0.06	0.08	0.11	0.19	0.29	0.12	0.23
	WBT_{avg}	0.60	0.10	0.33 - 0.82	0.59 , 0.60	0.47	0.53	0.62	0.66	0.74	0.13	0.27
Ambient pressure	250	0.12	0.05	0.00 - 0.28	0.12 , 0.12	0.06	0.08	0.11	0.15	0.19	0.07	0.13
	300	0.14	0.06	0.02 - 0.33	0.14 , 0.15	0.08	0.10	0.14	0.18	0.23	0.08	0.15
	400	0.21	0.08	0.02 - 0.45	0.20 , 0.21	0.11	0.14	0.20	0.26	0.32	0.12	0.22
	500	0.29	0.11	0.03 - 0.60	0.28 , 0.29	0.16	0.20	0.28	0.36	0.43	0.16	0.28
	600	0.39	0.14	0.08 - 0.80	0.38 , 0.40	0.22	0.29	0.38	0.49	0.58	0.20	0.36
	800	0.57	0.15	0.17 - 0.98	0.57 , 0.58	0.37	0.46	0.58	0.69	0.77	0.23	0.40
	1000	0.72	0.12	0.36 - 0.99	0.72 , 0.73	0.55	0.63	0.74	0.82	0.88	0.19	0.32
	1250	0.81	0.09	0.53 - 0.99	0.80 , 0.81	0.67	0.74	0.82	0.88	0.93	0.14	0.26
	1500	0.83	0.09	0.58 - 0.99	0.83 , 0.84	0.71	0.77	0.84	0.90	0.94	0.13	0.24
	2000	0.86	0.09	0.51 - 0.99	0.86 , 0.87	0.73	0.80	0.88	0.94	0.97	0.13	0.24
	2500	0.79	0.13	0.40 - 0.99	0.78 , 0.80	0.60	0.70	0.82	0.90	0.95	0.20	0.35
	3000	0.65	0.16	0.28 - 0.99	0.64 , 0.66	0.44	0.53	0.65	0.77	0.87	0.23	0.43
	4000	0.41	0.15	0.00 - 0.80	0.40 , 0.42	0.22	0.31	0.40	0.51	0.61	0.21	0.38
	5000	0.23	0.10	0.00 - 0.59	0.22 , 0.23	0.12	0.16	0.21	0.29	0.36	0.13	0.25
	6000	0.15	0.07	0.00 - 0.41	0.14 , 0.15	0.07	0.10	0.14	0.19	0.24	0.09	0.17
	8000	0.15	0.10	0.01 - 0.57	0.15 , 0.16	0.06	0.077	0.115	0.196	0.30	0.12	0.24
	WBT_{avg}	0.60	0.09	0.33 - 0.86	0.60, 0.61	0.48	0.53	0.6	0.66	0.71	0.13	0.23

4.4.1 Reliability measures of WBA obtained at peak and ambient pressure

Intra-class correlation coefficients (ICC) of the two-way mixed-effect model was administered to examine test-retest reliability and inter tester reliability. It was done on WBA at 16 frequencies (250 Hz to 8000 Hz) and WBT_{avg} data obtained in 120 ears from Group I participants for each reliability measures. After the first measurements (baseline), the repeated test was done immediately by removing and re-inserting the probe to the ear canal by the same tester for measuring the test-retest reliability and another qualified audiologist for measuring the inter-tester reliability measures. The results are shown in Table 4.6.

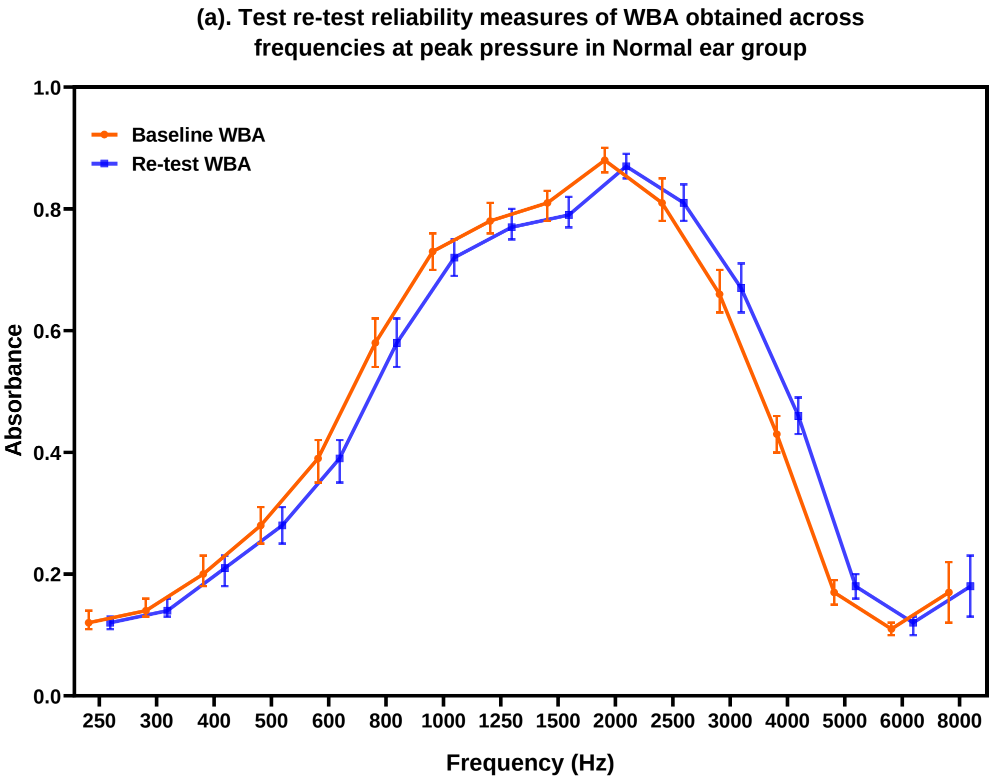
Table 4.6

Test-retest and inter-tester reliability measures (ICC coefficient at 95% CI) of WBA obtained at peak and ambient pressure in the Normal ear group

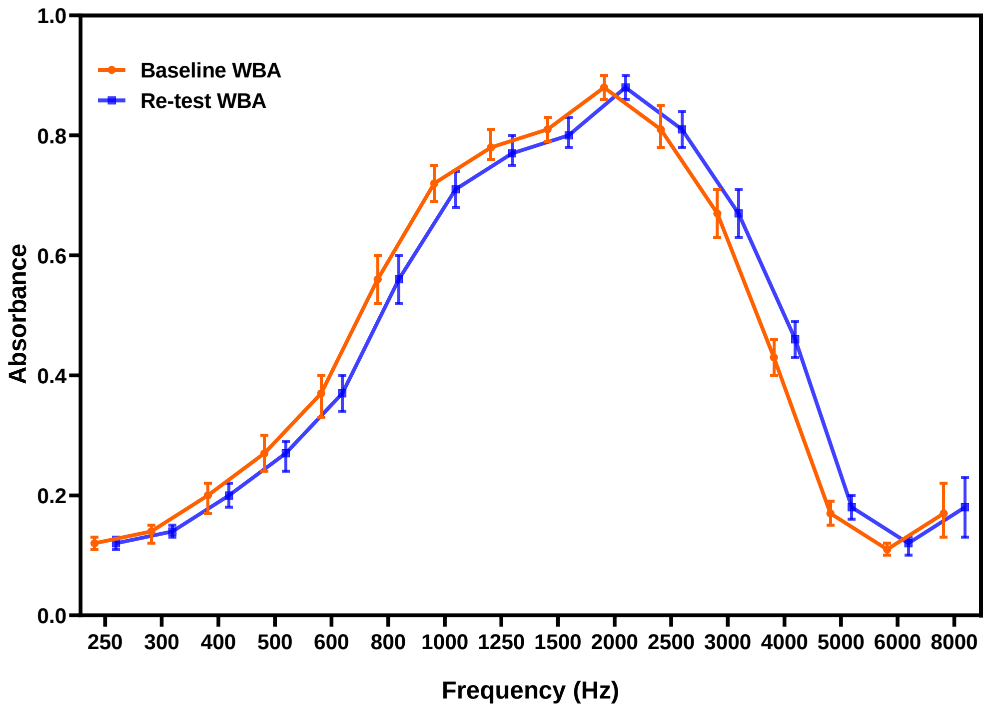
Frequency (Hz)	Test-retest reliability [n=120]				Inter-tester reliability [n =120]			
	Peak Pressure		Ambient pressure		Peak Pressure		Ambient pressure	
	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
250	0.77	0.60 - 0.87	0.75	0.56 - 0.85	0.68	0.44 - 0.82	0.60	0.36 - 0.75
300	0.78	0.62 - 0.87	0.76	0.58 - 0.86	0.73	0.53 - 0.84	0.61	0.38 - 0.77
400	0.79	0.63 - 0.88	0.75	0.57 - 0.86	0.73	0.53 - 0.84	0.62	0.35 - 0.78
500	0.79	0.63 - 0.88	0.75	0.56 - 0.85	0.70	0.49 - 0.83	0.59	0.39 - 0.77
600	0.79	0.63 - 0.88	0.76	0.59 - 0.86	0.72	0.51 - 0.84	0.59	0.39 - 0.76
800	0.72	0.52 - 0.84	0.72	0.52 - 0.84	0.84	0.71 - 0.91	0.66	0.41 - 0.80
1000	0.74	0.55 - 0.85	0.76	0.59 - 0.86	0.83	0.70 - 0.90	0.77	0.60 - 0.87
1250	0.85	0.74 - 0.91	0.87	0.77 - 0.92	0.73	0.53 - 0.84	0.66	0.42 - 0.81
1500	0.80	0.65 - 0.88	0.81	0.68 - 0.89	0.65	0.40 - 0.72	0.63	0.40 - 0.75
2000	0.72	0.52 - 0.84	0.72	0.52 - 0.84	0.62	0.38 - 0.78	0.66	0.41 - 0.80
2500	0.74	0.54 - 0.85	0.76	0.59 - 0.86	0.66	0.41 - 0.80	0.62	0.39 - 0.78
3000	0.62	0.35 - 0.78	0.64	0.37 - 0.79	0.58	0.38 - 0.76	0.58	0.36 - 0.74
4000	0.66	0.41 - 0.80	0.67	0.44 - 0.81	0.56	0.45 - 0.74	0.58	0.35 - 0.60
5000	0.76	0.58 - 0.86	0.76	0.58 - 0.86	0.60	0.37 - 0.77	0.60	0.38 - 0.77
6000	0.83	0.70 - 0.90	0.83	0.70 - 0.90	0.67	0.43 - 0.81	0.67	0.42 - 0.81
8000	0.86	0.75 - 0.91	0.86	0.76 – 0.91	0.78	0.62 - 0.87	0.79	0.63 - 0.88
WBT_{avg}	0.78	0.63 - 0.88	0.77	0.60 - 0.87	0.66	0.41 - 0.80	0.62	0.39 - 0.78

Note. ICC values <0.5 - Poor reliability; 0.5 to 0.75 - Moderate reliability; 0.75 to 0.9 - Good reliability; and > 0.90 - Excellent reliability

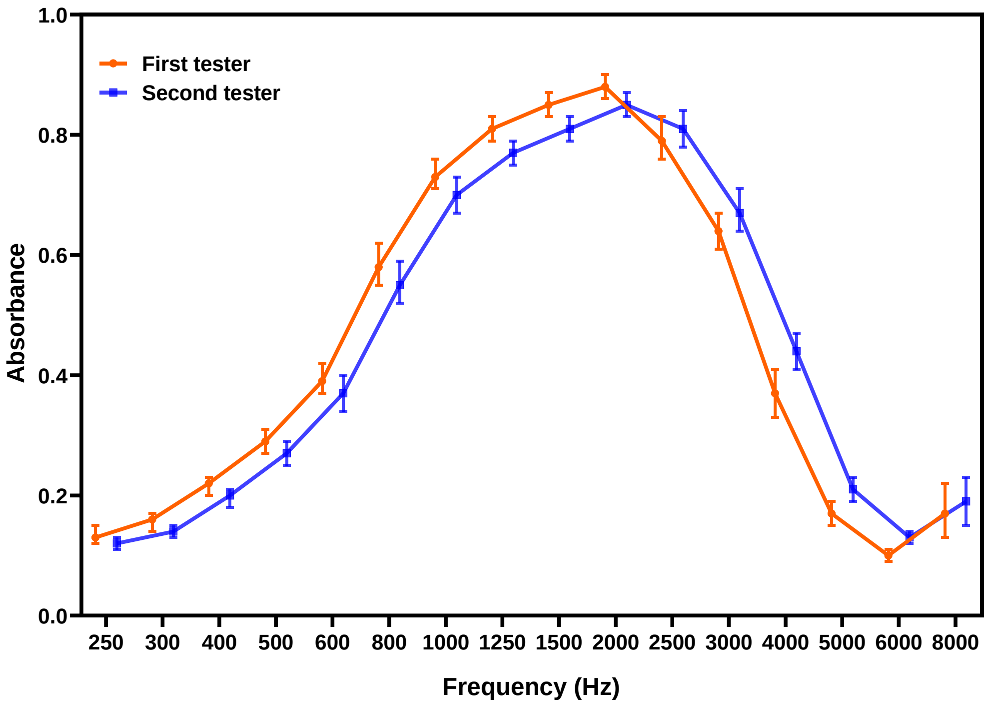
The results of the intra-class correlation coefficient showed significant (p<0.05) evidence of moderate to good reliability that ranged between 0.6 to 0.9 across the frequencies and WBT_{avg}, with most of the frequencies falling in the range of good reliability (>0.75) for test-retest reliability measures. This suggests good reliability of the test on multiple evaluations. Similarly, the inter-tester reliability measurement also showed moderate to good reliability that ranged between 0.6 to 0.9 across the frequencies and WBT_{avg}, with most of the frequencies falling in the range of moderate reliability (0.5 to 0.75). Figure 4.5 shows the reliability of WBA measured twice for both peak and ambient pressure across the frequencies.



(b). Test re-test reliability measures of WBA obtained across frequencies at ambient pressure in Normal ear group



(c). Inter-tester reliability measures of WBA obtained across frequencies at peak pressure in Normal ear group



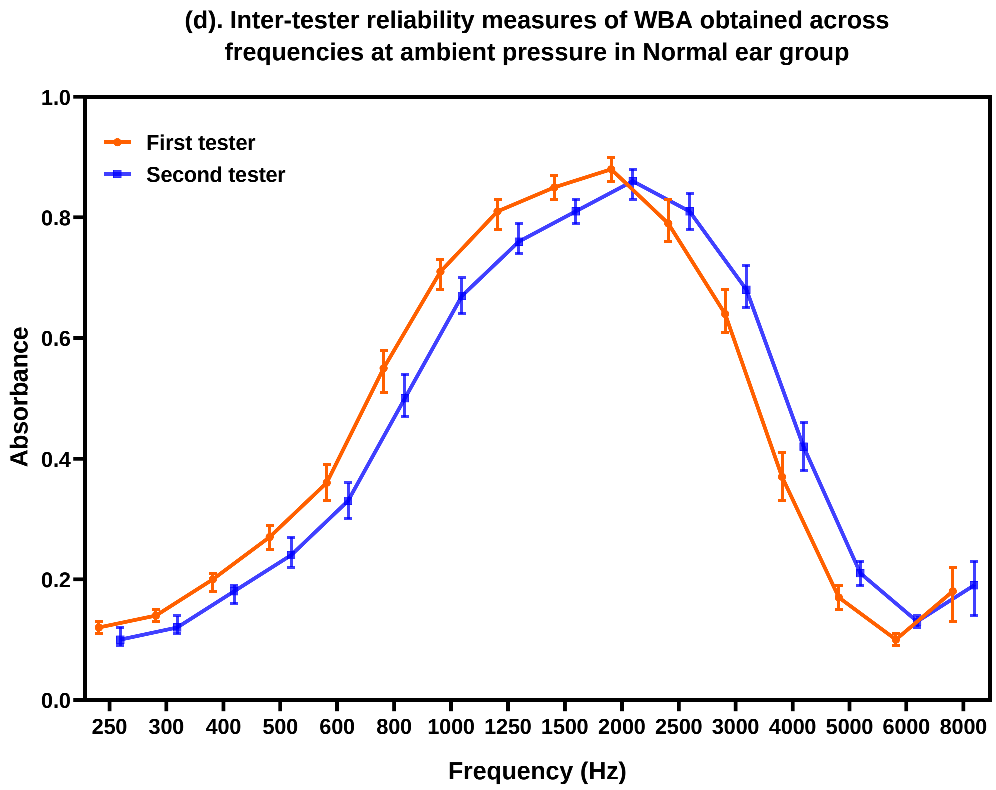


Figure 4.5 Graphical representation of Test re-test reliability measures of mean WBA [vertical lines denoting 95% CI] at (a) peak and (b) ambient pressure; and inter-tester reliability measures at (c) peak and (d) ambient pressure across the frequencies in the Normal ear group.

4.5 Comparison of WBA obtained in Normal ears with different ears with middle ear pathologies

To investigate the effect of pathological conditions on WBA, i.e. Group II (TmP, MEE, Otosclerosis and ETD) and Group III (SNHL), the WBA data of 150 randomly selected ears from Group I (Normal ear group) was compared with each of the pathological group. This random selection of Group I participants was performed to improve the statistical analysis's power and avoid the heterogeneity of variance due to unequal sample size (Rusticus & Lovato, 2014).

All the participants from the Normal ear group (Group I) had ‘A’ type tympanogram for 226 Hz probe tone with the mean static admittance of 0.66 ± 0.15

mmho (Range = 0.50 to 1.5 mmho), and tympanometric peak pressure (TPP) of -9.23 ± 14.06 daPa (Range = -69 to 46 daPa). The mean ear canal volume and the mean resonance frequency obtained were 1.21 ± 0.28 ml (Range = 0.60 to 1.90 ml) and 903.23 ± 74.58 Hz (Range = 801 to 1134 Hz) respectively. The TPP obtained in the WBT measurement was similar to the TPP of 226 Hz probe tone, with the mean TPP of -9.13 ± 12.70 daPa (Range = -55 to 48 daPa). Table 4.7 shows the descriptive statistics (mean, SD, and median) of the WBA measurements obtained at peak and ambient pressure in the Normal ear and pathological groups.

Table 4.7

Descriptive statistics (mean, SD, and median) of the WBA obtained at peak and ambient pressure conditions in Normal ear [n=150] and Pathological ear groups

Pressure conditions	**Frequency (Hz)**	**Normal Ear group**			**TmP group**			**MEE group**			**Otosclerosis group**			**ETD group**			**SNHL group**		
		Mean	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**
Peak pressure	250	0.14	0.04	0.14	0.08	0.04	0.07	0.08	0.06	0.06	0.06	0.03	0.05	0.11	0.06	0.10	0.11	0.04	0.11
	300	0.17	0.05	0.16	0.10	0.06	0.09	0.09	0.07	0.08	0.06	0.04	0.06	0.13	0.08	0.13	0.14	0.05	0.13
	400	0.24	0.06	0.24	0.17	0.10	0.15	0.13	0.09	0.11	0.09	0.06	0.08	0.20	0.10	0.19	0.20	0.08	0.19
	500	0.33	0.07	0.33	0.25	0.16	0.23	0.17	0.10	0.15	0.13	0.10	0.11	0.27	0.14	0.26	0.28	0.11	0.27
	600	0.45	0.07	0.45	0.37	0.23	0.32	0.20	0.10	0.19	0.18	0.12	0.16	0.36	0.17	0.34	0.38	0.13	0.37
	800	0.66	0.07	0.66	0.51	0.24	0.51	0.24	0.11	0.23	0.27	0.14	0.26	0.51	0.21	0.54	0.56	0.14	0.56
	1000	0.81	0.08	0.81	0.59	0.20	0.59	0.29	0.13	0.26	0.40	0.15	0.38	0.63	0.22	0.65	0.70	0.13	0.72
	1250	0.85	0.08	0.86	0.60	0.17	0.59	0.34	0.14	0.32	0.57	0.15	0.58	0.70	0.21	0.72	0.80	0.10	0.82
	1500	0.85	0.09	0.86	0.57	0.16	0.55	0.37	0.14	0.36	0.69	0.15	0.70	0.73	0.19	0.76	0.83	0.10	0.86
	2000	0.87	0.08	0.88	0.56	0.22	0.51	0.42	0.14	0.42	0.80	0.14	0.82	0.75	0.18	0.78	0.87	0.09	0.89
	2500	0.80	0.13	0.82	0.64	0.21	0.64	0.41	0.11	0.40	0.80	0.12	0.82	0.67	0.18	0.68	0.78	0.15	0.78
	3000	0.67	0.16	0.67	0.63	0.18	0.63	0.36	0.09	0.36	0.70	0.16	0.69	0.53	0.17	0.52	0.63	0.16	0.60
	4000	0.41	0.15	0.40	0.40	0.17	0.36	0.24	0.11	0.22	0.45	0.16	0.44	0.38	0.15	0.37	0.35	0.15	0.34
	5000	0.23	0.09	0.22	0.35	0.18	0.29	0.17	0.09	0.16	0.25	0.10	0.23	0.26	0.11	0.24	0.20	0.08	0.19
	6000	0.15	0.07	0.14	0.34	0.21	0.29	0.11	0.06	0.10	0.15	0.07	0.14	0.15	0.06	0.15	0.12	0.04	0.12
	8000	0.15	0.10	0.12	0.18	0.15	0.13	0.19	0.20	0.09	0.15	0.15	0.09	0.18	0.18	0.10	0.17	0.18	0.10
	WBT_{avg}	0.61	0.10	0.62	---	---	---	---	---	---	0.56	0.14	0.58	---	---	---	0.60	0.11	0.61
Ambient pressure	250	0.14	0.04	0.14	0.08	0.04	0.07	0.08	0.06	0.06	0.06	0.03	0.05	0.11	0.06	0.10	0.11	0.04	0.11
	300	0.17	0.05	0.16	0.10	0.06	0.09	0.09	0.07	0.08	0.06	0.04	0.06	0.13	0.08	0.13	0.14	0.05	0.13
	400	0.24	0.06	0.24	0.17	0.10	0.15	0.13	0.09	0.11	0.09	0.06	0.08	0.20	0.10	0.19	0.20	0.08	0.19
	500	0.33	0.07	0.33	0.25	0.16	0.23	0.17	0.10	0.15	0.13	0.10	0.11	0.27	0.14	0.26	0.28	0.11	0.27
	600	0.45	0.07	0.45	0.37	0.23	0.32	0.20	0.10	0.19	0.18	0.12	0.16	0.36	0.17	0.34	0.38	0.13	0.37
	800	0.66	0.07	0.66	0.51	0.24	0.51	0.24	0.11	0.23	0.27	0.14	0.26	0.51	0.21	0.54	0.56	0.14	0.56
	1000	0.81	0.08	0.81	0.59	0.20	0.59	0.29	0.13	0.26	0.40	0.15	0.38	0.63	0.22	0.65	0.70	0.13	0.72
	1250	0.85	0.08	0.86	0.60	0.17	0.59	0.34	0.14	0.32	0.57	0.15	0.58	0.70	0.21	0.72	0.80	0.10	0.82
	1500	0.85	0.09	0.86	0.57	0.16	0.55	0.37	0.14	0.36	0.69	0.15	0.70	0.73	0.19	0.76	0.83	0.10	0.86
	2000	0.87	0.08	0.88	0.56	0.22	0.51	0.42	0.14	0.42	0.80	0.14	0.82	0.75	0.18	0.78	0.87	0.09	0.89
	2500	0.80	0.13	0.82	0.64	0.21	0.64	0.41	0.11	0.40	0.80	0.12	0.82	0.67	0.18	0.68	0.78	0.15	0.78
	3000	0.67	0.16	0.67	0.63	0.18	0.63	0.36	0.09	0.36	0.70	0.16	0.69	0.53	0.17	0.52	0.63	0.16	0.60
	4000	0.41	0.15	0.40	0.40	0.17	0.36	0.24	0.11	0.22	0.45	0.16	0.44	0.38	0.15	0.37	0.35	0.15	0.34
	5000	0.23	0.09	0.22	0.35	0.18	0.29	0.17	0.09	0.16	0.25	0.10	0.23	0.26	0.11	0.24	0.20	0.08	0.19
	6000	0.15	0.07	0.14	0.34	0.21	0.29	0.11	0.06	0.10	0.15	0.07	0.14	0.15	0.06	0.15	0.12	0.04	0.12
	8000	0.15	0.10	0.12	0.18	0.15	0.13	0.19	0.20	0.09	0.15	0.15	0.09	0.18	0.18	0.10	0.17	0.18	0.10
	WBT_{avg}	0.61	0.10	0.62	---	---	---	---	---	---	0.56	0.14	0.58	---	---	---	0.60	0.11	0.61

The mean WBA obtained in the Normal ear group was minimal at 250 Hz, increased as a function of frequency to a maximum at 1250 Hz. Further, the WBA gradually increased to a second maximum at 2000 Hz and decreased moderately at the higher frequencies beyond 2000 Hz, reaching a minimum at 6000 Hz and above. Thus, it was observed that the WBA was least at 250 Hz, 6000 Hz and 8000 Hz; and highest at the mid-frequencies range between 1000 Hz and 2500 Hz. The WBA pattern was broader and relatively non-evident maximum peak at 1250 Hz and 2000 Hz, as shown in Figure 4.6. The WBA measured at peak pressure and ambient pressure was almost similar and followed a similar pattern across the frequencies. Similarly, the WBT_{avg} absorbance values are also similar between the peak pressure (0.61 ± 0.10 SD, range = 0.40 to 0.75) and ambient pressure (0.60 ± 0.06 SD, Range = 0.40 - 0.74).

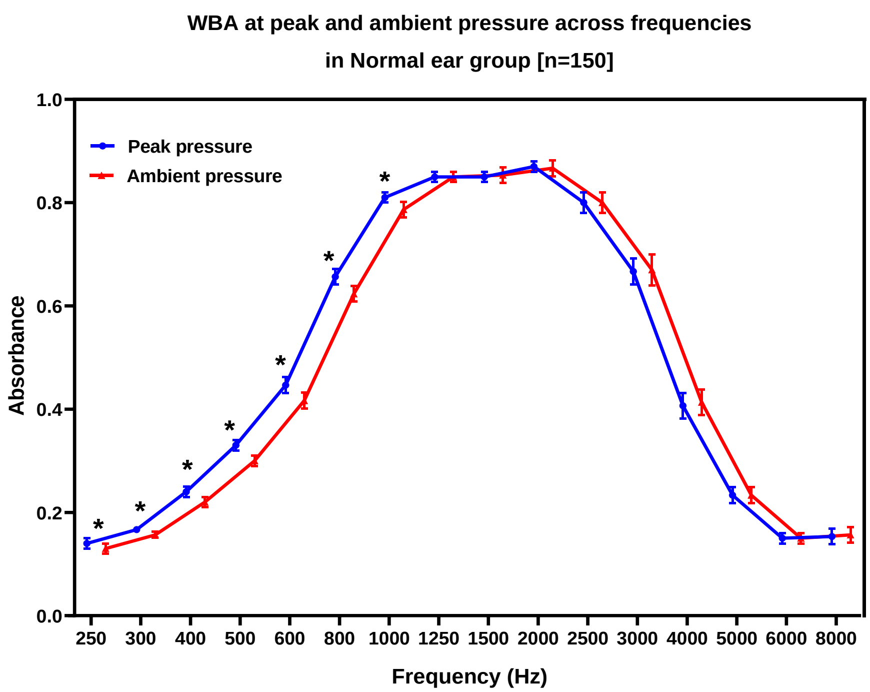


Figure 4.6 Graphical representation of mean WBA (vertical lines indicates 95% CI) measured at peak pressure and ambient pressure across frequencies in the Normal ear (n=150) group. *indicates corrected significant difference, $p<0.003$ with medium to large effect size.

Paired-T test with Bonferroni corrections showed a significant difference between the pressure conditions ($p<0.003$) for frequencies from 250 Hz to 1000 Hz in the Normal ear group, as shown in Table 4.8. The WBA at peak pressure showed slightly higher mean absorbance values than ambient pressure, as shown in Figure 4.6. While the WBT_{avg} measurements showed no significant difference between the pressure conditions ($p>0.05$) with identical mean WBT_{avg} values for peak and ambient pressure conditions. The equivalent non-parametric Wilcoxon signed-rank test between the pressure conditions in the Normal ear group also showed similar results and is shown in Annexure 4.4.

Table 4.8

Summary of Paired T-test results and its significant level of WBA obtained between the pressure conditions (peak, ambient) in the Normal ear [n=150] group

Frequency (Hz)	**t** *(df = 149)*	***p***	η_p^2
250	6.751	<0.001*	0.23
300	8.168	<0.001*	0.31
400	8.333	<0.001*	0.32
500	8.949	<0.001*	0.35
600	8.455	<0.001*	0.32
800	7.566	<0.001*	0.28
1000	5.702	<0.001*	0.18
1250	0.528	0.599	0.00
1500	-1.589	0.114	0.02
2000	0.272	0.786	0.00
2500	0.685	0.494	0.00
3000	-0.194	0.846	0.00
4000	-1.77	0.079	0.02
5000	-1.609	0.110	0.02
6000	-0.521	0.603	0.00
8000	-1.709	0.090	0.02
WBT_{avg}	2.838	0.005	0.05

Note. t- t-test statistics value, η_p^2 - Effect size of Partial Eta square values, and p - Significance level. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for $WBT_{avg,}$ and also the actual *p*-value was not depicted as the initial three values after the decimal were zero. The shaded region indicates a significant difference with medium to large effect size.

To study the effect of WBA across the study groups, i.e. Normal ear and Pathological groups, the Mixed ANOVA was performed with pressure conditions (02 levels) as within-subject factors, study group (06 levels) as between-subject factors and frequency (16 levels) as a repeated measure. The summary of the Mixed ANOVA results is tabulated in Table 4.9. The results indicated a significant main and interaction effect of group, pressure conditions and frequency ($p<0.05$) with large effect size ($\eta_p^2 \geq 0.14$). Hence, further analysis using two-way mixed ANOVA was performed between each of the pathological group (TmP, MEE, Otosclerosis, ETD and SNHL) with the Normal ear group to study the main and interaction effect as it is one of the main objectives of the study. This has also helped to obtained sensitivity and specificity to identify each pathological condition. If there is any significant main effect and/or interaction effect in the two-way mixed ANOVA, MANOVA and Paired-T test with Bonferroni correction were performed to see the significant difference between the groups and within-groups, respectively. The results are given under each of the pathological conditions.

Table 4.9

Mixed ANOVA results on WBA obtained from the independent variables (Group, pressure conditions) and dependent repeated-measure variable (frequency) across the groups

Variables	df, Error	F	*p*	η_p^2
Group	5, 761	30.59	<0.01*	0.63
Pressure conditions	1, 761	573.09	<0.01*	0.43
Pressure conditions * Group	5, 761	214.03	<0.01*	0.58
Frequency	5.02, 3822.71	2906.91	<0.01*	0.79
Frequency * Group	25.17, 3822.71	84.12	<0.01*	0.36
Pressure conditions * Frequency	4.48, 3399.34	153.85	<0.01*	0.17
Pressure conditions * Frequency * Group	22.33, 3399.34	54.29	<0.01*	0.26

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. Shaded region indicates significant difference with medium to large effect size.

With regard to the WBT_{avg} measurements, the average absorbance values were obtained for all the study groups in ambient pressure conditions. Whereas at peak pressure, the WBT_{avg} was obtained in only three study groups, i.e. Normal ear group, Otosclerosis group and SNHL group. Therefore, WBT_{avg} at peak and ambient pressure were analyzed in each pathological group in comparison to the Normal ear group. The results obtained are given under each pathological condition.

4.5.1 Tympanic membrane perforation (TmP)

The WBA data of 109 ears with tympanic membrane perforation was compared with 150 randomly selected normal ears from Group I. All the TmP group participants showed ‘B’ type tympanogram at 226 Hz probe tone frequency and had the average ear canal volume of 3.53 ± 0.97 ml (Range = 2.6 to 5.14 ml). However, the WBT measurement showed tympanometric peak pressure even for the ears with TmP, and the mean TPP was -269.22 ± 113.80 daPa (Range = -81 to -398 daPa). This could be due to the use of a wider frequency range stimulus for the measurement of WBA. Some variation in the change of transmission of sound energy took place with the change in ear canal pressure at the high frequencies. The external ear canal pressure at which maximum sound energy took place, the instrument probably considers it as a peak and displayed as peak pressure. Thus, the WBA obtained at the peak pressure, i.e. at TPP and the ambient pressure (0 daPa) across the frequencies in ears with TmP was considered for analysis. However, the WBT_{avg} could only be obtained at ambient pressure condition and not at peak pressure from the instrument. This could probably be due to the lack of changes in absorbance values with the variation of pressure in the ear canal at each of the frequencies (270 Hz and 2000 Hz) that are considered to calculate WBT_{avg}. This could be the limitation of the equipment.

The WBA measured at the peak and ambient pressure exhibited three maxima at 1000 Hz, 3000 Hz and 6000 Hz, as shown in Figure 4.7. A minimum mean absorbance value was observed at 250 Hz and also at 8000 Hz. Whereas a smooth and almost single broad peaked pattern was observed in the Normal ear group. Overall, the mean WBA observed in ears with TmP was lesser for low and mid frequencies up to 2500 Hz; identical at 3000 Hz, 4000 Hz, and 8000 Hz; and higher at 5000 Hz and 6000 Hz compared to the normal ear group. The mid-frequencies of 1000 Hz to 2500 Hz showed the largest difference in WBA values between the groups, as shown in Figure 4.6. The WBA obtained at the peak and ambient pressure was comparable for within-group comparison and followed a similar pattern for both TmP and Normal ear groups. The descriptive statistics (mean, SD and median) of WBA obtained in the TmP group was provided in Table 4.7.

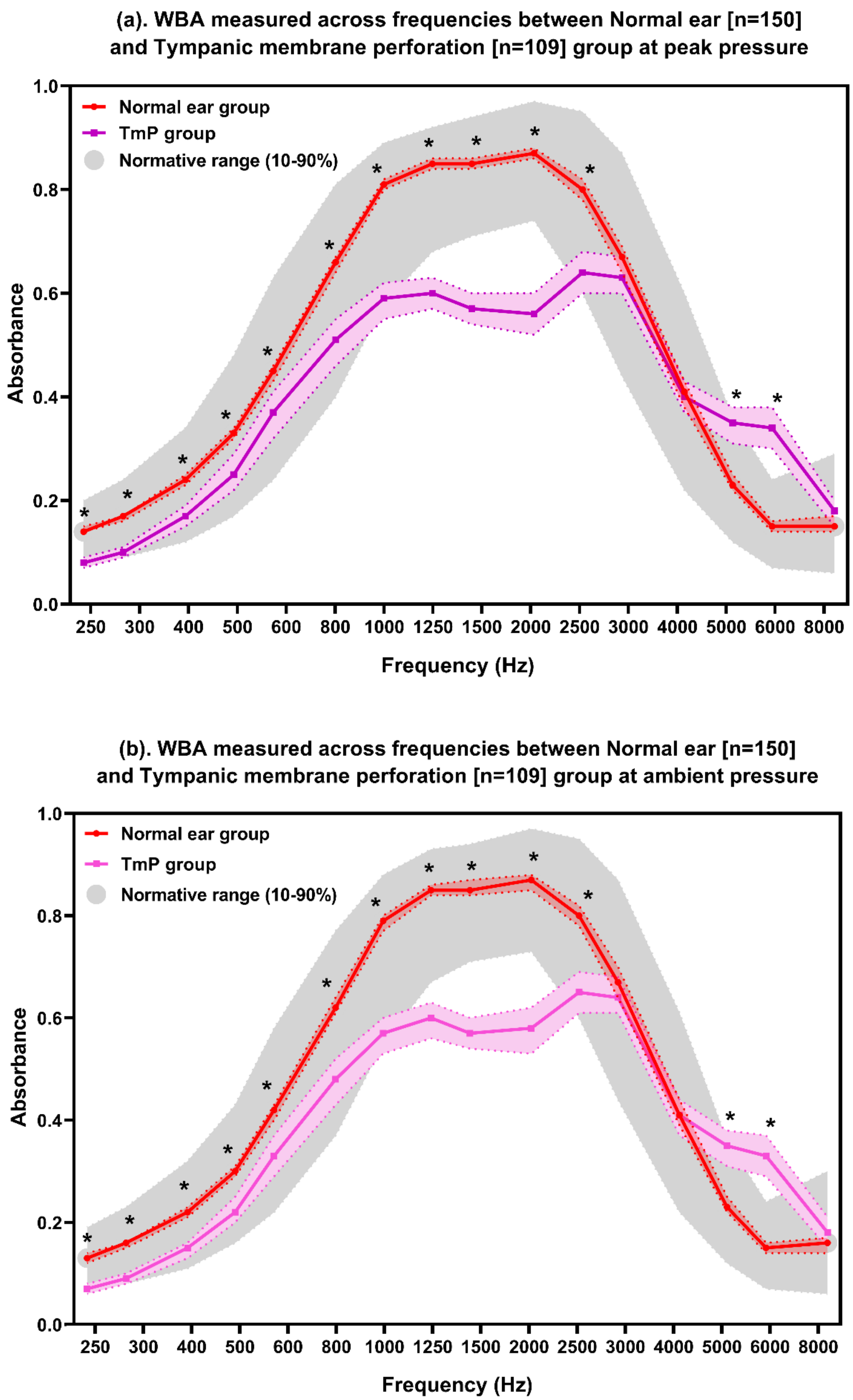


Figure 4.7 Mean WBA of Tympanic membrane perforation and Normal ear [n=150] groups obtained at (a) peak pressure and (b) ambient pressure across frequencies [Colour shaded region represents 95% CI; *indicates significant difference, *p*<0.05 with medium to large effect size].

The results of the mixed ANOVA showed a significant main effect of Group [F(1, 257)=130.025, $p<0.05$, $\eta_p^2=0.34$], pressure conditions [F(1, 257)=75.807, $p<0.05$, $\eta_p^2=0.23$] and frequency [F(6.33, 1626.739)=1002.205, $p<0.05$, $\eta_p^2=0.80$]. A significant interaction effect was seen between frequency and pressure conditions [F(5.136, 1319.968)= 32.885, $p<0.05$, $\eta_p^2=0.11$]; frequency and Group [F(6.33, 1626.739)= 78.381, $p<0.05$, $\eta_p^2=0.23$], demonstrating that WBA varied between the groups and pressure conditions across the frequency range. However, the interaction between pressure conditions and Group [F(1,257)=1.44, $p>0.05$, $\eta_p^2=0.00$] and the third-order interaction, i.e. pressure conditions, frequency and Group [F(5.136, 1319.968)= 2.076, $p>0.05$, $\eta_p^2=0.00$] were not significant.

The MANOVA was performed to study the significant difference in absorbance values at each frequency between the groups. The results did show a significant difference in mean absorbance values between the two groups at all frequencies ($p<0.05$), except for 3000 Hz and 4000 Hz ($p>0.05$) for both the pressure conditions, as shown in Table 4.10. As indicated earlier, the mean absorbance was lower for TmP group at low and mid-frequencies (250 Hz to 2500 Hz) and higher at 5000 and 6000 Hz. Similar results were also obtained using the non-parametric Mann-Whitney U test and are provided in Annexure 4.5.

Table 4.10

Summary of MANOVA results and its significant level of WBA obtained between TmP group and Normal ear group [n=150] at peak and ambient pressure conditions

Frequency (Hz)	**Peak pressure**			**Ambient pressure**		
	F *(df, error = 1, 257)*	***p***	**η_p^2**	**F** *(df, error = 1, 257)*	***p***	**η_p^2**
250	161.092	<0.01*	0.39	112.181	<0.01*	0.30
300	112.797	<0.01*	0.31	102.75	<0.01*	0.29
400	52.302	<0.01*	0.17	60.684	<0.01*	0.19
500	23.586	<0.01*	0.08	30.177	<0.01*	0.11
600	16.039	<0.01*	0.06	18.401	<0.01*	0.07
800	50.814	<0.01*	0.17	48.467	<0.01*	0.16
1000	155.174	<0.01*	0.38	152.448	<0.01*	0.37
1250	256.216	<0.01*	0.50	245.981	<0.01*	0.49
1500	321.932	<0.01*	0.56	325.23	<0.01*	0.56
2000	253.868	<0.01*	0.50	230.444	<0.01*	0.47
2500	58.22	<0.01*	0.19	46.922	<0.01*	0.15
3000	3.122	0.08	0.01	1.744	0.19	0.01
4000	0.057	0.81	0.00	0.025	0.87	0.00
5000	44.251	<0.01*	0.15	44.094	<0.01*	0.15
6000	105.996	<0.01*	0.29	100.844	<0.01*	0.28
8000	2.361	0.13	0.01	1.484	0.22	0.01

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Within-group comparison between the pressure conditions, the mean WBA measured at ambient pressure was lower for frequencies from 300 Hz to 1000 Hz and slightly higher at 2000 Hz to 3000 Hz than peak pressure in the TmP group. Other frequencies, i.e. 1250 Hz, 1500 Hz and above 3000 Hz, were similar for both the pressure conditions, as shown in Figure 4.8. Analysis using Paired-T test with Bonferroni corrections for TmP group showed a significant difference between peak and ambient pressure ($p<0.003$) only for frequencies from 300 to 1000 Hz, 2000 Hz and 2500 Hz with the effect size ranging from medium to large as shown in Table 4.11. The non-parametric Wilcoxon signed-rank test results for the TmP group also showed similar statistical significance results and are provided in the Annexure 4.6.

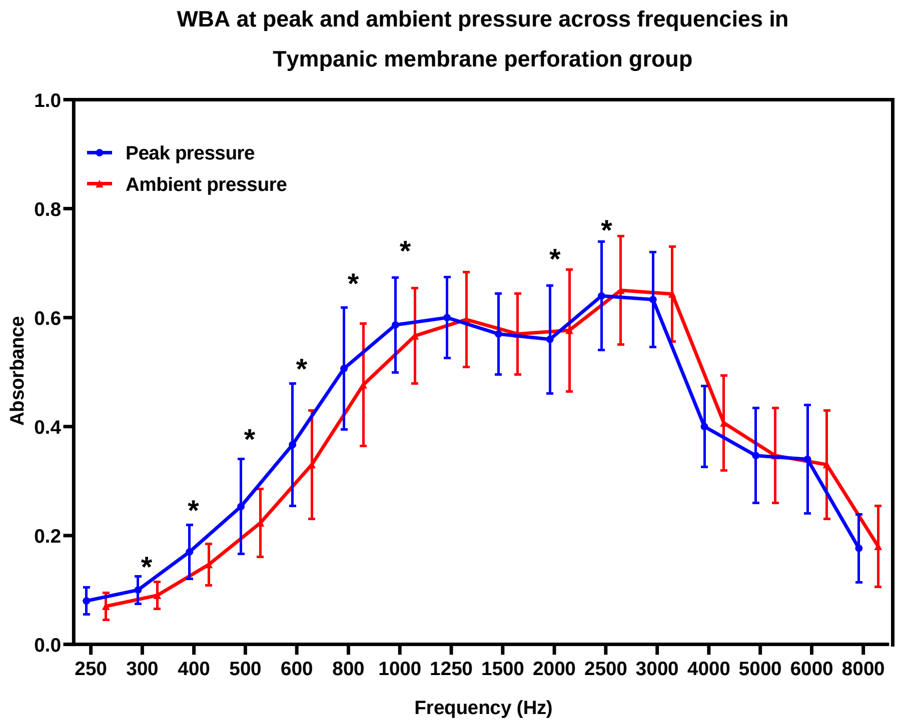


Figure 4.8 Graphical representation of mean WBA [vertical lines denoting 95% CI] measured at peak pressure and ambient pressure across frequencies in the Tympanic membrane perforation group. *indicates corrected significant difference, $p<0.003$ with medium to large effect size.

Table 4.11

Summary of Paired T-test results and its significant level of WBA obtained between the pressure conditions (peak, ambient) in Tympanic membrane perforation group

Frequency (Hz)	**t** *(df = 108)*	***p***	η_p^2
250	1.798	0.08	0.03
300	3.381	<0.001*	0.10
400	5.626	<0.001*	0.23
500	6.013	<0.001*	0.25
600	6.609	<0.001*	0.29
800	4.78	<0.001*	0.17
1000	3.751	<0.001*	0.12
1250	0.42	0.675	0.00
1500	-0.725	0.470	0.00
2000	-3.796	<0.001*	0.12
2500	-4.177	<0.001*	0.14
3000	-2.327	0.022	0.05
4000	-1.839	0.069	0.03
5000	-0.247	0.805	0.00
6000	1.056	0.293	0.01
8000	-0.339	0.736	0.00

Note. t- t-test statistics value, *p* - Significance level, η_p^2 - Effect size of Partial Eta square values. *corrected required significant level is $p<0.003$ for WBA across frequencies and also the actual *p*-value was not depicted as the initial three values after the decimal were zero. The shaded region indicates a significant difference with medium to large effect size.

Further, the WBT_{avg} measured only at ambient pressure for the TmP group was compared with the Normal ear group. The mean WBT_{avg} absorbance values of TmP group at ambient pressure was lower (0.45 ± 0.15 SD, Range: 0.09 - 0.82) compared to the Normal ear group (0.60 ± 0.06 SD, Range: 0.40 - 0.74). The results of the Independent-sample T-test showed a significant difference [$t(132.96) = 12.232$, $p<0.05$, $\eta_p^2 = 0.92$] between the TmP group and Normal ear group with large effect size. A similar result was also obtained using a non-parametric Mann-Whitney U test [U=2188, $p<0.05$, r=0.6].

Receiver Operating Characteristic (ROC) analysis was performed for those frequencies at which significant difference in absorbance values was observed between the groups, i.e. from 250 Hz to 2500 Hz, 5000 Hz, 6000 Hz in both pressure conditions and WBT_{avg} at ambient pressure. This analysis was performed to evaluate the test's accuracy in classifying the ear as normal or having a TmP. The ROC curve is plotted as a function of sensitivity and 1-specificity. Figure 4.9 shows the ROC obtained at 250 Hz to 2500 Hz, 5000 Hz, 6000 Hz for both pressure conditions and WBT_{avg} at ambient pressure. The ROC is passed over the diagonal line with an area under the ROC value >0.5, indicating perfect discrimination at all the significant frequencies. Among those, the frequencies of 250 Hz, 300 Hz, and 1000 Hz to 2000 Hz and WBT_{avg} at ambient pressure were closer to the top left-hand corner, indicating better prediction performance with a higher AUROC value of 0.8 and above.

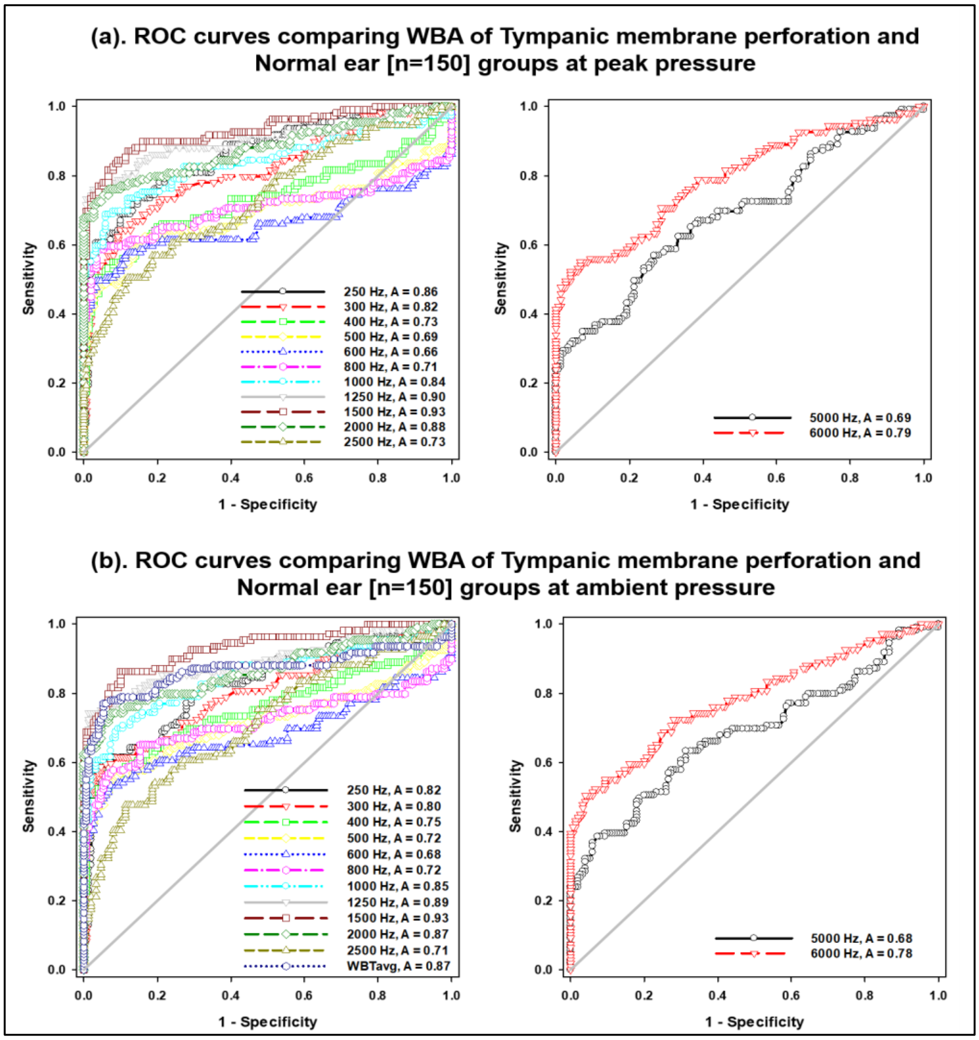


Figure 4.9 ROC curves and AUROC (A) values of WBA measured across the frequencies and WBT_{avg} at which significant difference has been obtained for (a) peak pressure and (b) ambient pressure between the Tympanic membrane perforation and Normal ear [n=150] groups.

The sensitivity and specificity calculated at the established criterion point based on the Youden index showed high specificity of greater than 88% in almost all frequencies. In contrast, the sensitivity was found to be below 88% in all these frequencies. The sensitivity and specificity were seen to be highest for mid-frequencies at 1250 Hz, 1500 Hz and 2000 Hz with identical cut-off criterion values for both the peak and ambient pressure conditions, as shown in Table 4.12.

Table 4.12

The AUROC, 95% CI, Youden Index point, criterion point with sensitivity and specificity measured at peak and ambient pressure across frequencies between the Tympanic membrane perforation and Normal ear [n=150] groups

Pressure conditions	**Frequency (Hz)**	**AUROC**	**95% CI**	**Youden Index (j)**	**Cut-off Criterion point**	**Sensitivity (%)**	**Specificity (%)**
Peak Pressure	250	0.86	0.81 - 0.91	0.59	≤0.10	68.81	90.00
	300	0.82	0.76 - 0.87	0.54	≤0.11	64.20	90.00
	400	0.73	0.66 - 0.80	0.47	≤0.15	55.05	92.00
	500	0.69	0.62 - 0.77	0.46	≤0.26	63.39	83.33
	600	0.66	0.58 - 0.74	0.46	≤0.30	49.54	96.67
	800	0.71	0.63 - 0.78	0.55	≤0.53	58.72	96.67
	1000	0.84	0.78 - 0.89	0.63	≤0.69	68.81	94.00
	1250	0.90	0.86 - 0.94	0.73	≤0.69	76.15	97.33
	1500	0.93	0.90 - 0.96	0.75	≤0.74	87.16	88.00
	2000	0.88	0.83 - 0.92	0.69	≤0.74	76.15	93.30
	2500	0.73	0.67 - 0.79	0.38	≤0.64	50.46	88.00
	5000	0.69	0.62 - 0.75	0.3	>0.27	56.88	73.30
	6000	0.79	0.73 - 0.85	0.48	>0.25	55.05	93.33
	WBT_{avg}	--	--	--	--	--	--
Ambient pressure	250	0.82	0.77 - 0.88	0.54	≤0.07	60.55	94.00
	300	0.80	0.75 - 0.86	0.54	≤0.09	58.72	95.33
	400	0.75	0.69 - 0.82	0.50	≤0.13	56.80	93.33
	500	0.72	0.65 - 0.79	0.48	≤0.20	55.05	93.33
	600	0.68	0.61 - 0.76	0.46	≤0.30	53.21	93.33
	800	0.72	0.64 - 0.79	0.50	≤0.48	56.88	93.33
	1000	0.85	0.79 - 0.90	0.60	≤0.66	69.72	90.67
	1250	0.89	0.85 - 0.94	0.73	≤0.66	74.31	98.67
	1500	0.93	0.90 - 0.96	0.76	≤0.74	86.24	90.00
	2000	0.87	0.82 - 0.92	0.67	≤0.74	74.31	92.67
	2500	0.71	0.65 - 0.78	0.36	≤0.63	47.71	88.67
	5000	0.68	0.61 - 0.75	0.32	>0.26	63.30	68.67
	6000	0.78	0.72 - 0.84	0.46	>0.28	50.46	96.00
	WBT_{avg}	0.87	0.81 - 0.92	0.72	≤0.54	77.06	94.67

Note. AUROC – Area under ROC curve, CI – Confidence interval. Shaded area indicates high sensitivity and specificity

In summary, the WBA measured at the peak, and ambient pressure was lesser at low and mid-frequencies up to 2500 Hz for ears with TmP compared to the normal ears. The pattern observed for WBA measurements across the frequencies showed three evident maxima for TmP group, compared to two relatively non-evident maximum peaks seen for the Normal ears. High sensitivity (>70%) and specificity (>90%) was observed in the mid-frequency region from 1250 Hz to 2000 Hz. Further, WBT_{avg} is obtained only at ambient pressure for ears with TmP and is lower than the Normal ears. The sensitivity and specificity of WBT_{avg} at ambient pressure was 77% and 95%, respectively, at a cut-off criterion point of ≤0.54 average absorbance value. The summary of the findings and the differential criteria compared to the normal ear is provided in Table 4.13.

Table 4.13

Summary of the findings of TmP group and differential criteria in comparison to Normal ear

Parameters		**TmP Group (in-comparison to Normal ear)**
WBA across frequencies from 250 Hz to 8000 Hz	-	Lower absorbance value up to 2500 Hz and higher at 5000 and 6000 Hz
WBA pattern	-	Three maxima peak at 1000 Hz, 3000 Hz and 6000 Hz, compared to 02 relatively non-evident broad peaks at 1250 Hz and 2000 Hz.
WBT_{avg}	-	Lower WBT_{avg} compared to the normal ear with 77% sensitivity and 95% specificity at ambient pressure.
Differential criteria		Mid-frequencies from 1250 Hz to 2000 Hz showed maximum sensitivity and specificity and suggested using these frequencies absorbance values for differentiation.

4.5.2 Middle ear effusion (MEE)

The middle ear effusion group consisted of 122 ears without having tympanic membrane perforation and are compared with 150 randomly selected normal ears from Group I. The details of the Normal ear group (Group I) have been given under section 4.4. A 226 Hz tympanometry for the MEE effusion group showed a 'B' type tympanogram and had the average ear canal volume of 1.16 ± 0.27 ml (Range = 0.57 - 1.94 ml). Though the TPP was not obtained for 226 Hz probe tone, WBT measurements showed a peak pressure (the reason has been given under 4.4a), and the mean TPP was -244 ± 179.83 (-395 to - 60 daPa). Thus, with the estimated TPP of WBT measurements, the WBA at peak pressure was extracted from the MEE group for all frequencies and compared with the Normal ear group along with ambient pressure conditions. Whereas the WBT_{avg} was obtained only at the ambient pressure condition and not at peak pressure. The descriptive statistics (mean, SD, and median) of the MEE group are shown in Table 4.7.

The MEE group exhibited a significant reduction in the absorbance values at all the frequencies compared to the Normal ear group for both the pressure conditions, as shown in Figure 4.10. The WBA of the MEE group was minimal at 250 Hz, 6000 Hz and 8000 Hz, similar to that of the Normal ear group. It increased gradually as the frequency increased to a maximum at around 2000 Hz and 2500 Hz. Beyond 2500 Hz, the WBA decreases sharply, reaching a minimum at 6000 Hz. The WBA obtained at the peak and ambient pressure is similar for the MEE group.

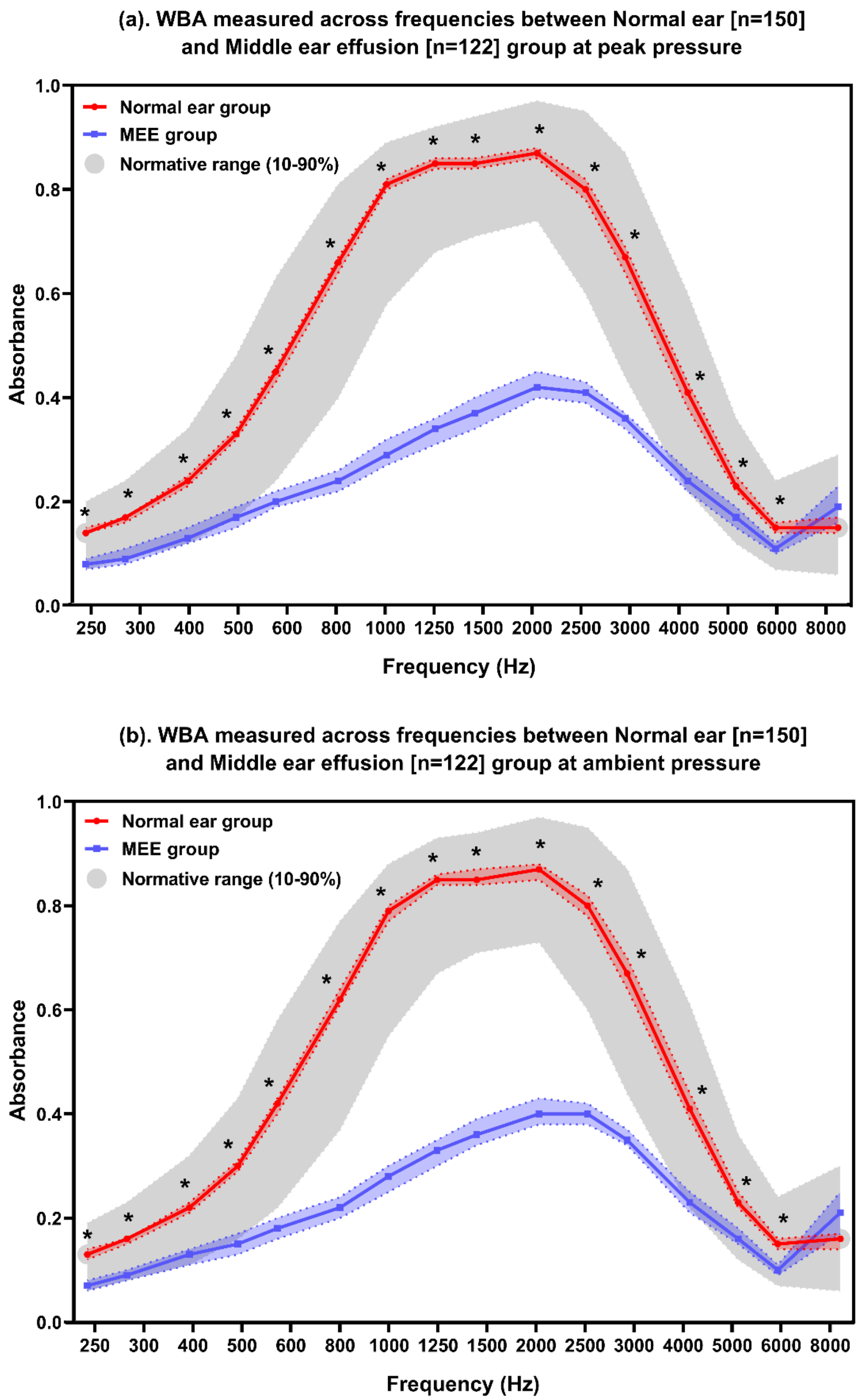


Figure 4.10 Graphical representation of mean WBA of Middle ear effusion and Normal ear [n=150] groups obtained at (a) peak pressure and (b) ambient pressure across frequencies [Colour shaded region represents 95% CI; *indicates significant difference, p<0.05 with medium to large effect size].

The mixed ANOVA was performed to study the effect of pressure conditions and groups. The results showed a significant main effect of Group [F(1,270)=2059.77, $p<0.05$, $\eta_p^2=0.88$], pressure conditions [F(1,270)=43.512, $p<0.05$, $\eta_p^2=0.14$] and frequency [F(4.409, 71.524)=1190.734, $p<0.05$, $\eta_p^2=0.82$]. A significant interaction effect was seen between frequency and Group [F(4.509,16.627)= 276.816, $p<0.05$, $\eta_p^2=0.51$]; Pressure conditions and frequency [F(4.254, 1148.613)= 18.554, $p<0.05$, $\eta_p^2=0.06$]; and between pressure conditions, frequency and Group [F(4.254, 1148.613)= 4.736 $p<0.05$, $\eta_p^2=0.02$], indicating that the WBA varied between the groups and pressure conditions across the frequency range. The interaction between pressure conditions and Group [F(1,270)=0.011, $p>0.05$, $\eta_p^2=0.00$] was not significant.

Further, the statistical analysis using the MANOVA was done to identify the frequencies at which there exists a significant difference in WBA between the groups. A statistically significant at all frequencies ($p<0.05$) was observed for both the pressure conditions, as shown in Table 4.14. However, the effect size was small for 8000 Hz indicating the difference between groups is not truly significant. The non-parametric Mann-Whitney U test also showed similar results and are provided in Annexure 4.7.

Table 4.14

Summary of MANOVA results and its significant level of WBA obtained between Middle ear effusion and Normal ear [n=150] groups at peak and ambient pressure conditions

Frequency (Hz)	**Peak pressure**			**Ambient pressure**		
	F *(df, error =1,270)*	***p***	η_p^2	**F** *(df, error =1,270)*	***p***	η_p^2
250	112.305	<0.01*	0.29	90.447	<0.01*	0.25
300	115.199	<0.01*	0.30	82.764	<0.01*	0.24
400	148.022	<0.01*	0.35	97.6	<0.01*	0.27
500	235.801	<0.01*	0.47	185.374	<0.01*	0.41
600	513.349	<0.01*	0.66	421.887	<0.01*	0.61
800	1427.605	<0.01*	0.84	1149.32	<0.01*	0.81
1000	1624.344	<0.01*	0.86	1507.974	<0.01*	0.85
1250	1435.475	<0.01*	0.84	1513.579	<0.01*	0.85
1500	1152.315	<0.01*	0.81	1145.586	<0.01*	0.81
2000	1065.726	<0.01*	0.80	1113.634	<0.01*	0.81
2500	709.075	<0.01*	0.72	696.451	<0.01*	0.72
3000	388.068	<0.01*	0.59	376.387	<0.01*	0.58
4000	107.25	<0.01*	0.28	117.078	<0.01*	0.30
5000	29.971	<0.01*	0.10	40.357	<0.01*	0.13
6000	24.182	<0.01*	0.08	36.469	<0.01*	0.12
8000	4.981	0.03	0.02	7.027	0.01	0.03

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Comparison of WBA obtained between the pressure conditions showed slightly lower absorbance at ambient pressure for low frequencies and slightly higher at high frequencies than peak pressure in the MEE group, as shown in Figure 4.11. A similar pattern is also observed in the Normal ear group (Figure 4.5). The Paired-T test with Bonferroni corrections showed a significant difference in absorbance values between the pressure conditions (p<0.003) for frequencies between 500 Hz and 1000 Hz having medium to large effect size as shown in Table 4.15. The non-parametric Wilcoxon signed-rank test also had similar results and are provided in Annexure 4.8.

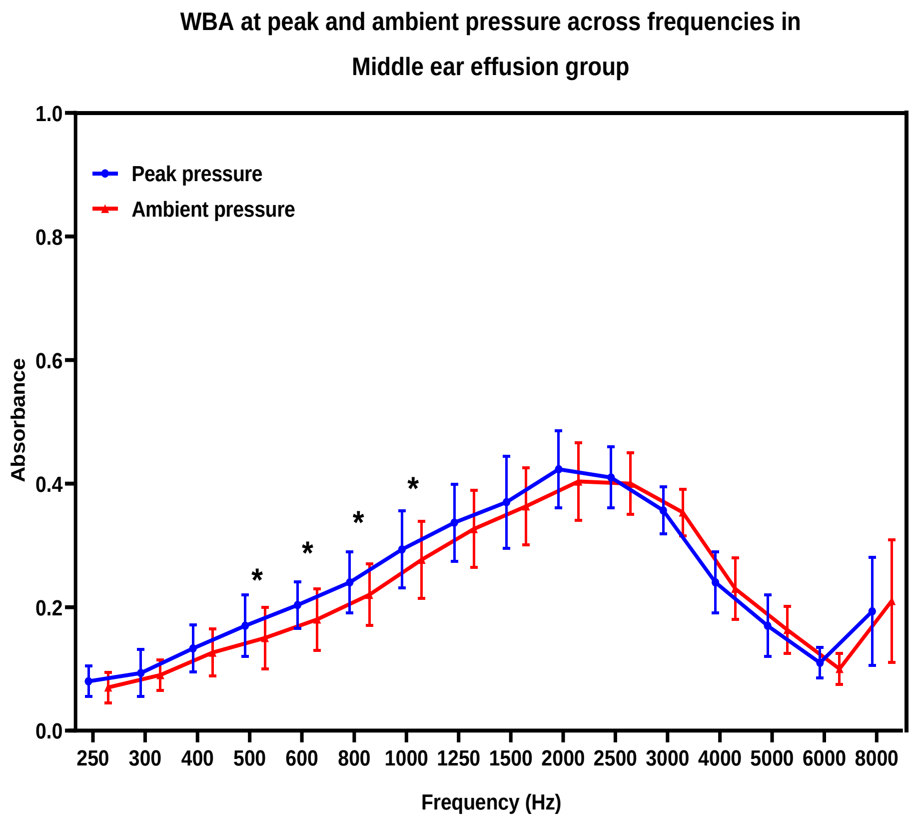


Figure 4.11 Graphical representation of mean WBA [vertical lines denoting 95% CI] measured at peak pressure and ambient pressure across frequencies in the Middle ear effusion group. *indicates corrected significant difference, $p<0.003$ with medium to large effect size.

Table 4.15

Summary of Paired T-test results and its significant level of WBA obtained between the pressure conditions (peak, ambient) in Middle ear effusion group

Frequency (Hz)	**t** *(df = 121)*	***p***	η_p^2
250	1.374	0.172	0.02
300	0.868	0.387	0.01
400	1.397	0.165	0.02
500	3.179	0.002	0.08
600	4.955	<0.001*	0.17
800	5.486	<0.001*	0.20
1000	4.726	<0.001*	0.16
1250	2.372	0.019	0.04
1500	1.397	0.165	0.02
2000	2.45	0.016	0.05
2500	2.242	0.027	0.04
3000	1.043	0.299	0.01
4000	2.509	0.013	0.05
5000	2.496	0.014	0.05
6000	3.442	0.001	0.09
8000	-1.859	0.065	0.03

Note. t- t-test statistics value, η_p^2 – Effect size of Partial Eta square values, and *p* - Significant level. *corrected required significant level is $p<0.003$ for WBA across frequencies and also the actual *p*-value was not depicted as the initial three values after the decimal were zero. The shaded region indicates a significant difference with medium to large effect size.

With regard to WBT_{avg}, the analysis was done only at the ambient pressure in ears with MEE. The MEE group had a lower WBT_{avg} absorbance value of 0.26 ± 0.09 (Range: 0.10 to 0.48) compared to the Normal group at ambient pressure. The Independent Sample T-test was used to evaluate the difference in WBT_{avg} absorbance value between the groups (MEE vs Normal group). The results showed a statistically significant WBT_{avg} absorbance values [$t(198.275) = 38.336$, $p<0.05$, $\eta_p^2=0.88$] between the groups with a large effect size ($\eta_p^2 \geq 0.14$). A similar result was also obtained using a non-parametric Mann-Whitney U test [U=14.00, $p<0.05$, r=0.9].

The sensitivity and specificity were established for the MEE group at a specific cut-off criterion point for all the frequencies identified as significant using the ROC analysis. WBA data considered were the frequencies from 250 Hz to 6000 Hz for both the pressure conditions and WBT_{avg} at ambient pressure. The ROC curve for each of the significant frequencies at both the pressure conditions was above the diagonal line with an AUROC value greater than 0.5, indicating good discrimination between the ear with MEE and without disorders, as shown in Figure 4.12. Among those, the frequencies from 800 Hz to 3000 Hz are most sensitive, having a high AUROC of 0.96 and above. These frequencies have high sensitivity and specificity of greater than 85% for both the pressure conditions in the estimated cut-off criterion point. While the frequencies from 250 Hz to 600 Hz and 4000 Hz has AUROC value that ranged between 0.8 and 0.9 with sensitivity and specificity of above 72%. However, the high frequencies at 5000 Hz and 6000 Hz has poor sensitivity of less than 55%, but the specificity is above 70%, with an AUROC value between 0.67 and 0.75. The cut-off criterion point estimated using the Youden index point for each frequency is shown in Table 4.16.

The WBT_{avg} absorbance value has an AUROC value of 1, indicating perfect discrimination between MEE and Normal ears. It has a 100% sensitivity and 98.67% specificity at a cut off criterion point of $\leq$0.48 absorbance value. As a whole, mid-frequencies from 800 Hz to 3000 Hz and WBT_{avg} were the most sensitive in identifying the MEE from the Normal ear group.

Table 4.16

The AUROC, 95% CI, Youden Index point, criterion point with sensitivity and specificity measured at peak and ambient pressure across frequencies between the Middle ear effusion and Normal ear [n=150] groups

Pressure conditions	**Frequency (Hz)**	**AUROC**	**95% CI**	**Youden Index (j)**	**Cut-off Criterion point**	**Sensitivity (%)**	**Specificity (%)**
Peak Pressure	250	0.84	0.79 - 0.88	0.64	≤0.11	78.69	85.33
	300	0.84	0.78 - 0.88	0.64	≤0.12	75.41	88.67
	400	0.84	0.79 - 0.88	0.64	≤0.17	74.60	89.33
	500	0.88	0.84 - 0.93	0.69	≤0.21	73.77	95.33
	600	0.96	0.92 - 0.97	0.79	≤0.30	86.07	92.67
	800	1.00	0.99 - 1.00	0.97	≤0.52	100.00	97.33
	1000	1.00	0.98 - 1.00	0.97	≤0.58	96.72	96.72
	1250	1.00	0.98 - 1.00	0.97	≤0.66	97.54	99.33
	1500	1.00	0.98 - 1.00	0.97	≤0.64	97.54	99.33
	2000	1.00	0.97 - 1.00	0.97	≤0.70	100.00	97.33
	2500	0.99	0.98 - 1.00	0.89	≤0.62	98.67	90.67
	3000	0.96	0.93 - 0.98	0.81	≤0.48	92.62	88.67
	4000	0.82	0.77 - 0.87	0.49	≤0.30	73.77	75.33
	5000	0.70	0.64 - 0.77	0.33	≤0.16	52.46	80.67
	6000	0.67	0.61 - 0.74	0.29	≤0.11	54.92	74.00
Ambient pressure	250	0.80	0.75 - 0.85	0.59	≤0.07	64.75	94.00
	300	0.80	0.75 - 0.85	0.60	≤0.08	64.75	95.33
	400	0.80	0.74 - 0.86	0.60	≤0.13	63.93	96.00
	500	0.86	0.81 - 0.90	0.65	≤0.23	78.70	86.00
	600	0.95	0.92 - 0.97	0.79	≤0.30	86.07	92.67
	800	1.00	0.98 - 1.00	0.98	≤0.45	100.00	98.00
	1000	1.00	0.99 - 1.00	0.95	≤0.56	96.72	98.67
	1250	1.00	0.99 - 1.00	0.97	≤0.64	97.54	99.33
	1500	1.00	0.98 - 1.00	0.95	≤0.64	96.72	98.67
	2000	1.00	0.98 - 1.00	0.95	≤0.68	99.18	96.00
	2500	0.99	0.96 - 1.00	0.90	≤0.62	100.00	90.00
	3000	0.96	0.94 - 0.98	0.81	≤0.49	93.44	88.00
	4000	0.83	0.78 - 0.87	0.53	≤0.34	84.43	68.67
	5000	0.75	0.69 - 0.80	0.40	≤0.18	65.57	74.00
	6000	0.72	0.66 - 0.77	0.35	≤0.10	56.56	78.00
	WBT_{avg}	1.00	0.99 - 1.00	0.99	≤0.48	100.00	98.67

Note. AUROC – Area under ROC curve, CI – Confidence interval. Shaded area indicates high sensitivity and specificity

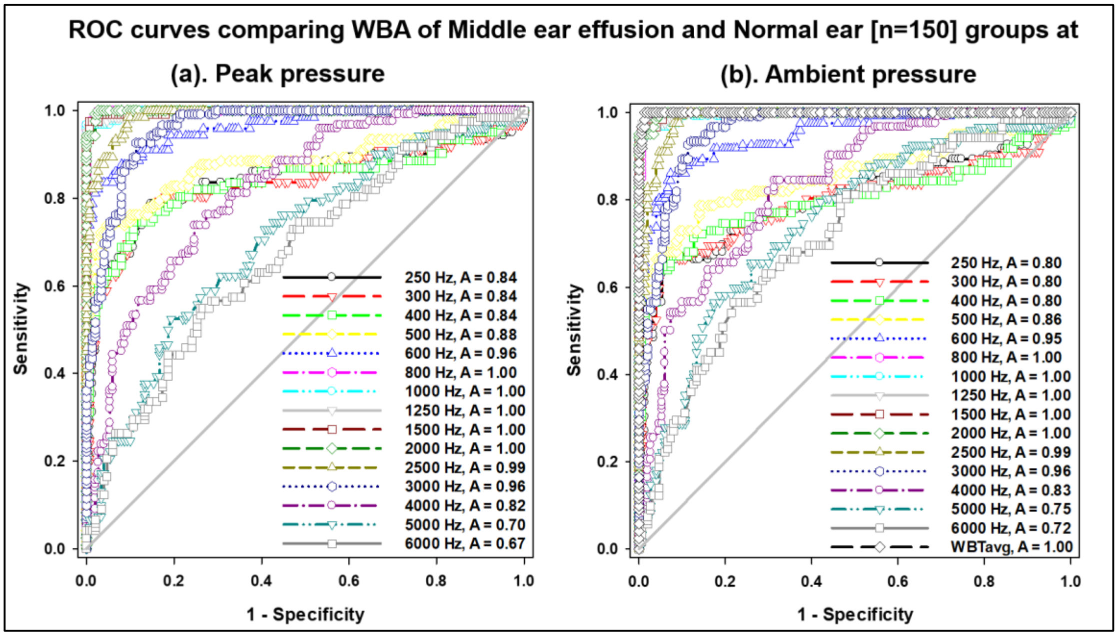


Figure 4.12 ROC curves and AUROC (A) values of WBA measured across the frequencies and WBT_{avg} at which significant difference has been obtained for (a) peak pressure and (b) ambient pressure between the Middle ear effusion and Normal ear [n=150] groups.

In summary, the MEE group had a lower WBA for all the frequencies than the normal ear in both pressure conditions. However, the WBA pattern was almost similar for both groups, with a broad and higher absorbance pattern for the Normal ear group and a gradually rising pattern for the MEE group. High sensitivity and specificity of greater than 70% were seen for all the frequencies except at 5000 Hz and 6000 Hz, with the most sensitive being mid-frequencies from 600 Hz to 3000 Hz. Further, WBT_{avg} at ambient pressure for ears with MEE found to have high sensitivity and specificity of greater than 98%. The summary of the findings and the differential criteria compared to the Normal ear group is provided in Table 4.17.

Table 4.17

Summary of the findings of Middle ear effusion group and differential criteria in comparison to Normal ear group

Parameters	MEE group (in comparison to the Normal ear)
WBA across frequencies from 250 Hz to 8000 Hz	- Drastic reduction of absorbance for all the frequencies compared to the Normal ear group.
WBA pattern	- A gradually rising pattern with a single peak around 2000 Hz and 2500 Hz, compared to 02 relatively non-evident broad peaks at 1250 Hz and 2000 Hz.
WBT_{avg}	- Lower WBT_{avg} compared to the normal ear at ambient pressure with the sensitivity and specificity of 100% and 98.67%.
Differential criteria	- Mid-frequencies from 800 Hz to 3000 Hz showed maximum sensitivity and specificity and suggested using these frequencies absorbance values for differentiation.

4.5.3 Otosclerosis

The WBA data of 140 ears of having Otosclerosis were compared with 150 randomly selected normal ears from the Group I. All the participants in the Otosclerosis group had 'As' type tympanogram, with the mean static admittance of 0.30 ± 0.35 mmho (Range= 0.15 to 0.48 mmho), TPP of -10.84 ± 21.28 daPa (Range = -97 to 47 daPa) and the mean ear canal volume of 0.96 ± 0.24 ml (Range=0.51 to 1.92 ml). The mean TPP obtained in WBT measurements for ears with Otosclerosis was -9.87 ± 20.28 daPa (Range = -85 to 50 daPa).

Figure 4.13 shows the WBA measured across frequencies in ears with Otosclerosis compared to the Normal ear group. The Otosclerosis group showed a minimum absorbance at 250 Hz and gradually increased steeply with an increase in frequency to a maximum absorbance value around 2000 Hz and 2500 Hz. Further, the absorbance values decreased steeply to a minimum value at 6000 Hz and beyond. Moreover, the WBA pattern observed in the Otosclerosis group is a single narrow peak with a maximum absorbance seen at 2000 Hz and 2500 Hz. Table 4.7 showed the descriptive statistics (mean, SD, and median) of the Normal ear group and the Otosclerosis group. The Otosclerosis group has lower absorbance value for the low and mid-frequencies up to 2000 Hz compared to the Normal ear group. At high frequencies above 2000 Hz, the absorbance values were similar for both the groups. Also, the WBA data obtained at peak pressure and ambient pressure were similar for both the groups.

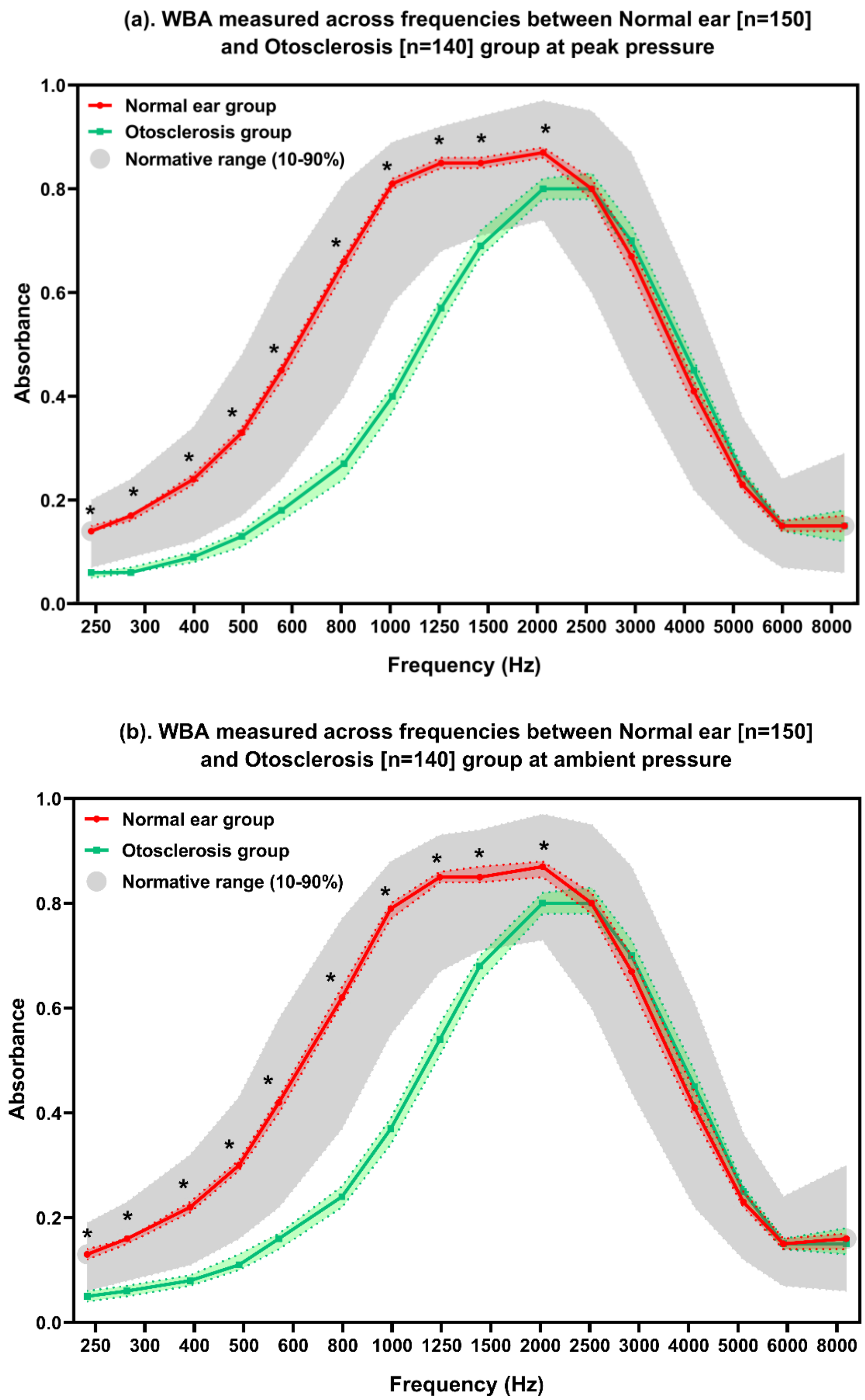


Figure 4.13 Graphical representation of mean WBA of Otosclerosis and Normal ear [n=150] groups obtained at (a) peak pressure and (b) ambient pressure across frequencies [Colour shaded region represents 95% CI; *indicates significant difference, *p*<0.05 with medium to large effect size].

Using a mixed ANOVA, the significant main effect of the group [$F(1,288)=569.667$, $p=0.00$, $\eta_p^2=0.66$], pressure [$F(1,288)=112.784$, $p<0.05$, $\eta_p^2=0.28$] and frequency [$F(3.6, 1036.809) = 2167.754$, $p<0.05$, $\eta_p^2=0.88$] was observed. A significant interaction effect was also seen between frequency and pressure [$F(3.597, 1036.23)=39.066$, $p<0.05$, $\eta_p^2=0.12$]; and frequency and Group [$F(3.6, 1036.809)= 160.468$, $p=0.00$, $p<0.05$, $\eta_p^2=0.36$]; demonstrating that WBA varied with pressure and group across the frequency. Though the third-order interaction between pressure conditions, frequency and Group [$F(3.597, 1036.23)= 8.696$, $p<0.05$, $\eta_p^2=0.00$] was significant, the effect size is too small to be considered as truly significant. Whereas, the interaction between pressure conditions and Group [$F(1,288)=0.319$, $p>0.05$, $\eta_p^2=0.00$] was not significant.

To investigate the frequencies at which a significant group difference occurred, MANOVA was performed. The results showed statistically significant differences in absorbance values between the two groups for low and mid-frequencies up to 2000 Hz for both pressure conditions. The effect size for those significant frequencies from 250 Hz to 1500 Hz was large, and the medium effect size was seen at 2000 Hz, as shown in Table 4.18. Similar results were obtained using the non-parametric Mann-Whitney U test with significant difference seen up to 2000 Hz. The statistical results of the Mann-Whitney U test are provided in Annexure 4.9.

Table 4.18

Summary of MANOVA results and its significant level of WBA obtained between the Otosclerosis and Normal ear [n=150] groups at peak and ambient pressure conditions

	Peak pressure			Ambient pressure		
Frequency (Hz)	**F** *(df, Error =1, 288)*	***p***	**η_p^2**	**F** *(df, Error =1, 288)*	***p***	**η_p^2**
250	408.715	<0.01*	0.59	337.013	<0.01*	0.54
300	414.268	<0.01*	0.59	367.649	<0.01*	0.56
400	481.6	<0.01*	0.63	475.572	<0.01*	0.62
500	415.237	<0.01*	0.59	462.819	<0.01*	0.62
600	534.234	<0.01*	0.65	569.577	<0.01*	0.66
800	904.296	<0.01*	0.76	904.058	<0.01*	0.76
1000	921.86	<0.01*	0.76	921.265	<0.01*	0.76
1250	404.092	<0.01*	0.58	473.856	<0.01*	0.62
1500	117.317	<0.01*	0.29	139.758	<0.01*	0.33
2000	27.047	<0.01*	0.09	24.654	<0.01*	0.08
2500	0.07	0.79	0.00	0.138	0.71	0.00
3000	3.002	0.08	0.01	2.632	0.11	0.01
4000	4.184	0.06	0.01	4.094	0.06	0.01
5000	1.857	0.17	0.01	1.296	0.26	0.00
6000	0.039	0.84	0.00	0.084	0.77	0.00
8000	0.003	0.96	0.00	0.126	0.72	0.00
WBT_{avg}	9.591	0.00	0.03	661.504	<0.01*	0.697

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

In comparing pressure conditions (peak, ambient) within the Otosclerosis group, a lower WBA at lower frequencies and higher absorbance values at high frequencies were observed in the ambient pressure compared to the peak pressure conditions and is similar to that has been observed in Normal ear group. However, such a difference was seen only at low and mid-frequencies of up to 1500 Hz, as shown in Figure 4.14. Paired T-test with Bonferroni corrections showed a significant difference ($p<0.003$) in absorbance values between the pressure conditions up to 1250 Hz for the Otosclerosis group, as shown in Table 4.19. The effect size was large ($\eta_p^2 \geq 0.14$) for frequencies from 250 Hz to 1000 Hz and medium at 1250 Hz ($\eta_p^2 \geq 0.06$). The non-parametric Wilcoxon signed-rank test showed similar statistical significance results ($p<0.003$), and the results are tabulated in Annexure 4.10.

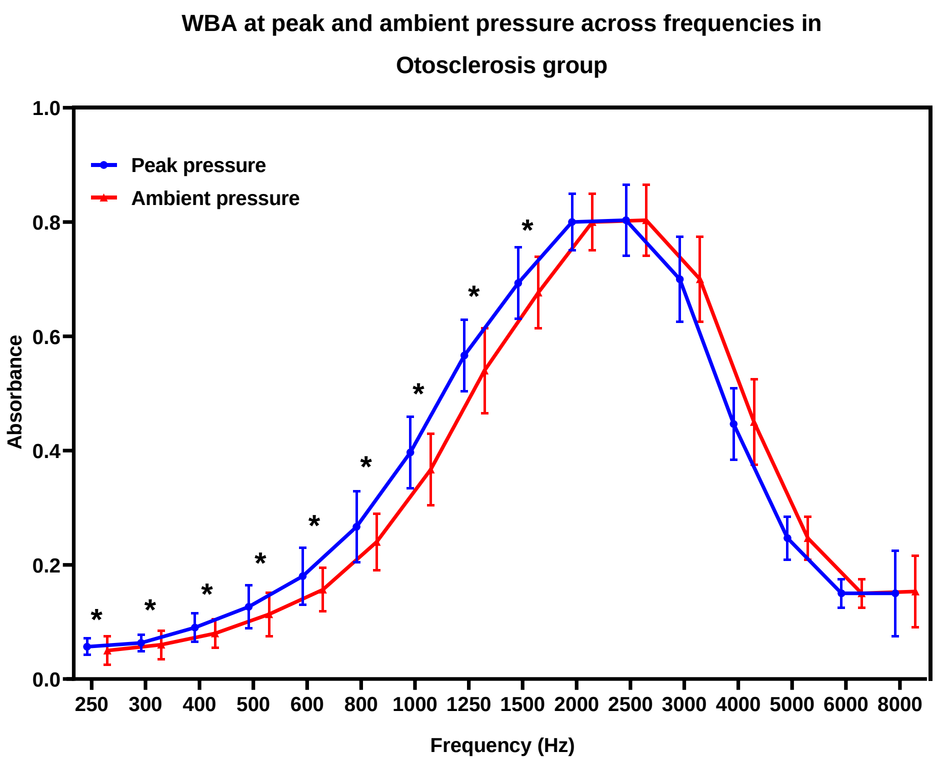


Figure 4.14 Graphical representation of mean WBA (vertical lines denoting 95% CI) measured at peak pressure and ambient pressure across frequencies in the Otosclerosis group. *indicates corrected significant difference, $p<0.003$ with medium to large effect size.

Table 4.19

Summary of Paired T-test results and its significant level of WBA obtained between the pressure conditions (peak, ambient) in the Otosclerosis group

Frequency (Hz)	**t** *(df =139)*	***p***	η_p^2
250	4.38	<0.001*	0.12
300	4.565	<0.001*	0.13
400	5.144	<0.001*	0.16
500	5.079	<0.001*	0.16
600	5.126	<0.001*	0.16
800	6.497	<0.001*	0.23
1000	7.303	<0.001*	0.28
1250	6.616	<0.001*	0.24
1500	3.871	<0.001*	0.10
2000	-0.657	0.512	0.00
2500	-0.029	0.977	0.00
3000	0.819	0.414	0.00
4000	-3.044	0.003	0.06
5000	0.753	0.453	0.00
6000	0.947	0.345	0.01
8000	-2.743	0.007	0.05
WBT_{avg}	-7.728	<0.001*	0.17

Note. t- t-test statistics value, η_p^2 – Effect size of Partial Eta square, and *p* - Significant level. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for WBT_{avg}, and also the actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Apart from WBA across the frequencies, the WBT_{avg} measurements obtained at peak and ambient pressure were compared between the Otosclerosis and Normal ear group. The mean WBT_{avg} of Otosclerosis group at peak pressure and ambient pressure was 0.56 ± 0.14 (Range = 0.16 to 0.75) and 0.37 ± 0.10 (Range= 0.09 to 0.78) respectively. The Independent Sample T-test was used to evaluate the difference in WBT_{avg} absorbance value between the groups (Otosclerosis vs Normal ear group) at peak pressure and ambient pressure conditions. The results showed a statistically significant in WBT_{avg} absorbance values at peak pressure [$t(217.093) = 25.269$, $p<0.05$, $\eta_p^2 = 0.04$] having small effect size ($\eta_p^2 < 0.06$) and at ambient pressure [$t(242.179) = 3.057$, $p<0.05$, $\eta_p^2 = 0.75$] with a large effect size ($\eta_p^2 \geq 0.14$). The mean WBT_{avg} was lower for Otosclerosis group than the Normal ear group with a greater mean difference of 0.26 seen at ambient pressure and 0.05 at peak pressure. A similar result was also obtained using a non-parametric Mann-Whitney U test at peak pressure [U=8996.5, $p<0.05$, r=0.1] and ambient pressure [U=618.50, $p<0.05$, r=0.8].

Within-group comparison using paired T-test showed a significant difference between pressure conditions [$t(139) = -14.08$, $p<0.05$, $\eta_p^2 = 0.59$] having a large effect size in the Otosclerosis group. The mean WBT_{avg} of the Otosclerosis group was lower at ambient pressure compared to the peak pressure. Similar results were obtained using the non-parametric Wilcoxon signed-rank test [Z= -6.626, $p<0.05$, = 0.6].

A ROC analysis was performed to investigate the sensitivity and specificity and establish a cut-off criterion point. The WBA data considered for ROC analysis were from 250 Hz to 2000 Hz and WBT_{avg} at peak and ambient pressure. The ROC curve for all the frequencies (250 Hz to 2000 Hz) and also for WBT_{avg} lying above the diagonal line indicating good discrimination ($p<0.05$) between the groups, i.e. Normal ear and Otosclerosis group (Figure 4.15). However, high sensitivity and specificity were seen for frequencies up to 1250 Hz. Table 4.20 shows the AUROC value with 95% CI obtained for WBA for each of the frequencies and WBT_{avg} at peak and ambient pressure conditions. The sensitivity and specificity obtained at specific cut-off criteria based on the Youden index point are also shown in Table 4.20.

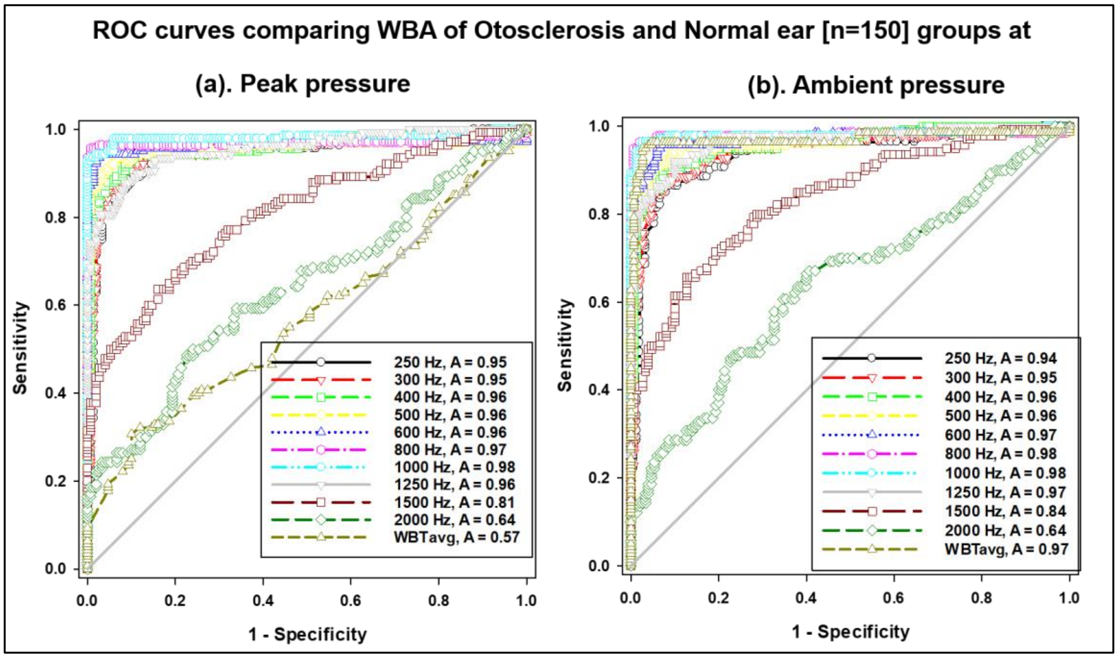


Figure 4.15 ROC curves and AUROC (A) values of WBA measured across the frequencies and WBT_{avg} at which significant difference has been obtained for (a) peak pressure and (b) ambient pressure between the Otosclerosis and Normal ear [n=150] groups.

The AUROC value was high that ranged above 0.95 for frequencies from 250 Hz to 1250 Hz in both the pressure conditions with high sensitivity and specificity of greater than 80%. However, reduced sensitivity and specificity with lower AUROC values (<0.8) were seen at 1500 and 2000 Hz. The cut-off criterion point estimated using the Youden index was identical in peak and ambient pressure conditions at 300 Hz and from 800 Hz to 1250 Hz, as shown in Table 4.20. The cut-off criterion points for other frequencies are almost similar in both pressure conditions. Similarly, ROC measured for WBT_{avg} showed high accuracy (>95% sensitivity and specificity) in differentiating the Otosclerosis from the normal ear with a high AUROC value (0.97) at ambient pressure.

Table 4.20

The AUROC, 95% CI, Youden Index point, criterion point with sensitivity and specificity measured at peak and ambient pressure across frequencies and WBT_{avg} between the Otosclerosis and Normal ear [n=150] groups

Pressure conditions	Frequency (Hz)	AUROC	95% CI	Youden Index (j)	Cut-off Criterion point	Sensitivity (%)	Specificity (%)
Peak Pressure	250	0.95	0.92 - 0.97	0.82	≤0.12	94.29	87.33
	300	0.95	0.92 - 0.98	0.84	≤0.14	87.86	96.00
	400	0.96	0.93 - 0.98	0.87	≤0.22	92.14	94.67
	500	0.96	0.93 - 0.99	0.92	≤0.30	94.29	97.33
	600	0.96	0.94 - 0.99	0.95	≤0.47	95.71	99.33
	800	0.97	0.95 - 1.00	0.93	≤0.61	93.57	99.33
	1000	0.98	0.96 - 1.00	0.79	≤0.73	87.86	91.33
	1250	0.96	0.93 - 0.98	0.48	≤0.75	83.57	84.00
	1500	0.81	0.58 - 0.70	0.26	≤0.82	50.00	76.00
	2000	0.64	0.45 - 0.56	0.06	≤0.85	66.00	41.00
	WBT_{avg}	0.57	0.50 - 0.64	0.21	≤0.47	30.71	90.00
Ambient pressure	250	0.94	0.91 - 0.97	0.80	≤0.09	85.00	95.33
	300	0.95	0.92 - 0.97	0.83	≤0.14	90.71	92.67
	400	0.96	0.94 - 0.98	0.85	≤0.18	90.71	94.67
	500	0.96	0.94 - 0.99	0.89	≤0.26	95.71	93.33
	600	0.97	0.95 - 0.99	0.94	≤0.43	96.43	98.00
	800	0.98	0.96 - 1.00	0.94	≤0.61	96.43	97.33
	1000	0.98	0.96 - 1.00	0.82	≤0.73	92.14	90.00
	1250	0.97	0.95 - 0.99	0.53	≤0.75	85.71	87.33
	1500	0.84	0.79 - 0.88	0.26	≤0.87	67.14	59.33
	2000	0.64	0.58 - 0.71	0.06	≤0.87	60.00	33.33
	WBT_{avg}	0.97	0.95 - 0.99	0.21	≤0.50	95.00	97.33

Note. AUROC – Area under ROC curve, CI – Confidence interval, the shaded area indicates high sensitivity and specificity

In summary, the WBA at low and mid-frequencies from 250 Hz to 2000 Hz was lower for the Otosclerosis group than the Normal ear group. However, the high sensitivity and specificity were seen up to 1250 Hz for both the pressure conditions. The Otosclerosis group has a single maximum at 2000 Hz with a narrow peak around 2000 to 2500 Hz.

Further, WBT_{avg} measured at ambient pressure has high accuracy at a cut-off point of 0.50 absorbance value and can be considered for differentiating Otosclerosis from the Normal ears. The summary of the findings and the differential criteria compared to the normal ear is provided in Table 4.21.

Table 4.21

Summary of the findings of Otosclerosis group and differential criteria in comparison to Normal ear group

Parameters	Otosclerosis group (in-comparison to Normal ear)
WBA across frequencies from 250 Hz to 8000 Hz	- Lower absorbance value up to 2000 Hz
WBA pattern	- A single sharp maximum peak at around 2000 Hz and 2500 Hz in the Otosclerosis group
WBT_{avg}	- Lower absorbance only at ambient pressure condition with a sensitivity of 95% and specificity of 97.33%.
Differential criteria	- High sensitivity and specificity at lower and mid-frequencies up to 1250 Hz can be considered for differential diagnosis of Otosclerosis from the normal ear.

4.5.4 Eustachian tube dysfunction (ETD)

In the ETD group, data were obtained from 106 ears and compared with 150 randomly selected normal ears from Group I. All the participants in the ETD group had 'C' type tympanogram having the mean TPP of -191.28 ± 78.84 daPa (Range = -397 to -101 daPa), and the mean static admittance of 0.64 ± 0.39 mmho (Range = 0.24 to 1.46 mmho) obtained using 226 Hz probe tone frequency. The average ear canal volume obtained in ETD group was 1.12 ± 0.25 cc (Range = 0.52 to 1.88 cc). Also, the TPP recorded during WBT measurements for the ETD group was -157.18 ± 144.14 daPa (Range = -399 to -101 daPa).

The descriptive statistics (mean, median, and SD) of WBA across the frequencies and WBT_{avg} measured in the ETD group for both the pressure conditions are shown previously in Table 4.7. The WBA obtained in the ETD group was minimal at 250 Hz and increased as the frequencies increased up to 2000 Hz. The WBA reached a maximum at 2000 Hz and thereon reduced further, reaching a minimum absorbance value at 6000 Hz and above. Though the WBA pattern was similar to that of the Normal ear group, reduced absorbance was seen for all frequencies up to 3000 Hz in both the pressure conditions, as shown in Figure 4.16. However, the reduction in absorbance in the ETD group was more at ambient pressure conditions compared to the peak pressure. At high frequencies above 3000 Hz, the WBA are nearly similar between the groups in both pressure conditions.

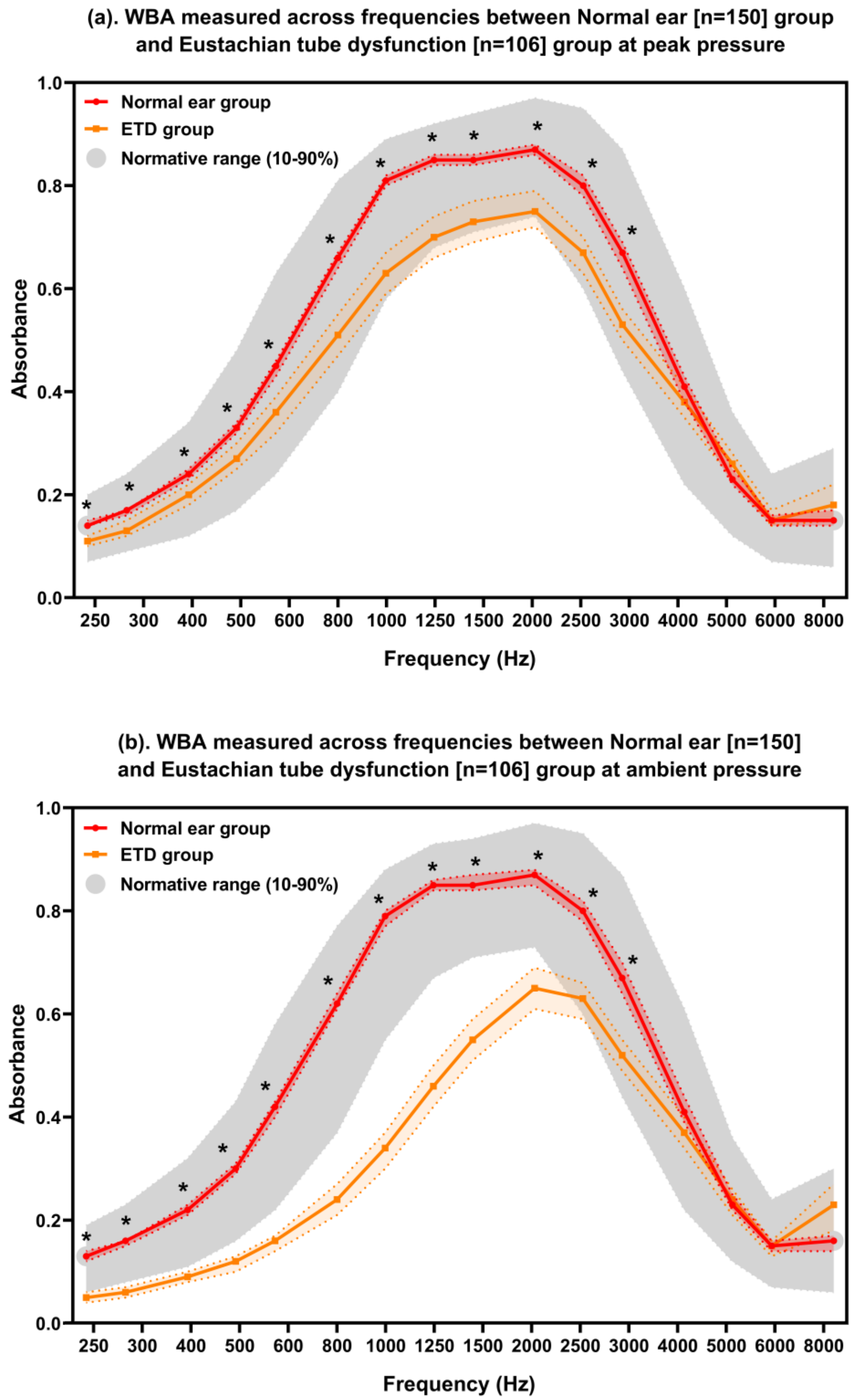


Figure 4.16 Graphical representation of mean WBA of Eustachian tube dysfunction and Normal ear [n=150] groups obtained at (a) peak pressure and (b) ambient pressure across frequencies [Colour shaded region represents 95% CI; *indicates significant difference, $p<0.05$ with medium to large effect size].

A mixed ANOVA was performed to study the effect of pressure conditions as within-subject conditions and groups as between-subject conditions. There were a significant main effect of Group [F(1,254)=335.47, $p<0.05$, $\eta_p^2=0.57$], pressure conditions [F(1,254)= 476.3, $p<0.05$, $\eta_p^2=0.65$] and frequency [F(3.767, 956.801)= 1414.464, $p<0.05$, $\eta_p^2=0.87$]. Further, the analyses showed a significant second order interaction effect between frequency and Group [F(3.767, 956.801)= 65.399, $p<0.05$, $\eta_p^2=0.21$]; Group and pressure conditions [F(1,254)= 344.809, $p<0.05$, $\eta_p^2=0.58$]; and the frequency and pressure conditions [F(3.964, 1006.745)= 121.122, $p<0.05$, $\eta_p^2=0.21$]. Also, the third order interaction effect between frequency, pressure conditions and Group [F(3.964, 1006.745) = 86.768, $p<0.05$, $\eta_p^2=0.26$] was significant.

Further analysis was done to see whether there exists a significant difference in absorbance values between the groups in each of the frequencies. Analysis using the MANOVA showed a significant difference for all frequencies ($p<0.05$) except the higher frequencies from 4000 Hz and above, for both the pressure conditions, as shown in Table 4.22. At peak pressure condition, the effect size was medium ($\eta_p^2 \geq 0.06$) for the lower frequencies from 250 Hz to 600 Hz and large ($\eta_p^2 \geq 0.14$) for mid frequencies from 800 Hz to 3000 Hz. A large effect size ($\eta_p^2 \geq 0.14$) was seen in ambient pressure condition for all those significant frequencies (250 Hz to 3000 Hz). As indicated earlier, the mean absorbance values were lower for the ETD group for the low and mid-frequencies up to 3000 Hz, with a large difference seen at ambient pressure conditions. The non-parametric Mann-Whitney U test also showed similar results of significant difference, and the statistical values are provided in Annexure 4.11.

Table 4.22

Summary of MANOVA results and its significant level of WBA obtained between the Eustachian tube dysfunction and Normal ear [n=150] groups at peak and ambient pressure conditions

Frequency (Hz)	**Peak pressure**			**Ambient pressure**		
	F *(df, error =1,254)*	***p***	**η_p^2**	**F** *(df, error =1,254)*	***p***	**η_p^2**
250	28.697	<0.01*	0.10	205.286	<0.01*	0.45
300	21.273	<0.01*	0.08	215.404	<0.01*	0.46
400	17.417	<0.01*	0.06	278.235	<0.01*	0.52
500	17.807	<0.01*	0.07	406.021	<0.01*	0.62
600	31.714	<0.01*	0.11	564.704	<0.01*	0.69
800	66.065	<0.01*	0.21	696.730	<0.01*	0.73
1000	81.951	<0.01*	0.24	656.380	<0.01*	0.72
1250	71.143	<0.01*	0.22	411.621	<0.01*	0.62
1500	46.019	<0.01*	0.15	224.997	<0.01*	0.47
2000	46.102	<0.01*	0.15	139.067	<0.01*	0.35
2500	45.191	<0.01*	0.15	75.846	<0.01*	0.23
3000	47.556	<0.01*	0.16	53.491	<0.01*	0.17
4000	1.796	0.18	0.01	4.803	0.06	0.02
5000	3.84	0.06	0.02	0.030	0.86	0.00
6000	0.332	0.57	0.00	0.145	0.70	0.00
8000	2.787	0.10	0.01	11.261	0.07	0.04

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

The WBA measured at peak pressure and ambient pressure was compared within the group to check which of the frequencies showed significant differences. In the ETD group, the Paired-T test with Bonferroni corrections showed a significant difference between the pressure conditions from 250 Hz to 2500 Hz (p<0.003) having large effect size up to 2000 Hz ($\eta_p^2 \geq 0.14$) and medium effect at 2500 Hz ($\eta_p^2 \geq 0.06$) as shown in Table 4.23. There exhibits a drastic reduction of absorbance at ambient pressure condition from 250 Hz to

2500 Hz compared to the peak pressure in the ETD group (Figure 4.17). However, the mean difference of WBA obtained at the peak and ambient pressure in the ETD group was larger than the observed WBA in the Normal ear group. It ranged from 0.15 to 0.29, especially at the low and mid-frequencies between 500 Hz and 1500 Hz. Similar results were also obtained from the non-parametric Wilcoxon signed-rank test, and the statistical results are shown in Annexure 4.12.

Table 4.23

Summary of Paired T-test results and its significant level of WBA obtained between the pressure conditions (peak, ambient) in Eustachian tube dysfunction group

Frequency (Hz)	**t** *(df = 105)*	***p***	η_p^2
250	10.584	<0.001*	0.52
300	11.175	<0.001*	0.54
400	13.205	<0.001*	0.62
500	13.445	<0.001*	0.63
600	13.824	<0.001*	0.65
800	15.606	<0.001*	0.70
1000	15.375	<0.001*	0.69
1250	12.306	<0.001*	0.59
1500	8.938	<0.001*	0.43
2000	6.158	<0.001*	0.27
2500	3.831	<0.001*	0.12
3000	0.833	0.407	0.01
4000	3.106	0.004	0.04
5000	5.029	0.006	0.03
6000	3.258	0.004	0.05
8000	-3.051	0.005	0.03

Note. t- t-test statistics value, η_p^2 – Effect size of Partial Eta square values, and *p*—Significance level. *corrected required significant level is $p<0.003$ for WBA across frequencies, and also the actual *p*-value was not depicted as the initial three values after the decimal were zero. The shaded region indicates a significant difference with medium to large effect size.

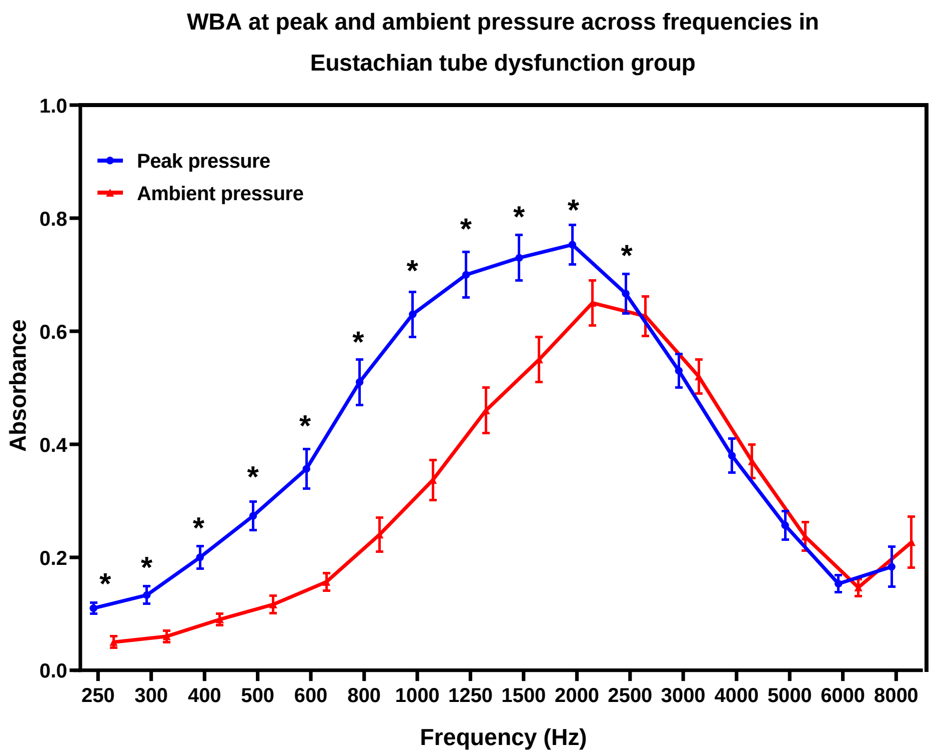


Figure 4.17 Graphical representation of mean WBA (vertical lines denoting 95% CI) measured at peak pressure and ambient pressure across frequencies in the Eustachian tube dysfunction group. *indicates corrected significant difference, $p<0.003$ with medium to large effect size.

For the measurement of WBT_{avg}, the absorbance value was obtained for all the ears (n=106 ears) at ambient pressure conditions whereas, only16 ears (out of 106 ears) had absorbance values at the peak pressure in the ETD group. This may be because of the increase in middle ear stiffness due to decreased pressure in the middle ear (Kim & Koo, 2015). This would restrict the low-frequency energy transmission to the middle ear and allow the high-frequency energy transmission that could probably be above 2000 Hz. Thus, the absorbance values obtained from WBT_{avg} at ambient pressure was only considered for analysis.

The mean absorbance value of WBT_{avg} at ambient pressure condition was 0.34 ± 0.14 SD (Range = 0.04 to 0.71). The mean WBT_{avg} absorbance value was lower in the ETD group compared to the normal ear group. Between-group comparison using independent sample T-test showed a significant difference [$t(131.242) = 19.889$, $p<0.05$, $\eta_p^2 = 0.75$], with large effect size. A similar result was also obtained using a non-parametric Mann-Whitney U test [U=693.5, p<0.05, r=0.8].

ROC analysis was performed for those frequencies that are found to be significant between the Normal ear group and ETD group, i.e. from 250 Hz to 3000 Hz at peak and ambient pressure conditions, and the results are depicted in Figure 4.18. The AUROC values for WBA measured at peak pressure ranged from 0.66 to 0.75. The ROC curves fall closer to the diagonal line, indicating the moderate accuracy in differentiating ETD from the Normal ears. In contrast, the AUROC values for WBA at ambient pressure were above 0.75 with the ROC lines that lie to the top left corner of the ROC curve, indicating the good accuracy in detecting ETD from the Normal ears.

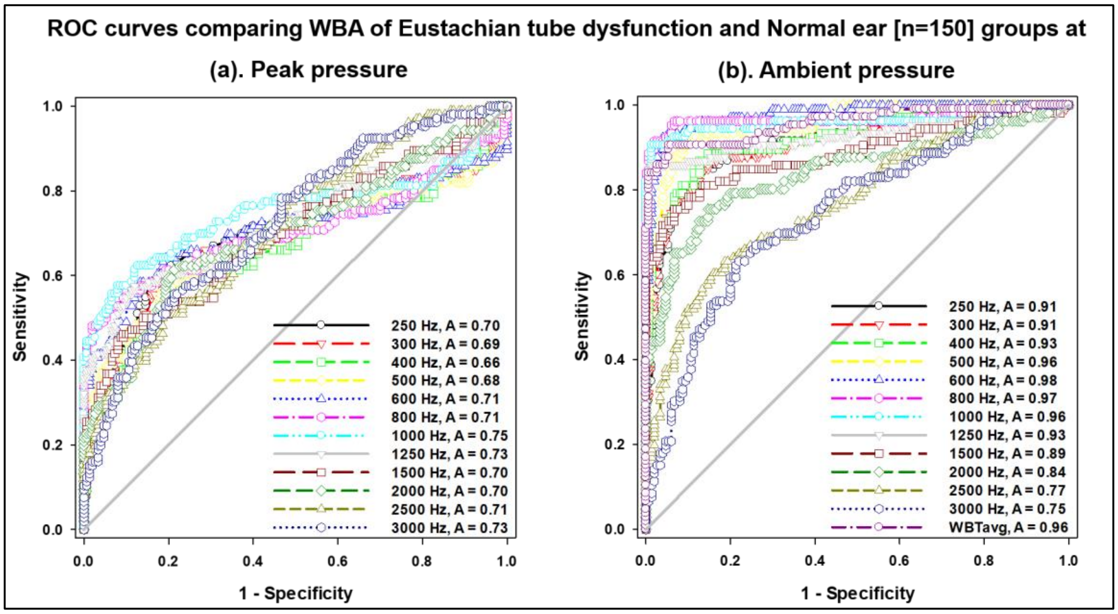


Figure 4.18 ROC curves and AUROC (A) values of WBA measured across frequencies and WBT_{avg} at which significant difference has been obtained for (a) peak pressure and (b) ambient pressure between the Eustachian tube dysfunction and Normal ear [n=150] groups.

Further, the sensitivity and specificity were calculated at a cut-off criterion point based on the Youden index and are shown in Table 4.24. Though the specificity was above 78%, the sensitivity was lower (<62%) for all the frequencies measured at peak pressure. In contrast, the sensitivity and specificity were higher for WBA measured at ambient pressure. Among those, the mid-frequencies from 500 Hz to 1000 Hz showed high sensitivity and specificity of greater than 90%. Whereas the low frequencies from 250 Hz to 400 Hz had a sensitivity and specificity of around 85%. The sensitivity was reduced for frequencies (<75%) above 2000 Hz, with specificity ranging between 78% and 86%, as shown in Table 4.24.

The WBT_{avg} obtained at ambient pressure had an AUROC value of 0.96 with the ROC curve that falls closer to the top-left corner, indicating high accuracy in differentiating the ETD from normal ears (Figure 4.18). The sensitivity and specificity at the cut-off criterion point of 0.55 average absorbance value was 90.57% and 94.67%, respectively.

Table 4.24

The AUROC, 95% CI, Youden Index point, criterion point with sensitivity and specificity of WBA measured at peak and ambient pressure across frequencies and WBT_{avg} at ambient pressure between the Eustachian tube dysfunction and Normal ear [n=150] groups

Pressure conditions	Frequency (Hz)	AUROC	95% CI	Youden Index (j)	Cut-off Criterion point	Sensitivity (%)	Specificity (%)
Peak Pressure	250	0.70	0.63 - 0.77	0.42	≤0.11	57.55	84.67
	300	0.69	0.62 - 0.76	0.40	≤0.13	55.66	84.00
	400	0.66	0.59 - 0.74	0.36	≤0.20	57.55	78.00
	500	0.68	0.60 - 0.75	0.38	≤0.27	56.60	81.33
	600	0.71	0.63 - 0.78	0.45	≤0.36	58.49	86.67
	800	0.71	0.64 - 0.78	0.47	≤0.55	53.83	94.00
	1000	0.75	0.68 - 0.82	0.50	≤0.72	62.26	87.33
	1250	0.73	0.66 - 0.80	0.46	≤0.76	57.55	88.00
	1500	0.70	0.63 - 0.77	0.37	≤0.73	46.23	90.67
	2000	0.70	0.63 - 0.77	0.40	≤0.81	61.32	78.67
	2500	0.71	0.64 - 0.78	0.32	≤0.68	50.94	81.33
	3000	0.73	0.67 - 0.79	0.34	≤0.54	55.66	78.67
Ambient pressure	250	0.91	0.87 - 0.95	0.71	≤0.09	84.91	86.00
	300	0.91	0.87 - 0.95	0.71	≤0.11	86.79	84.67
	400	0.93	0.89 - 0.96	0.74	≤0.15	86.79	87.33
	500	0.96	0.94 - 0.98	0.82	≤0.21	90.57	91.33
	600	0.98	0.96 - 0.99	0.88	≤0.27	92.45	96.00
	800	0.97	0.94 - 0.99	0.90	≤0.48	96.23	93.33
	1000	0.96	0.92 - 0.99	0.89	≤0.57	90.57	98.67
	1250	0.93	0.88 - 0.97	0.85	≤0.66	85.85	98.67
	1500	0.89	0.84 - 0.93	0.69	≤0.70	75.47	93.33
	2000	0.84	0.79 - 0.90	0.61	≤0.78	75.47	85.33
	2500	0.77	0.71 - 0.83	0.45	≤0.68	63.21	81.33
	3000	0.75	0.68 - 0.81	0.41	≤0.55	63.21	78.00
	WBT_{avg}	0.96	0.93 - 0.98	0.85	≤0.55	90.57	94.67

Note. AUROC – Area under ROC curve, CI – Confidence interval, shaded area indicates high sensitivity and specificity

In summary, the ETD group had lower absorbance values from 250 Hz to 3000 Hz than the normal ears for both the pressure conditions. However, the drastic reduction was seen for WBA at ambient pressure condition. The mean WBA difference between peak and ambient pressure was greater for the ETD group than the Normal ear group. Further, the sensitivity and specificity were higher for WBA measurements at ambient pressure conditions. Thus, the use of WBA measured at ambient pressure with a greater difference and lesser difference at peak pressure could hint the presence of ETD. The summary of the findings and the differential criteria compared to the Normal ear is provided in Table 4.25.

Table 4.25

Summary of the findings of Eustachian tube dysfunction group and differential criteria in comparison to Normal ear group

Parameters	**ETD group (in comparison to the Normal ear)**
WBA across frequencies from 250 Hz to 8000 Hz	- Drastic reduction of absorbance only for ambient pressure condition from 250 Hz to 3000 Hz, compared to the Normal ear group.
WBA pattern	- Identical WBA pattern with reduced absorbance compared to the normal group.
WBT_{avg}	- Lower WBT_{avg} at ambient pressure with sensitivity and specificity of 90.57% and 94.67% respectively.
Differential criteria	- Mid-frequencies from 500 Hz to 1000 Hz showed maximum sensitivity and specificity only at ambient pressure. These frequencies can be considered for differential diagnosis of the ETD from the normal ear.

4.6 Comparison of WBA obtained in healthy ears and ears with Sensorineural hearing loss

WBA measurements from 140 ears with SNHL were compared with 150 randomly selected normal ears from Group I. Conventional 226 Hz tympanometry for ears with SNHL showed 'A' or 'A_s' type tympanogram with the mean static admittance of 0.51 ± 0.22 mmho (Range= 0.31 to 1.59 mmho) and the mean TPP of -11.01 ± 14.79 daPa (-44 to 38 daPa). The mean resonance frequency obtained in the SNHL group was 971.60±227.63 Hz (Range = 803 Hz to 1287 Hz) and had an ear canal volume of 1.11 ± 0.24 ml (Range = 0.68 – 1.9 ml). The TPP obtained in WBA measurement was -7.67 ± 13.67 daPa (-39 to 43 daPa). The WBA measurements were performed at the peak pressure and ambient pressure, and the descriptive statistics (mean, median, and SD) is given previously in Table 4.7.

The WBA measured at the peak and ambient pressure is depicted in Figure 4.19. It can be clearly seen that the WBA pattern of the SNHL group is identical to that of the Normal ear group with marginally lower absorbance values at all the frequencies except at 2000 Hz and 8000 Hz. The SNHL group showed minimal absorbance at 250 Hz and increased as the frequency increased, reaching a maximum at 1250 Hz and 2000 Hz, similar to that of the Normal ear group. Further, the WBA decreased beyond 2000 Hz, reaching a minimum value at 6000 Hz and 8000 Hz. The WBA measured at peak pressure and ambient pressure were also similar for both groups.

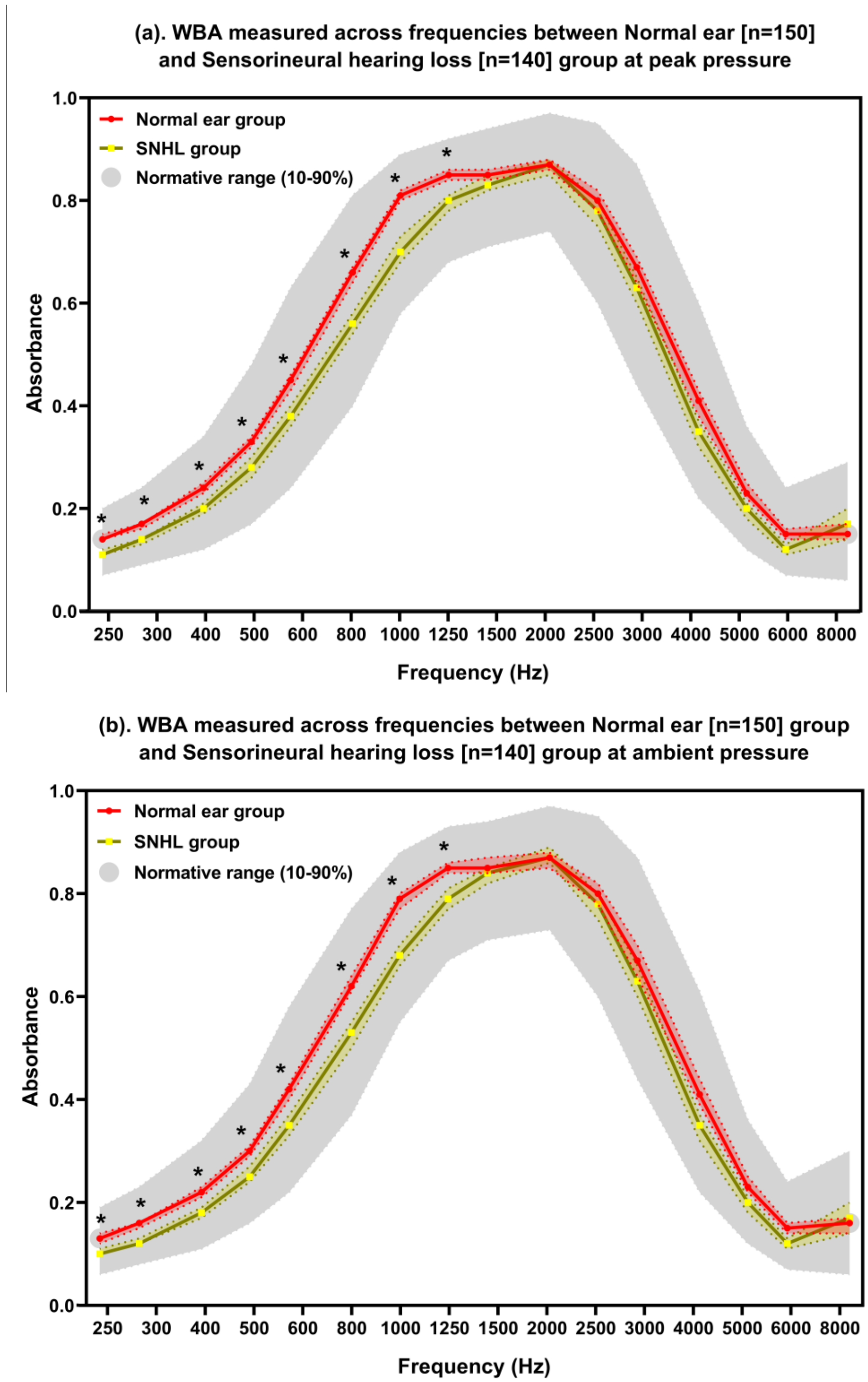


Figure 4.19 Graphical representation of mean WBA of Sensorineural hearing loss and Normal ear [n=150] groups obtained at (a) peak pressure and (b) ambient pressure across frequencies [Colour shaded region represents 95% CI; *indicates significant difference, p<0.05 with medium to large effect size].

A mixed ANOVA was performed to observe the main effect of group (between-subject factor), pressure condition (within-subject factor) and the frequencies on WBA. Results showed a significant main effect of group [F(1,288)=61.146, $p<0.05$, $\eta_p^2=0.18$], pressure conditions [F(1,288)=120.794, $p<0.05$, $\eta_p^2=0.30$] and frequency [F(3.56, 1025.306) = 2538.665, $p<0.05$, $\eta_p^2=0.90$]. A significant interaction effect was seen between the frequency and pressure conditions [F(3.427, 987.053)=56.763, $p<0.05$, $\eta_p^2=0.17$] having large effect size ($\eta_p^2\geq0.14$); and between frequency and Group [F(3.56, 1025.306)= 7.872, $p<0.05$, $\eta_p^2=0.03$] with small effect size ($\eta_p^2<0.06$). However, no significant interaction effect was found between pressure conditions and Group [F(1,288)=0.35, $p>0.05$, $\eta_p^2=0.00$]; and between pressure conditions, frequency and group [F(3.427, 987.053)=0.764, $p>0.05$, $\eta_p^2=0.00$].

Further analysis was performed to investigate the significant difference of WBA between the group in each of the frequencies. The results of the MANOVA showed a significant difference between the groups for frequencies from 250 Hz to 1250 Hz and from 3000 Hz to 6000 Hz ($p<0.05$) as shown in Table 4.26. The mean absorbance value of the SNHL group in those frequencies was significantly lower than the Normal ear groups. However, the effect size is small for high frequencies ($\eta_p^2<0.06$), indicating the difference in WBA between the group is not truly significant. The non-parametric Mann-Whitney U test also showed identical results with the parametric test result, and the statistical results are shown in Annexure 4.13.

Table 4.26

Summary of MANOVA results and its significant level of WBA obtained between Sensorineural hearing loss group and Normal ear [n=150] group at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure			Ambient pressure		
	F *(df, Error =1, 288)*	***p***	η_p^2	**F** *(df, Error =1, 288)*	***p***	η_p^2
250	35.855	<0.01*	0.11	28.822	<0.01*	0.09
300	32.997	<0.01*	0.10	29.979	<0.01*	0.09
400	27.667	<0.01*	0.09	24.052	<0.01*	0.08
500	19.677	<0.01*	0.06	21.746	<0.01*	0.07
600	25.047	<0.01*	0.08	24.788	<0.01*	0.08
800	54.488	<0.01*	0.16	47.313	<0.01*	0.14
1000	70.759	<0.01*	0.20	61.421	<0.01*	0.18
1250	25.946	<0.01*	0.08	29.853	<0.01*	0.09
1500	1.793	0.18	0.01	2.563	0.11	0.01
2000	0.007	0.94	0.00	0.143	0.71	0.00
2500	2.291	0.13	0.01	1.899	0.17	0.01
3000	4.956	0.03*	0.02	4.814	0.03	0.02
4000	11.6	<0.01*	0.04	11.852	<0.01*	0.04
5000	11.429	<0.01*	0.04	12.013	<0.01*	0.04
6000	18.757	<0.01*	0.05	18.625	<0.01*	0.05
8000	1.716	0.19	0.01	0.921	0.34	0.00
WBT_{avg}	0.317	0.57	0.001	5.611	0.07	0.01

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

The comparison of WBA between the pressure conditions (peak, ambient) within the group showed slightly lower WBA at ambient pressure condition than the peak pressure in the SNHL group, which is similar to that observed in the normal ear group as shown in Figure 4.20. Paired T-test with Bonferroni corrections showed a significant difference (p<0.003) between the pressure conditions up to 1000 Hz with a large effect size ($\eta_p^2 \geq 0.14$)

as shown in Table 4.27. The non-parametric Wilcoxon signed-rank test showed similar results in terms of statistical significance (p<0.003), and the results are shown in Annexure 4.14.

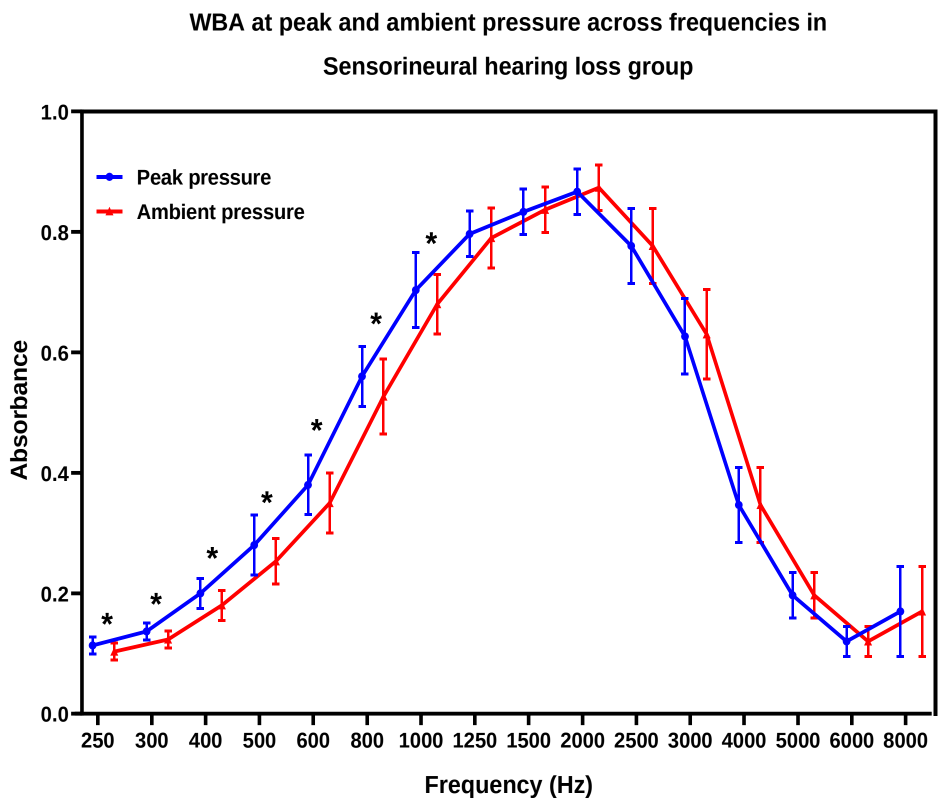


Figure 4.20 Graphical representation of mean WBA (vertical lines denoting 95% CI) measured at peak pressure and ambient pressure across frequencies in the Sensorineural hearing loss group. *indicates corrected significant difference, p<0.003 with medium to large effect size.

Table 4.27

Summary of Paired T-test results and its significant level of WBA obtained between the pressure conditions (peak, ambient) in Sensorineural hearing loss group

Frequency (Hz)	**t** *(df =139)*	***p***	η_p^2
250	6.317	<0.001*	0.22
300	6.796	<0.001*	0.25
400	5.67	<0.001*	0.19
500	8.42	<0.001*	0.34
600	8.018	<0.001*	0.32
800	7.713	<0.001*	0.30
1000	5.151	<0.001*	0.16
1250	2.183	0.004	0.03
1500	-0.264	0.792	0.00
2000	-1.655	0.100	0.02
2500	-0.108	0.914	0.00
3000	-0.86	0.391	0.01
4000	-2.974	0.004	0.06
5000	0.238	0.812	0.00
6000	-0.358	0.721	0.00
8000	0.131	0.896	0.00
WBT_{avg}	-1.82	0.071	0.02

Note. t- t-test statistics value, η_p^2 – Effect size of Partial Eta square, and *p*—Significance level. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for WBT_{avg} and also the actual *p*-value was not depicted as the initial three values after the decimal were zero. Shaded region indicates significant difference with medium to large effect size.

Further, the WBT_{avg} measurements obtained at peak and ambient pressure were compared between the SNHL and the Normal ear group. The mean WBT_{avg} absorbance value at peak pressure was identical for both the groups, i.e. 0.60 ± 0.11 (Range = 0.30 to 0.82) for the SNHL group and 0.61 ± 0.10 (Range = 0.40 to 0.75) for the Normal ear group. Similarly, the mean WBT_{avg} absorbance value at ambient pressure for SNHL group was 0.58 ± 0.09 (Range = 0.28 to 0.77) and 0.60 ± 0.06 (Range= 0.40 to 0.74) for the Normal ear group.

The Independent Sample T-test was used to evaluate the difference in WBT_{avg} absorbance value between the groups (SNHL vs Normal ear group) at peak pressure and ambient pressure conditions. The results showed a no significant difference in WBT_{avg} absorbance values at peak pressure [$t(271.598) = 0.560$, $p>0.05$, $\eta_p^2 = 0.00$] and at ambient pressure [$t(236.503) = 1.885$ $p>0.05$, $\eta_p^2 = 0.01$]. Similar results were obtained using non-parametric Mann-Whitney U test for both peak [$U=10152.00$, $p>0.05$, $r = 0.0$] and ambient pressure [$U=6448.50$, $p>0.05$, $r = 0.1$]. Within-group comparison using paired T-test did not showed any significant difference between pressure conditions [$t(139) = -1.82$, $p>0.05$, $\eta_p^2=0.02$] in the SNHL group. The mean WBT_{avg} of SNHL group was slightly lower at ambient pressure compared to the peak pressure. Similar results were obtained using the non-parametric Wilcoxon signed-rank test [$Z= -1.455$, $p>0.05$, = 0.1].

ROC analysis was performed to investigate the sensitivity and specificity and establish a cut-off criterion point based on the Youden index. The WBA data considered for ROC analysis were from 250 Hz to 1250 Hz. The ROC curve for these frequencies passes closer and above the diagonal line, as shown in Figure 4.21. The AUROC value ranged between 0.66 and 0.75 for both pressure conditions indicating a moderate differentiation level between the groups. The AUROC value with 95% CI, Youden index value and the cut-off criterion point with sensitivity and specificity measured at peak and ambient pressure are tabulated in Table 4.28.

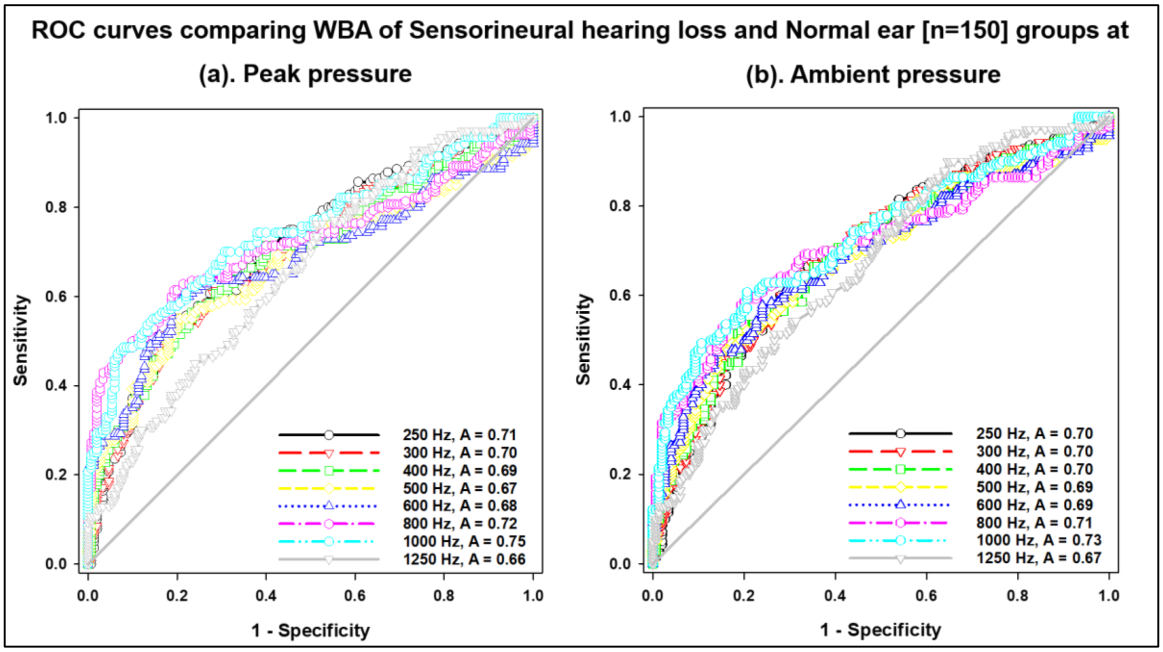


Figure 4.21 ROC curves and AUROC (A) values of WBA measured across frequencies and WBT_{avg} at which significant difference has been obtained for (a) peak pressure and (b) ambient pressure between Sensorineural hearing loss and Normal ear [n=150] groups.

The sensitive and specificity were calculated at a cut-off point based on the Youden index. The specificity was better (>70%) for both the pressure conditions, while the sensitivity is lower by around 50% in most of the frequencies, as shown in Table 4.28. This suggests that WBA values across frequencies are likely to be similar.

Table 4.28

The AUROC, 95% CI, Youden Index point, criterion point with sensitivity and specificity of WBA measured at peak and ambient pressure across frequencies between the Sensorineural hearing loss and Normal ear [n=150] groups

Pressure conditions	**Frequency (Hz)**	**AUROC**	**95% CI**	**Youden Index (j)**	**Cut-off Criterion point**	**Sensitivity (%)**	**Specificity (%)**
Peak Pressure	250	0.71	0.65 - 0.77	0.35	≤0.12	55.00	80.00
	300	0.70	0.64 - 0.76	0.35	≤0.13	51.43	83.33
	400	0.69	0.63 - 0.75	0.33	≤0.21	56.43	76.67
	500	0.67	0.61 - 0.74	0.35	≤0.28	55.00	80.00
	600	0.68	0.62 - 0.74	0.39	≤0.39	57.86	81.33
	800	0.72	0.66 - 0.78	0.42	≤0.60	61.43	80.67
	1000	0.75	0.69 - 0.80	0.41	≤0.71	48.57	92.00
	1250	0.66	0.59 - 0.72	0.25	≤0.86	76.43	48.67
Ambient pressure	250	0.70	0.64 - 0.76	0.34	≤0.11	63.57	70.00
	300	0.70	0.64 - 0.76	0.33	≤0.13	62.14	70.67
	400	0.69	0.63 - 0.76	0.34	≤0.18	53.57	80.00
	500	0.69	0.63 - 0.75	0.33	≤0.23	47.14	86.00
	600	0.69	0.63 - 0.75	0.34	≤0.36	57.86	76.00
	800	0.71	0.65 - 0.77	0.39	≤0.55	59.29	79.33
	1000	0.73	0.68 - 0.78	0.40	≤0.73	60.71	79.33
	1250	0.67	0.61 - 0.73	0.25	≤0.90	90.00	35.33

Note. AUROC – Area under ROC curve, CI – Confidence interval.

In summary, the WBA at low and mid-frequencies from 250 Hz to 1250 Hz was slightly lower for the SNHL group than the Normal ear group. However, the WBA pattern was almost identical between the groups with no difference between the pressure conditions. ROC analysis showed low to moderate accuracy in differentiating the SNHL group from the Normal ears. The WBT_{avg} were not significantly different between the groups. The summary of the findings and the differential criteria compared to the normal ear is provided in Table 4.29.

Table 4.29

Summary of the findings of Sensorineural hearing loss group and differential criteria in comparison to Normal ear group

Parameters		**Sensorineural hearing loss group (in comparison to the Normal ear)**
WBA across frequencies from 250 Hz to 8000 Hz	-	Marginally lower absorbance value up to 1250 Hz, compared to the Normal ear group.
WBA pattern	-	Identical WBA pattern for both the pressure conditions.
WBT_{avg}	-	Identical WBT_{avg} for both the pressure conditions between the groups.
Differential criteria	-	Findings of SNHL group were almost similar to that of the Normal ear group. There exists no good demarcation to distinguish between the SNHL and Normal ear group.

4.7 Comparison of WBA obtained between the pathological group

The current study results, as delineated under each of the pathological conditions above, showed that WBA obtained at low and mid-frequencies up to 3000 Hz are significantly lower than the normal ears. Among these, the frequencies from 250 Hz to 1250 Hz are significantly lower, having a larger effect size for all the middle ear pathological groups than the Normal ear group. In the current section, an attempt has been made to compare the WBA data across frequencies and WBT_{avg} obtained in different pathological ears and established the pattern among the pathological groups, i.e. middle ear disorders and SNHL groups. The WBA obtained across frequencies and WBT_{avg} in different pathological ears were tabulated, and descriptive statistics (mean, SD and median) are given in Table 4.7.

The multivariate analysis (MANOVA) was performed to study at which frequencies the absorbance values are significantly different among the study groups (Pathological conditions and Normal ear group) at both the peak and ambient pressure conditions. The results indicated a statistically significant difference in WBA among the study groups at peak pressure [$F (64, 2339.43) = 31.42$, $p < 0.05$; Wilk's $\Lambda = 0.088$, $\eta_p^2 = 0.45$] and at ambient pressure [$F (64, 2339.43) = 31.56$, $p < 0.05$; Wilk's $\Lambda = 0.088$, $\eta_p^2 = 0.46$] across the frequencies. Further analysis in each of the frequencies at peak and ambient pressure conditions showed a significant difference ($p<0.05$) for all the frequencies from 250 Hz to 6000 Hz, as shown in Table 4.30. At 8000 Hz, a significant difference was seen only at ambient pressure condition with a small effect size ($\eta_p^2<0.06$), indicating the difference is not truly significant. The equivalent non-parametric Kruskal-Wallis test for WBA obtained at the peak and ambient pressure among the study groups also showed similar results. The significant values were tabulated in Annexure 4.15.

Table 4.30

Summary of MANOVA results and its significant level of WBA obtained between the pathological group at peak and ambient pressure conditions

Frequency (Hz)	**Peak pressure**			**Ambient pressure**		
	F *(df, Error =4, 612)*	***p***	**η_p^2**	**F** *(df, Error = 4, 612)*	***p***	**η_p^2**
250	31.46	<0.01*	0.17	30.65	<0.01*	0.17
300	32.85	<0.01*	0.18	30.39	<0.01*	0.17
400	39.01	<0.01*	0.20	36.84	<0.01*	0.19
500	40.56	<0.01*	0.21	46.27	<0.01*	0.23
600	53.27	<0.01*	0.26	66.65	<0.01*	0.30
800	97.34	<0.01*	0.39	115.63	<0.01*	0.43
1000	134.60	<0.01*	0.47	148.01	<0.01*	0.49
1250	155.08	<0.01*	0.50	151.40	<0.01*	0.50
1500	175.52	<0.01*	0.53	151.24	<0.01*	0.50
2000	177.21	<0.01*	0.54	177.66	<0.01*	0.54
2500	126.78	<0.01*	0.45	135.42	<0.01*	0.47
3000	94.53	<0.01*	0.38	100.88	<0.01*	0.40
4000	35.13	<0.01*	0.19	36.94	<0.01*	0.19
5000	38.76	<0.01*	0.20	40.42	<0.01*	0.21
6000	92.63	<0.01*	0.38	92.12	<0.01*	0.38
8000	1.01	0.40	0.01	3.13	0.01	0.02

Note. df—Degrees of freedom, F—F-test statistic, *p* - Significant level, and η_p^2 - Effect size of Partial Eta square values. *The actual *p*-value was not depicted as the initial three values after the decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Further, multiple pairwise comparisons using Tukey's HSD post-hoc tests were performed for WBA across the frequencies to study the significant difference between the study groups at both pressure conditions. The results are tabulated in Table 4.31. In comparing the TmP group with other pathological groups, significant higher WBA absorbance values were seen in the TmP group at all the frequencies except at 250 Hz, 300

Hz and 8000 Hz in both the pressure conditions compared to the MEE group. Similarly, with the Otosclerosis group, a significant higher mean absorbance values up to 1250 Hz, and at 5000 Hz and 6000 Hz; whereas lower absorbance values were observed from 1500 Hz to 3000 Hz for both the pressure conditions in the TmP group. Compared to the ETD group, the mean WBA values for the TmP group were significantly lower at 250 Hz, 300 Hz, 1250 Hz to 2000 Hz and significantly higher at 3000 Hz, 5000 Hz and 6000 Hz at the peak pressure. Whereas at ambient pressure, the TmP group showed significantly higher WBA absorbance at 250 Hz to 400 Hz, 1000 Hz to 1500 Hz, 2500 Hz to 4000 Hz and significantly lower absorbance at 2000 Hz and 8000 Hz. With the SNHL group, the TmP group showed a significant lower WBA value at 250 Hz to 400 Hz and 1000 Hz to 2500 Hz, whereas higher WBA values were observed from 4000 Hz to 6000 Hz for both peak and ambient pressure conditions.

A significantly lowered mean absorbance was observed in most frequencies in the MEE group than other pathological groups. With the Otosclerosis group, a significantly lower mean WBA value was obtained at all frequencies for both pressure conditions except at 600 Hz, 800 Hz and 8000 Hz in the MEE group. Likewise, with the ETD group, significantly lower mean absorbance values were observed at peak pressure in all the frequencies except at 8000 Hz for the MEE group. In ambient pressure conditions, lower mean absorbance was seen from 250 Hz to 400 Hz and 1000 Hz to 6000 Hz. With the SNHL, the MEE group showed a significantly reduced WBA from 250 Hz to 4000 Hz for both pressure conditions.

Similarly, the Otosclerosis group at peak pressure conditions had significantly reduced mean absorbance value up to 1250 Hz and higher absorbance values at 3000 Hz and 4000 Hz compared to the ETD group. At ambient pressure, a significant higher absorbance was observed for the Otosclerosis group for frequencies from 1250 Hz to 4000 Hz and lower absorbance at 8000 Hz, compared to the ETD group. Between Otosclerosis and SNHL group, a significant difference was seen for all frequencies in both the pressure conditions except at 2500 Hz, 6000 Hz and 8000 Hz. The mean absorbance values were lower for the Otosclerosis group up to 2000 Hz and higher beyond 2000 Hz, compared to the SNHL group. Comparing the ETD group with SNHL showed a significant difference with lower WBA values obtained for the ETD group from 1000 Hz to 3000 Hz and higher WBA values at 5000 Hz at the peak pressure condition. Significant lower WBA values were seen at ambient pressure from 250 Hz to 3000 Hz for the ETD group compared to the SNHL group.

Similarly, the study also measured the WBT_{avg} at peak and ambient pressure in most of the group. Among the pathological group, the WBT_{avg} was observed only for the Otosclerosis group and the SNHL group at peak pressure conditions. The TmP group, MEE group and ETD group did not elicit the WBT_{avg} values at peak pressure. Thus, an Independent Sample T-Test was performed to evaluate the difference in WBT_{avg} absorbance value at peak pressure between the Otosclerosis and SNHL group. The results showed a statistically significant in WBT_{avg} absorbance values [$t(266.627) = -2.385$, $p < 0.05$, $\eta_p^2 = 0.02$] between the two, but with a small effect size indicating the difference is not truly significant. Mann-Whitney U test showed no significant difference between Otosclerosis and SNHL group [$U = 8658.500$, $p > 0.05$, $r = 0.10$].

At ambient pressure conditions, the WBT_{avg} absorbance was observed for all the pathological groups. Hence, the Univariate ANOVA was performed for each of the pressure conditions. The results of the Univariate ANOVA at ambient pressure [F(5,761)=236.115, p<0.05, η_p^2=0.61] showed a significant difference between the groups. The post hoc analysis using Tukey's HSD test was performed for WBT_{avg} at ambient pressure to study the significant difference between the pathological groups. The results are tabulated in Table 4.31.

The TmP group showed significantly higher WBT_{avg} average absorbance values than the MEE group, Otosclerosis group, and ETD group. Whereas significant lower WBT_{avg} average values are seen compared to the SNHL group. With regard to the MEE group, the WBT_{avg} absorbance values were significantly lower compared to all other pathological conditions (Otosclerosis, ETD, and SNHL). In the Otosclerosis group, the WBT_{avg} was significantly lower compared to the SNHL group. However, there exhibit no significant difference between the Otosclerosis and ETD group. Likewise, the ETD group showed lower average absorbance values than the SNHL group.

An equivalent non-parametric Post hoc Mann-Whitney U test was also done, and a similar result was obtained. The details are given in Annexure 4.16.

Table 4.31

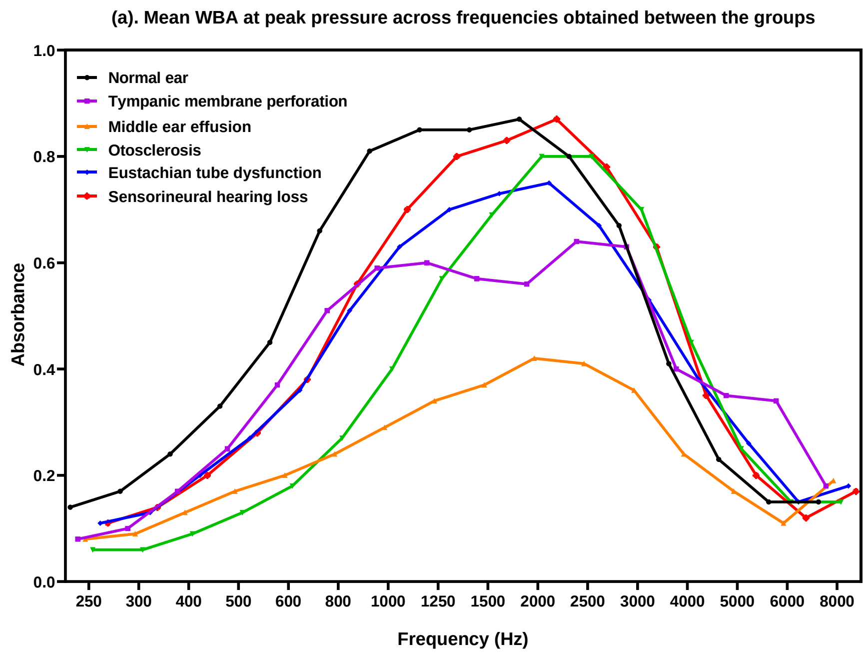
A Post hoc Tukey's HSD test results of WBA obtained between the pathological groups at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure				Groups	Ambient pressure			
	TmP	MEE	Otosc-lerosis	ETD		TmP	MEE	Otosc-lerosis	ETD
250	NS				MEE	NS			
	**	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	NS	
	**	**	**	NS	SNHL	**	**	**	**
300	NS				MEE	NS			
	**	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	NS	
	**	**	**	NS	SNHL	**	**	**	**
400	**				MEE	NS			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	**	**	NS	
	**	**	**	NS	SNHL	**	**	**	**
500	**				MEE	**			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	**	NS	NS	
	NS	**	**	NS	SNHL	NS	**	**	**
600	**				MEE	**			
	**	NS			Otosclerosis	**	NS		
	NS	**	**		ETD	**	NS	NS	
	NS	**	**	NS	SNHL	NS	**	**	**
800	**				MEE	**			
	**	NS			Otosclerosis	**	NS		
	NS	**	**		ETD	**	NS	NS	
	NS	**	**	NS	SNHL	NS	**	**	**
1000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	**	**	NS	
	**	**	**	**	SNHL	**	**	**	**
1250	**				MEE	**			
	NS	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	**	
	**	**	**	**	SNHL	**	**	**	**
1500	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	NS	**	**	
	**	**	**	**	SNHL	**	**	**	**

2000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	**	**	**	
	**	**	**	**	SNHL	**	**	**	**
2500	**				MEE	**			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	NS	**	**	
	**	**	NS	**	**	**	**	NS	**
3000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	**	
	NS	**	**	**	SNHL	NS	**	**	**
4000	**				MEE	**			
	NS	**			Otosclerosis	NS	**		
	NS	**	**		ETD	NS	**	**	
	**	**	**	NS	SNHL	**	**	**	NS
5000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	**	**	NS	
	**	NS	**	**	SNHL	**	NS	**	NS
6000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	**	**	NS	
	**	NS	NS	NS	SNHL	**	NS	NS	NS
8000	NS				MEE	NS			
	NS	NS			Otosclerosis	NS	NS		
	NS	NS	NS		ETD	NS	NS	**	
	NS	NS	NS	NS	SNHL	NS	NS	NS	NS
WBT_{avg}	--				MEE	**			
	--	--			Otosclerosis	**	**		
	--	--	--		ETD	**	**	NS	
	--	--	**	--	SNHL	**	**	**	**

Note. ** indicates significant different at $p<0.05$; NS – Not significant at $p\geq0.05$.

As a whole, it is important to note that there are no unique common WBA values that can differentiate between the groups in any of the frequencies at both the peak and ambient pressure conditions. Thus, the results suggest that there are no common WBA criteria to differentiate the conditions. However, the WBA pattern was different among the disorders and is unique compared to the normal ear group and different from each pathological condition, as shown in Figure 4.22. This difference was mostly seen at low and mid-frequencies, whereas the high frequencies were almost similar across the groups for both the peak and ambient pressure conditions.



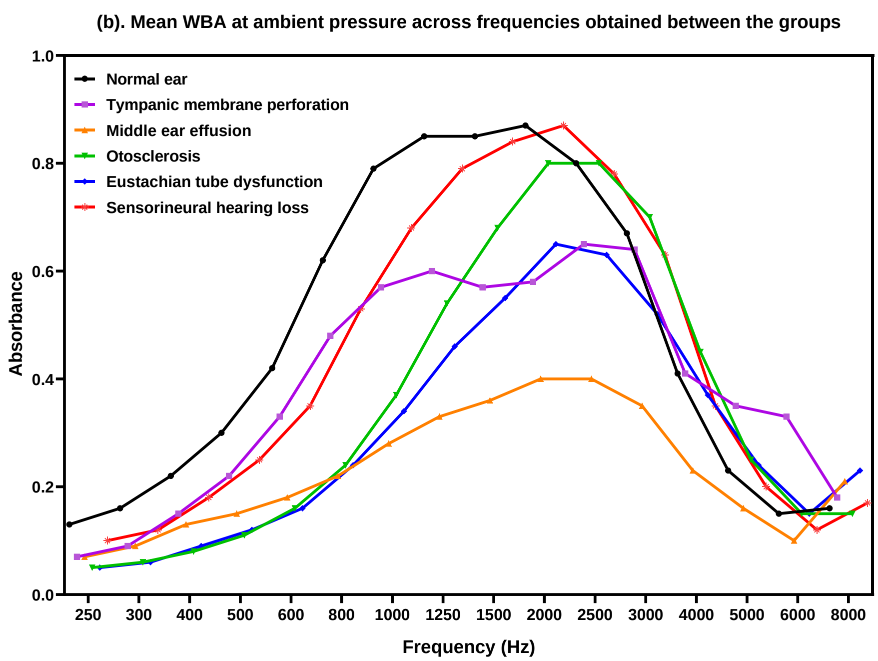


Figure 4.22 Graphical representation of mean WBA pattern across the frequencies at (a) peak and (b) ambient pressure among the groups.

It is evident in Figure 4.22 that the WBA of the normal ear group is wider and having higher absorbance across frequencies with a relatively non-prominent two peaks compared to any other pathological group. The SNHL also followed a similar pattern but narrower WBA pattern having a single peak. Whereas in the Otosclerosis group, the WBA pattern further narrowed down, having maxima at a relatively higher frequency region than any other group. In the TmP group, the WBA values reduced significantly across frequencies with three broader peaks that differentiate TmP from other conditions. Whereas the ETD and MEE showed a similar pattern having single broader peaks with reduced absorbance. However, the MEE group showed the least absorbance values among all the groups considered in the study, making it unique from other groups. Further, it is observed that the WBA obtained at peak and ambient pressure is similar for all the groups except the ETD group showing the difference in patterns between the two.

On the other hand, the middle ear disordered group showed lower average absorbance values of less than 0.5, whereas the Normal ear and SNHL groups showed absorbance of greater than 0.5. However, there exhibit a lot of overlap in WBT_{avg} values for all the study groups, and hence, WBT_{avg} at ambient pressure is not suitable for differentiating the disorders

Chapter 5

DISCUSSION

The purpose of the current study was to determine the normative values of wideband absorbance measurements and to evaluate its clinical utility while assessing the middle ear function in adults having middle ear disorders and SNHL. This study measured the WBA at peak pressure and ambient pressure across the frequencies from a sample of 1127 ears with a normal hearing having normal middle ear function. Prior to the establishment of normative, the study investigated the effect of gender (male, female), ear (right, left) and pressure conditions (peak, ambient) on WBA measurements in the normal ear group. Further, the study also evaluated the diagnostic accuracy of the WBA measurement in detecting middle ear disorders (Tympanic membrane perforation, Middle ear effusion, Otosclerosis and Eustachian tube dysfunction) and SNHL on a total sample of 617 pathological ears in comparison with the Normal ear group. A mixed ANOVA and ROC curve analyses were performed for statistical comparison and its significance to meet these objectives. The results of the study are discussed under the following categories:

1. Effect of ears on WBA in Normal ears
2. Effect of gender on WBA in Normal ears
3. Effect of pressure conditions on WBA in Normal ears
4. WBA norms at peak and ambient pressure
5. Reliability measures of WBA in Normal ears
6. Effect of middle ear disorders on WBA
    a. Tympanic membrane perforation

b. Middle ear effusion

c. Otosclerosis

d. Eustachian tube dysfunction

7. Effect of WBA on Sensorineural hearing loss

8. Effect of between-pathological groups comparison on WBA

5.1 Effect of ears on WBA in Normal ears

With regard to the ear differences, the mean WBA for the left ear was marginally lower than the right ear for frequencies from 250 Hz to 1500 Hz; no difference at 2000, 2500 and 3000 Hz, and increased for frequencies above 4000 Hz was seen. Whereas identical WBT_{avg} absorbance values between the right and left ear were obtained at peak and ambient pressure. However, no significant differences in WBA across frequencies and WBT_{avg} were seen between the ears at both peak and ambient pressure in the present study. The results of the current study are consistent with the previously reported WBA studies (Burdiek & Sun, 2014; Feeney & Sanford, 2004; Kenny, 2013; Liu et al., 2008; Shahnaz & Bork, 2006; Shaw, 2009; Sliwa et al., 2020) where the significant difference was not reported. Similarly, Jaffer (2016) compared the WBA between the ears and found that the mean absorbance values did not differ significantly at either the peak or ambient pressure.

Few studies contradict the current findings showing significant differences between ears (Feeney et al., 2004, 2014; Rosowski et al., 2012; Werner et al., 2010). In Werner et al. (2010) study, the energy reflectance measured in both infants and adults at ambient pressure showed a significant high reflectance value (low absorbance) across the frequencies in the right ear than the left ear. A few investigations have shown lower absorbance values in the right than the left ear in specific frequencies measured at ambient pressure, i.e. at 300 Hz in the middle-aged adults (Rosowski et al., 2012) and 6350 Hz in the older adults (Feeney et al., 2004). This distinction was ascribed to the physiological contrast between the ears where the right ear is somewhat stiffer than the left ear (Feeney & Sanford, 2004; Rosowski et al., 2012; Werner et al., 2010). On the other hand, Feeney et al. (2014) reported lower absorbance values across the frequencies in the left ear compared to the right ear in adults. With regard to WBT_{avg}, to my knowledge, this is the first study to determine the effect of the ear, showing identical average absorbance values with no significant difference between the ears at peak and ambient pressure conditions.

There is a discrepancy in WBA measurements between the ears, with most studies that do not significantly differ. Though few studies have shown significant differences in WBA values, the difference was relatively small between the ears (Feeney et al., 2004; Werner et al., 2010). This indicates that the transmission of sound energy through the middle ear is similar, whether it is right or left. This means that ear-specific WBA measurements are likely not a significant factor in WBA measurements and may have little or no impact on setting WBA norms.

5.2 Effect of gender on WBA in Normal ears

The current results showed that males have higher WBA values than females at lower frequencies (250 -1000 Hz) and above 5000 Hz. On the other hand, females showed high mean WBA values at frequencies from 2500 Hz to 4000 Hz. Although these distinctions are significant, the effect size was too little to be considered as critical. Nonetheless, indistinguishable mean WBA values were seen at frequencies from 1250 to 2000 Hz and at 5000 Hz. Similarly, WBT_{avg} had significant differences, with males showing higher absorbance values than females with a small effect size.

The results of this study are consistent with the results of the previous studies that found statistically significant differences in WBA measurements at the higher and or lower frequencies between the genders (Feeney & Sanford, 2004; Keefe et al., 2000; Mazlan et al., 2015; Polat et al., 2015; Rosowski et al., 2012; Shahnaz et al., 2013; Shahnaz & Bork, 2006). Generally, higher absorbance for males below 1000 Hz and lower absorbance between 2000 and 4000 Hz than females were reported in the literature (Margolis et al., 1999; Shahnaz & Bork, 2006), which is similar to that of our findings. Mazlan et al. (2015) also have noted lower WBA in frequencies ranging from 2830 to 4490 Hz in males compared to females and higher WBA in males at frequencies below 1000 Hz.

In contrast, there are inconsistencies in the WBA results showing variation across frequencies. Feeney and Sanford (2004) observed higher absorbance values at 794 Hz and 1000 Hz and lower absorbance values at 5040 Hz for males, while higher admittance (absorbance) were reported in males between 1781 Hz and 2367 Hz (Shahnaz & Bork, 2006). Feeney et al. (2014) study showed a lower mean absorbance pattern down to 3000 Hz at ambient pressure and higher mean absorbance values above 3000 Hz for males than for females. Few studies have shown a significant difference only at high frequencies of having increased absorbance in females than in males (Jaffer, 2016; Polat et al., 2015; Rosowski et al., 2012). However, these difference were not consistent across the frequencies, i.e. Polat et al. (2015) reported higher absorbance in females than in males from 3100 to 6900 Hz; Jaffer (2016) reported similar findings at 4000 and 5000 Hz; and Rosowski et al. (2012) indicated only at 4000 Hz. Whereas, few other studies have not shown any significant differences between males and females in adults (Shahnaz & Bork, 2006; Sliwa et al., 2020) and children (Beers et al., 2010; Hunter, Tubaugh, et al., 2008) at ambient pressure conditions.

Researchers have indicated that such a trend could be because of the lesser stiffness-dominated middle ear system for males than females (Margolis et al., 1999; Mazlan et al., 2015). Margolis et al. (1999) found that males had less stiffness dominated eardrums, higher middle ear resistance below 1000 Hz and lowered middle ear resistance between 2000 and 4000 Hz relative to females. This increased stiffness in females makes a larger contribution to impedance, thereby decreasing absorbance at low-frequencies and increasing absorbance at high frequencies (Allen et al., 2005; Beers et al., 2010; Feeney et al., 2014). The changes in the middle ear characteristics between the gender were attributed to differences in body size, head circumference, size of the middle ear cavity, and the ear canal size (Brucker et al., 2003; Jaffer, 2016). Males found to have a larger body size than females that could probably have larger ear canal volume and middle ear cavity, leading to an increase in the middle ear's mass component. This could increase the impedance, thereby reducing absorbance at high frequencies in males (Allen et al., 2005; Shahnaz & Bork, 2006). This could possibly explain the observed differences in WBA between genders in the current study.

With respect to the WBT_{avg}, the present study showed no significant difference between gender at peak pressure having identical mean average absorbance values. Whereas at ambient pressure, slightly higher average absorbance values were seen for males than females, which was significant with smaller effect sizes. There is very limited research that had demonstrated WBT_{avg} on gender difference and had shown higher averaged absorbance values in males than females at both the peak and ambient pressure

(Jaffer, 2016). The difference was attributed to the smaller middle ear cavities and ear canal sizes in females generating more stiffness, resulting in lesser absorbance at low and mid-frequencies (Kenny, 2013; Shahnaz & Bork, 2006; Shahnaz & Davies, 2006; Wan & Wong, 2002). As the average WBT is the derivative of WBA elicited at low and mid-frequencies, thus reducing WBT_{avg} absorbance in females compared to males (Jaffer, 2016). However, more research on WBT_{avg} is necessary for conclusive remarks.

In summary, higher absorbance was seen for males below 1000 Hz consistently and lower absorbance at high frequencies anywhere between 4000 to 6300 Hz, compared to females and identical WBT_{avg} absorbance values. Although a similar trend was noticed in the present study, the effect size is small, demonstrating that the significance is insignificant. Hence, the null hypothesis stating that "There is no significant difference in wideband absorbance in normal (healthy) ears between males and females" is partially accepted.

5.3 Effect of pressure conditions on WBA in Normal ears

The study evaluated the WBA measures obtained at the peak and ambient pressure conditions in the Normal ear group. WBA measure at peak pressure was slightly higher but significant at 250 Hz to 1000 Hz than the WBA values obtained at ambient pressure. The present study did not find significant differences in WBA between two pressure conditions at high frequencies. These findings are in-line with the previously reported studies (Aithal, Aithal, Kei, & Manuel, 2019; Feeney et al., 2017; Keefe et al., 2015; Liu et al., 2008; Sun, 2016).

Several studies have investigated the WBA at peak pressure and ambient pressure across the frequencies, but only limited studies reported differences between the two

(Burdiek & Sun, 2014; Kenny, 2011; Liu et al., 2008; Shaw, 2009). Aithal et al. (2019) reported differences in WBA obtained between peak and ambient pressure conditions at frequencies ranging from 250 Hz to 1500 Hz for Caucasian children and 1250 Hz for aboriginal children. Similarly, a large difference of WBA up to 2000 Hz has been observed in young people (Feeney et al., 2017; Liu et al., 2008; Sun, 2016). As in the present study, Margolis et al. (1999) also showed reduced absorbance at ambient pressure in the lower frequencies and unchanged at higher frequencies. However, despite the difference in WBA values, the pattern obtained at peak and ambient pressure was comparable (Jaffer, 2016; Wali et al., 2017), similar to the present study's findings.

In general, the pressurized WBA measurements, i.e., peak pressure, exhibit the eardrum's greatest mobility (Schlagintweit, 2018). This improves the sound energy transmission to the middle ear and predicts the middle ear status (Onusko, 2004). Thus, the WBA at peak pressure allows the maximum energy into the middle ear, and it gets reflected as a greater absorbance in the WBA measurements. At ambient pressure, reduced absorbance was usually seen at the lower frequencies than the peak pressure (Aithal, Aithal, Kei, & Manuel, 2019; Feeney et al., 2017; Keefe et al., 2015; Liu et al., 2008; Sun, 2016).

During the ambient pressure condition, the ear canal pressure is not equal to the middle ear pressure (Sun, 2016). There can either be positive or negative pressure induced in the ear canal with reference to the middle ear pressure (Liu et al., 2008; Shaver & Sun, 2013). This will result in pulling or pushing the tympanic membrane, resulting in increased stiffness and generating larger impedance, thereby reflecting more energy to the ear canal (Allen et al., 2005; Feeney & Keefe, 1999; Robinson et al., 2016). Due to this, there is a reduction in the absorbance at low frequencies in the ambient pressure conditions than the peak pressure (Feeney et al., 2017; Shaver & Sun, 2013; Voss et al., 2012).

Further, the study indicated no significant difference in WBA obtained at peak and ambient pressure conditions at high frequencies. This could be because the transmission of sound energy at high frequencies is not stiffness controlled (Pickles, 2012). Hence, the WBA values at those frequencies are not affected and have a similar pattern despite the pressure difference. Irrespective of the difference in WBA between the pressure conditions, studies have shown the importance of measuring WBA at peak pressure (Liu et al., 2008; Margolis et al., 1999) along with the ambient pressure. Margolis et al. (1999) suggested that a WBA measurement at peak pressure could correctly identify middle ear pathology in those whose WBA appeared normal at ambient pressure.

Regarding WBT_{avg}, the present study showed a slight difference between the pressure conditions with slightly lower average absorbance at ambient pressure than peak pressure and are insignificant. However, limited studies in the literature had reported the difference in wideband average absorbance tympanometry between the pressure conditions (peak, ambient) in adults. Jaffer (2016) had measured wideband average absorbance tympanometry averaged between 375 and 2000 Hz at peak and ambient pressure for both Chinese and Caucasians. Though the study did not compare the WBA between the pressure conditions, the mean average absorbance values for peak pressure were slightly higher than the ambient pressure for both ethnic groups. The authors attributed this difference to the increase in the eardrum's stiffness due to pressurization (Gaihede, 1996). Thus, this would have resulted in the differences in WBT_{avg} between the pressure condition observed in the current study. While no significant difference between peak and ambient pressure on WBT_{avg} was reported on the Indian population (Karuppannan & Barman, 2020, 2021a).

In summary, there is a significant difference in WBA at low frequencies obtained between the pressure condition across frequencies. This could be due to slight variation in-ear canal pressure compared to middle ear pressure when measured in ambient pressure condition. However, the difference in WBA is likely to be negligible in Normal ears, and if it exists, it would be only at the low frequencies. Hence, the null hypothesis as "There is no significant difference in wideband absorbance measured at peak pressure to that of ambient pressure in healthy ears", is partially rejected. **The null hypothesis as "There is no significant difference in wideband average absorbance measured at peak pressure to that of ambient pressure in normal (healthy) ears", is accepted.**

5.4 WBA norms at peak and ambient pressure

Normative WBA data at peak and ambient pressure were developed in the adult population aged 22 to 50 years. The present study did not affect the ears or gender for peak and ambient pressure conditions significantly. However, a significant difference was observed for the WBA between the pressure conditions (peak, ambient) at low frequencies. Considering all these and the importance of measuring WBA at peak pressure and ambient pressure (Margolis et al., 1999), a separate normative was established for WBA at peak and ambient pressure, as recommended by researchers (Burdiek & Sun, 2014; Kenny, 2013; Liu et al., 2008; Shaw, 2009).

The current investigation demonstrated that the mean WBA values were minimum at 250 Hz, and absorbance values increased with the increase in frequencies, reaching a maximum at 1250 Hz. It remains almost constant with a second maximum absorbance value obtained at 2000 Hz. Further, the absorbance values progressively decreased with increased frequency, thereafter having a minimum value at 6000 Hz and above. In the current study, maximum absorbance values were obtained between 1000 Hz and 2500 Hz. On the other hand, a gradual reduction in absorbance values is observed on either side of these frequencies. This reflects the middle ear's nature dominated by either stiffness or mass properties of the middle ear (Kim & Koo, 2015).

Reduced absorbance for lower frequencies below 1000 Hz could be due to the stiffness controlled middle ear system generated by the tympanic membrane, middle ear cavity, and the annular ligament (Lynch et al., 1982). As the frequencies increases, i.e. at higher frequencies, the middle ear system is mass dominated generated by the ossicles (Allen et al., 2005). The stiffness and mass component can cause high impedance mismatch in the middle ear, where most of the sound energy (below 1000 Hz and above 3000 Hz) is reflected in the ear canal (Allen et al., 2005; Puria & Allen, 1998). At mid-frequencies between 1000 and 3000 Hz, both stiffness and mass component equals and cancel each other, thereby allowing most of the energy into the middle ear (Allen et al., 2005). This trend is evidently seen in the WBA pattern, where high absorbance was seen at mid-frequencies. Keefe et al. (1993) also reported that transmission of sound energy to the middle ear is most efficient at mid-frequencies, and generally, these frequencies are important for speech perception.

Due to the middle ear's mass and stiffness properties, the WBA values obtained were lower at low frequencies, higher at mid-frequencies and lower at high frequencies (Margolis et al., 1999, 2001; Shahnaz, Bork, et al., 2009). This pattern is in line with previous research showing that the WBA at ambient and peak pressure reaches a maximum anywhere from 1000 to 4000 Hz in adults and lowest at the extreme low and high frequencies (Feeney & Sanford, 2004; Keefe et al., 1993; Kenny, 2013; Margolis et al., 1999; Sanford & Feeney, 2008; Shahnaz & Bork, 2006; Shaver, 2010; Shaw, 2009; Wang et al., 2019; Werner et al., 2010). A study by Wang et al. (2019) on Chinese individuals showed two maxima, at 1000 Hz and around 2500 to 3000 Hz, for both the peak and ambient pressure conditions, similar to the current findings. Further, there is no significant difference in WBA between Chinese and Indians reported in the literature, as the average height and weight are comparable between Chinese and Indians (Wali & Mazlan, 2018). Similarly, other studies on WBA measured at ambient pressure condition showed high absorbance values at 1200 Hz and 3500 Hz (Feeney et al., 2004; Keefe et al., 1993; Voss & Allen, 1994). However, there are reports of variation in WBA norms established among the adult population. The absorbance measured in Caucasian and Chinese young adults showed a single maximum around 3000 Hz in the Chinese individuals whereas, two maxima (around 1617 Hz and 3164 Hz) in the Caucasian individuals (Shahnaz & Bork, 2006).

Several reasons can be attributed to the difference in WBA among the studies available in the literature. However, the major one could be the racial difference among the subjects (Kenny, 2013; Shahnaz & Bork, 2006; Shaw, 2009; Wang et al., 2019). Such difference could be due to the disparity in body size between races, with Caucasians having a greater average height and weight than Chinese people (Shahnaz & Bork, 2006). Larger body size tends to have a larger ear canal cross-sectional area, differing ear canal geometries, and longer ear canal lengths at the probe's measurement location (Rosowski et al., 2013). Thus, the middle ear's acoustic transfer function is influenced by such anatomical and physiological differences of the conductive pathway (Hunter & Shanaz, 2014; Shahnaz, 2010).

Another reason could be the differences in the methodology, such as the age of subjects, procedures used to measure WBA, the type of probe microphone and ear tip used, and the calibration method for ear-canal volume (Feeney et al., 2004). Due to the variation in WBA among the races and difference in the method used, several researchers had suggested determining the ethnically homogeneous sets of normative data for WBA (Beers et al., 2010; Shahnaz et al., 2013; Shahnaz & Bork, 2006). Henceforth, the present study had developed WBA norms at the peak and ambient pressure conditions in the Indian adult population using Interacoustic Titan WBT equipment and the same was used for clinically validating the WBA in the pathological conditions.

Similarly, the study attempted to develop norms for wideband average absorbance tympanometry calculated between 375 Hz and 2000 Hz at peak pressure and ambient pressure conditions. The present study showed that the average absorbance is similar for both pressure conditions, ranging between 0.33 and 0.82 for peak pressure; and 0.34 and 0.86 for ambient pressure. Similar findings were also reported in the literature (Karuppannan & Barman, 2020, 2021; Kaya et al., 2019; Kim et al., 2019; Sliwa et al., 2020; Terzi et al., 2015). Jaffer (2016) had reported the mean WBT_{avg} of 0.47 in Caucasians and 0.44 in Chinese at peak pressure, whereas 0.44 in Caucasians and 0.42 in Chinese at ambient pressure. These differences were attributed to the difference in body size, middle ear cavities and the ear canal sizes among the ethnic groups (Jaffer, 2016; Kenny, 2013; Shahnaz & Bork, 2006; Wang et al., 2019), as similar to the difference obtained for WBA across frequencies. However, some studies used different bandwidth based on absorbance obtained at different frequencies to measure WBT_{avg} (Neto et al., 2014; Niemczyk et al., 2019; Sliwa et al., 2020). Thus, there exists no consistency in the average absorbance measurements that are reported in the literature.

The wideband average absorbance tympanometry is advantageous as it contains information of wider frequency range depicted in a single-peaked tympanogram and is easy to interpret compared to WBA across frequencies (Jaffer, 2016). Also, studies pointed out that WBA at mid-frequencies is more sensitive in identifying conductive hearing loss than the 226 Hz tympanometry (Piskorski et al., 1999). Thus, further studies on wideband average absorbance tympanometry are warranted with fixed frequency bandwidth to measure the average absorbance and to differentiate with the middle ear pathologies.

5.5 Reliability measures of WBA in Normal ears

The test-retest and inter-tester reliability were examined for the WBA measured across frequencies at peak and ambient pressure conditions. The study revealed that the intraclass correlation coefficient (ICC) values of WBA measured at peak and ambient pressure conditions were comparable. The ICC values across the frequencies for absorbance values and WBT_{avg} in the test-retest reliability measures ranged between 0.62 and 0.85, with the most frequencies showing good reliability (>0.75). However, the ICC values were slightly lower than the inter-tester reliability measures that ranged between 0.56 and 0.84.

The test-retest reliability measures were similar for all the frequencies in the present study, which is similar to that reported in the previous studies obtained in adults (Feeney et al., 2014; Rosowski et al., 2012; Werner et al., 2010). A study on test-retest reliability of WBA in an adult population with and without the reinsertion of probe tube indicated a small difference in WBA at high frequencies above 1000 Hz in the reinsertion method (Vander Werff et al., 2007). A similar observation was reported in Beers et al. (2010) study, where they showed no significant difference following reinsertion. While the test-reliability correlations measured in adults after two weeks' gap showed high reliability at low and mid-frequencies and low reliability at frequencies above 3000 Hz (Werner et al., 2010). Reliability measures were also performed on the middle ear disorders subjects, and they had reported lower reliability measures, i.e. large change in WBA was observed in those with middle ear disorders conditions compared to those with intact middle ear (Beers et al., 2010; Vander Werff et al., 2007). The differences could be due to the change in the severity of the pathological conditions.

Although previous studies have shown a small difference in test-retest measures, they are attributed to changes in static ear canal pressure or fluid levels between tests (Voss et al., 2013). The proper probe fit, especially the probe's orientation in the ear canal during the reinsertion, might also lead to the difference in WBA measures (Beers et al., 2010; Voss et al., 2013). However, no studies on inter-tester reliability of WBA have been performed previously in the Normal ears or the pathological conditions. The test-retest measures have shown good test-retest reliability and inter-test reliability measures for most frequencies at the peak and ambient pressure conditions in the present study. Hence, it can be used as a clinical tool beyond doubt.

5.6 Effect of middle ear disorders on WBA

The present study compared the difference in WBA across frequencies and wideband average absorbance tympanometry in different middle ear disorders with Normal ear group (Group I) at peak and ambient pressure conditions. The middle ear disorders included in the study were Tympanic membrane perforation, Middle ear effusion without perforation, Otosclerosis, and Eustachian tube dysfunction.

5.6.1 Tympanic membrane perforation

The study compared the WBA across frequencies and WBT_{avg} absorbance value obtained at peak and ambient pressure in the TmP group with the Normal ear group. TmP group demonstrated lower absorbance values at low and mid-frequencies with significant reduction up to 2500 Hz and higher absorbance beyond 4000 Hz. Further, the WBA pattern of TmP group had three maxima at 1000 Hz, 3000 Hz and 6000 Hz, with the greatest reduction seen in the mid-frequency region (1000 – 2500 Hz).

The present study's findings are consistent with Kim et al. (2019) study demonstrating lower absorbance values in the low and mid-frequency region, mostly below 1000 Hz. At high frequencies, studies had shown a wide range of variability with few reporting of near-normal reflectance/absorbance at higher frequencies above 1000 Hz (Feeney et al., 2003; Voss et al., 2012) and few others reporting higher transmittance (absorbance) above 4000 Hz (Allen et al., 2005; Jeng et al., 2008), as similar to the current findings. However, the reason for having three maxima in the present study is not clear. One would expect either increase in absorbance values at low or high-frequency region depending upon pathological changes, making the middle ear either mass or stiffness dominated system. However, it is a unique finding and pattern observed in ears with Tympanic membrane perforation and the reason for the same need to be explored.

Contradictory findings are also reported in the literature of reduced reflectance, i.e., increased absorbance for frequencies between 250 Hz and 1000 to 2000 Hz in-ears with perforations (Allen et al., 2005; Feeney et al., 2003; Jeng et al., 2008; Voss et al., 2012).

The authors attributed this difference to the increase in the middle ear's mass component due to tympanic membrane perforation that allows low-frequency energy into the middle ear (Kim & Koo, 2015). Further, the studies had shown that the size of the perforation alters the absorbance, with the smallest perforations showing the largest effect below 1000 Hz, while no change at high frequencies above 2000 Hz (Allen et al., 2005; Feeney et al., 2003; Voss et al., 2001c, 2001b, 2001a, 2012). This could be because of the presence of low-frequency mass generating lower impedance, thereby reducing the reflectance (increase in absorbance) at lower frequencies (Nakajima et al., 2013; Voss et al., 2012). As the diameter of the perforation enlarges, most of the energy is absorbed by the middle ear cavity and conducted into ossicles (Jeng et al., 2008). However, the present study did not consider the perforation's size while measuring the WBA across frequencies, which could have influenced the WBA results.

Generally, there exist no measurable peak in ears with tympanic membrane perforation. In the present study, WBA measurements were obtained even at peak pressure for the TmP group. This could probably be due to the use of a wider frequency range stimulus in the WBA measurements. Some variation in the change of transmission of sound energy took place with the change in ear canal pressure at the high-frequency region. The instrument probably considers this change as maximum sound energy reaching the middle ear as a peak and displayed as peak pressure. There are no studies that had measured WBA at peak pressure to support our findings. The present study showed a significant difference between peak and ambient pressure conditions with lower absorbance from 300 Hz to 1000 Hz and higher values between 2000 Hz and 3000 Hz. This increase in absorbance at peak pressure could be because of the contribution of stiffness due to pressurization (Shahnaz et al., 2013) in addition to the existing mass component due to the TmP (Voss et al., 2012).

Interestingly, the WBT_{avg} was not present at peak pressure in ears with the TmP group. This could probably be due to the lack of changes in the absorbance with the variation of pressure in the ear canal at each frequency (375 Hz and 2000 Hz) that is considered to calculate WBT_{avg}. Thus, the WBA across frequencies and WBT_{avg} results indicate that the variation in the transmission of sound energy took place beyond 2000 Hz with the change in ear canal pressure, which could be a limitation of the equipment. However, no study in the literature had commented on WBT_{avg} at peak pressure for the TmP group. Kim et al. (2019) had reported TPP in ears with TmP in the wideband average tympanometry measurements. Although the TPP was reported, the study measured WBT_{avg} only at ambient pressure indicating a ‘B’ type pattern without any measurable peaks and absorbance. In the present study, the WBT_{avg} obtained at ambient pressure was significantly lower than the Normal ear group. Thus, further studies are warranted to understand the difference in average absorbance obtained between the studies.

Receiver Operating Characteristic (ROC) analysis performed in the TmP group showed a high sensitivity (>74%) and specificity (>85%) for frequencies at 1250 Hz, 1500 Hz and 2000 Hz in both the pressure conditions and WBT_{avg} at ambient pressure. This is because there is a significant reduction in absorbance values at these frequencies due to high impedance, resulting in better differentiation between the TmP from the Normal ear. However, there are no supporting studies to compare the findings of the present study.

It can be concluded that WBA is useful for distinguishing tympanic membrane perforation from the Normal ear with high accuracy at mid-frequencies (1250 Hz, 1500 Hz and 2000 Hz) and WBT_{avg} in ambient pressure conditions. **Thus, the null hypothesis stating that "There is no significant difference in wideband absorbance between Tympanic membrane perforation and normal (healthy) ears" has been rejected.**

5.6.2 Middle ear effusion

The effect of middle ear effusion without perforation was compared with the normal ear. The result showed a significant reduction in absorbance from 250 Hz to 6000 Hz in both pressure conditions. The findings of this study are in agreement with previous research that has used reflectance measurements (Allen et al., 2005; Feeney et al., 2003; Piskorski et al., 1999; Voss et al., 2012) and absorbance measurements (Beers et al., 2010; Ellison et al., 2012; Keefe et al., 2012; Terzi et al., 2015). Studies had shown increased reflectance, i.e. decreased absorbance at all frequencies, mostly seen below 3000 Hz, and it depends on the amount of fluid-filled in the middle ear (Dai et al., 2008; Voss et al., 2012). Experiments on cadaver ears had indicated that the reflectance was increased (decreased absorbance) at all frequencies when the middle ear space was filled with fluid with the significant reduction seen below 3000 Hz (Voss et al., 2012). Also, the magnitude of change in absorbance was constant across frequencies, especially below 4000 Hz, indicating the effect of fluid is independent of frequencies (Jeng et al., 2008). The findings were supported by umbo-displacement studies where there was a reduction in the umbo velocity across the frequencies when the fluid was filled in the middle ear space (Gan et al., 2006; Ravicz et al., 2004).

Similar findings of reduced absorbance were also reported on human ears with MEE (Allen et al., 2005; Beers et al., 2010; Feeney et al., 2003; Keefe et al., 2012; Piskorski et al., 1999; Terzi et al., 2015) with few reporting a notch between 4000 and 7000 Hz (Feeney et al., 2003). Although the present study did not account for the amount of fluid present inside the middle ear cavity, it was observed that the reduction was seen across the frequencies up to 3000 Hz. No significant difference was observed at 8000 Hz, similar to the Terzi et al. (2015) study.

Further, the study had measured the WBA across frequencies at peak and ambient pressure. The reason for the occurrence of WBA at peak pressure and not for WBT_{avg} in ears with middle ear effusion has been discussed earlier in section 5.5.1. A significant reduction in absorbance at ambient pressure than peak pressure for frequencies between 500 Hz and 1000 Hz, similar to the Normal ear group, was observed in the present study. This difference could be due to the pressurization at the tympanic membrane that slightly increases the middle ear's stiffness and improves the sound energy entering the middle ear at peak pressure (Liu et al., 2008; Shahnaz & Bork, 2006; Wang et al., 2019). In contrast, Keefe et al. (2012) had reported no significant difference in absorbance across frequencies measured between ambient and pressure sweep method in ears with conductive hearing loss due to Otitis media with effusion. While Terzi et al. (2015) had indicated that only a few of the ears with MEE were able to elicit TPP, the WBA measurements at peak pressure were not considered in their study.

Regarding WBT_{avg}, a significant reduction in average absorbance at ambient pressure was observed in the MEE group than the Normal ear group. Similar results were reported in the literature showing reduced average absorbance for otitis media group and lowest for otitis media with effusion group at ambient pressure compared to the normal ear (Terzi et al., 2015). While WBT_{avg} was not elicited at peak pressure and the reasons has been discussed earlier in section 5.5.1.

Reduction in absorbance across frequencies and WBT_{avg} absorbance values was attributed to an increase in the middle ear cavity impedance due to loss of aeration and accumulation of fluids in the middle ear space (Nakajima et al., 2013). This accumulation of fluid in the middle ear reduces the middle ear space, resulting in reduced tympanic membrane stiffness (Von Unge et al., 1995) and umbo velocity (Ravicz et al., 2004). This could be one of the possible reason for reduced absorbance at low frequencies. At high frequencies, the reduction of absorbance was due to the increased mass caused by the fluid attached to the tympanic membrane in the middle ear and reduction in umbo velocity (Gan et al., 2006; Ravicz et al., 2004).

The accuracy of WBA measurements in MEE has been investigated previously on infants and children. Studies have shown good sensitivity and specificity in identifying the MEE (Beers et al., 2010; Ellison et al., 2012; Terzi et al., 2015). Beers et al. (2010) and Terzi et al. (2015) had shown the higher AUROC in the frequency range of 1000 to 1600 Hz, while Ellison et al. (2012) had suggested 800 Hz to 2000 Hz as the most sensitive frequencies in identifying MEE. A similar observation was seen in the present study showing high AUROC in the broader frequency range of 800 Hz to 3000 Hz having high sensitivity (>92%) and specificity (>88%) for both the pressure conditions. Further, the WBT_{avg} measurements at ambient pressure also had higher AUROC with 100% sensitivity and 98% specificity in differentiating MEE from the normal ear. The findings were supported by Terzi et al. (2015) where 100% sensitivity and 94% specificity on WBT_{avg} measurements was reported.

It can be concluded that WBA is effective in distinguishing MEE from the Normal ear with high accuracy at mid-frequencies (800 Hz to 3000 Hz) for both pressure conditions and WBT_{avg} in ambient pressure condition. Thus**, the null hypothesis stating that "There is no significant difference in wideband absorbance between Middle ear effusion and normal (healthy) ears" has been rejected.**

5.6.3 Otosclerosis

The present study compared the WBA across frequencies of the Otosclerosis group with that of the Normal ear group at both pressure conditions. The WBA of the Otosclerosis group measured at the peak and ambient pressure showed reduced absorbance at low and mid-frequencies up to 2000 Hz significantly and slightly higher absorbance values beyond 2000 Hz for both pressure conditions compared to the Normal ear group.

The present study results were in agreement with previously reported studies (Allen et al., 2005; Feeney et al., 2003; Karuppannan & Barman, 2020, 2021a; Kelava et al., 2020; Shahnaz, Bork, et al., 2009; Sliwa et al., 2020), which showed a decrease in absorbance values at frequencies below 1000 Hz for the Otosclerosis group. This reduction depends largely on the severity of the Otosclerosis and can be observed even at frequencies higher than 1000 Hz (Karuppannan & Barman, 2021a; Neto et al., 2014; Shahnaz, Bork, et al., 2009). The decline in WBA at low frequencies may be due to the annular ligament stiffening, which generates a large impedance and reflects much of the energy below 2000 Hz into the ear canal (Allen et al., 2005; Feeney & Keefe, 1999; Sliwa et al., 2020). In contrast, the transmission is not stiffness-controlled at higher frequencies, i.e., above 2000 Hz, and hence, WBA values are less affected (Pickles, 2012). However, the absorbance values at high frequencies are highly variable (Allen et al., 2005). Few studies had indicated reduced absorbance at 1260 Hz, 2520 Hz, and 4000 Hz in-ears with otosclerosis (Feeney et al., 2003; Wang et al., 2019). In contrast, increased absorbance was also reported from

5000 Hz to 8000 Hz (Kelava et al., 2020; Wang et al., 2019). However, the present study did not observe any significant difference in absorbance values at higher frequencies, although slightly higher values were observed in the Otosclerosis group.

Studies on changes in pressure in the middle ear have shown a significant effect on absorbance patterns in the Normal ear group with a lower WBA at ambient pressure than peak pressure (Karuppannan & Barman, 2020, 2021a; Liu et al., 2008; Shahnaz & Bork, 2006; Wang et al., 2019). However, limited studies have compared the WBA obtained at peak pressure with ambient pressure in the Otosclerosis group (Karuppannan & Barman, 2020, 2021a). Karuppannan and Barman (2021) showed reduced absorbance at ambient pressure for lower frequencies up to 1500 Hz than at peak pressure in-ears with otosclerosis. Similar results were obtained in the present study. This could be because of the stiffening of the tympanic membrane as a result of pressurization, i.e. at peak intensity, which increases sound energy transmission to the middle ear (Shahnaz et al., 2013). While at ambient pressure, there is a minimal amount of pressure gradient between the ear canal and middle ear, resulting in a slight change in the tympanic membrane's stiffness and thus influencing the WBA measurements (Kenny, 2013). In contrast, Karuppannan and Barman (2020) reported no significant difference between pressure conditions (peak, ambient), and this could be because of the limited sample size considered in their study.

Studies on WBT_{avg} in the literature are limited. Kim et al. (2019) had measured WBT_{avg} at ambient pressure (375 Hz – 2000 Hz), and it was found that the average wideband absorbance pattern was relatively low in Otosclerosis compared to the Normal ear. The current study showed a similar pattern with a significant reduction in average absorbance values at ambient pressure for Otosclerosis than in the Normal ear group. While at peak pressure, although the WBT_{avg} measurement was lower than the Normal ear group, it did not show any significant differences. Similar findings were also reported in the literature with reduced WBT_{avg} at ambient pressure in Otosclerosis compared to the Normal ear, while no significant difference at peak pressure (Karuppannan & Barman, 2020, 2021a).

However, few studies have reported average absorbance measured from the WBA measurements in different bandwidth across frequencies (250 Hz to 8000 Hz). Niemczyk et al. (2019) averaged the WBA obtained between 250 Hz and 4000 Hz in Otosclerosis and had reported the average absorbance value that ranged between 0.64 and 0.92. Whereas Neto et al. (2014) had shown a higher overall reflectance rate (>70%), i.e. lower absorbance rate in ears with Otosclerosis, averaged between 250 Hz and 6000 Hz. Though there are variations in average absorbance values between studies, it cannot be directly compared to the current study results due to the difference in frequency range considered and the method used to obtain the average absorbance values.

In addition, no study has compared the wideband average absorbance values between peak and ambient pressure conditions. However, the present study showed significantly reduced average absorbance at ambient pressure compared to peak pressure. This could be due to a change in the tympanic membrane's stiffness that would further reduce the transmission of sound to the middle ear across low frequencies (Allen et al., 2005; Feeney & Keefe, 1999), resulting in lower WBT_{avg}. A greater decrease in ambient pressure than peak pressure may be due to the further increase in stiffness at the tympanic membrane due to pressurization, which improves the transmission of sound to the middle ear at peak pressure condition (Shahnaz et al., 2013). However, more studies are warranted to understand whether WBT_{avg} could be a useful tool in detecting Otosclerosis.

ROC analysis was performed to measure the sensitivity and specificity in detecting Otosclerosis from the Normal ear. In the present study, ROC analysis of WBA at peak pressure and ambient pressure showed high sensitivity and specificity of > 80%, especially at low and mid-frequencies (250 Hz to 1250 Hz). Among those, the frequencies from 400 Hz to 800 Hz at peak pressure and 300 Hz to 1000 Hz at ambient pressure showed the highest sensitivity and specificity (>90%). This is because there is a significant reduction in absorbance values at these frequencies due to high impedance, resulting in better differentiation between the otosclerosis ear from the normal ear. Also, ambient pressure conditions showed better sensitivity than peak pressure due to a more significant reduction of absorbance.

Only limited studies had performed ROC analysis and had reported the sensitivity and specificity. Shanaz et al. (2009) had shown a significant difference in low and mid frequencies below 1000 Hz, exhibiting high AUROC values at 315 Hz and 500 Hz with a sensitivity of 82% at 500 Hz in Otosclerosis ears. Similarly, Karuppannan and Barman (2021) indicated that low frequencies up to 1500 Hz are sensitive in identifying otosclerosis with a high diagnostic value of >90% at 1000 Hz. While Kelava et al. (2020) have shown high AUROC values in the frequency range >0.5 to ≤ 1 kHz and >4 to ≤ 8 kHz to identify Otosclerosis. Śliwa et al. (2020) had suggested that WBA measurements are highly effective, for frequency centred around 650 Hz, with a sensitivity and specificity of over 85%. Hence, the low and mid-frequencies are found to be sensitive in the identification of Otosclerosis.

Karuppannan and Barman (2020) on WBT_{avg} measurements had indicated high accuracy in detecting otosclerosis at ambient pressure conditions at a criterion point of 0.45 having high sensitivity (92.2%) and specificity (97%). While the study had shown low accuracy at peak pressure. However, no other studies had performed WBT_{avg} measurements with a frequency range of 375 Hz to 2000 Hz. Nakajima et al. (2012) study had averaged the absorbance values between 600 Hz and 1000 Hz at peak pressure in combination with an air-bone space at an average of 1000 to 4000 Hz, showed high sensitivity (86%) and specificity (100%) to differentiate otosclerosis. The sensitivity and specificity of WBT for frequencies averaged between 226 Hz and 2000 Hz were observed to be lower (80%) compared to the average absorbance values between 500 and 700 Hz (Sliwa et al., 2020). The present study indicated high accuracy (>95% sensitivity and specificity) in detecting Otosclerosis only at ambient pressure conditions. The difference in WBT_{avg} only at ambient pressure and not at peak pressure needs to be speculated through further research on WBT_{avg} measurements.

Thus, it can be concluded that low and mid frequencies are efficient in differentiating Otosclerosis ears from Normal ears. Therefore**, the null hypothesis stating that "There is no significant difference in wideband absorbance between Otosclerosis and normal (healthy) ears" is partially rejected.**

5.6.4 Eustachian tube dysfunction

In the present study, the comparison of the ETD group with that of the Normal ear group showed a similar WBA pattern with a significant reduction of absorbance values across frequencies up to 3000 Hz for both the pressure conditions. Further, the reduction was more significant at ambient pressure than at peak pressure conditions. At high frequencies above 3000 Hz, the WBA was almost similar between the groups except at 8000 Hz showing higher absorbance.

ETD is one of the most commonly occurring middle ear disorders that affect the transmission of sound to the inner ear due to abnormal negative middle ear pressure (Shaver & Sun, 2013). Limited studies had reported a change in WBA measured in abnormal middle ear pressure and are performed mostly in young children (Aithal, Aithal, Kei, Anderson, et al., 2019; Beers et al., 2010; Hunter, Tubaugh, et al., 2008; Sanford & Brockett, 2014). Few efforts have been made to examine the effect of negative middle ear pressure on WBAs in adults (Karuppannan & Barman, 2021b). Most of these studies were based on the stimulated conditions in healthy individuals/cadaveric ears (Margolis et al., 1999, 2001).

Studies had shown that any alteration in middle ear pressure increased the energy reflectance (decrease in absorbance) below 3000 to 4000 Hz and decreased (increase in absorbance) at higher frequencies beyond 4000 Hz (Karuppannan & Barman, 2021b; Margolis et al., 1999; Shaver & Sun, 2013; Voss et al., 2012). Our findings appear to be well supported by earlier studies on negative middle ear pressure showing a reduction in absorbance for frequencies below 3000 Hz. The mid-frequencies showed a considerable reduction in WBA at peak pressure compared to the lower frequencies, as similar to the findings reported in the literature (Karuppannan & Barman, 2021b; Shaver & Sun, 2013). The amount of reduction in absorbance at low and mid-frequencies could be because of the increased stiffness of the middle ear due to the presence of negative middle ear pressure (Robinson et al., 2016). This generates a large impedance at the level of the tympanic membrane due to the increase in stiffness, thereby reflecting most of the energy to the ear canal (Allen et al., 2005; Feeney & Keefe, 1999; Robinson et al., 2016). Further, it is supported by the umbo-displacement measurement studies performed in the negative middle ear pressure conditions where reduced umbo movements for frequencies below about 2000 Hz was reported (Gan et al., 2006; Murakami et al., 1997).

In general, studies have reported a large reduction in absorbance below 1000 Hz with minimal changes above 2000 Hz with varying degrees of negative middle ear pressure (Karuppannan & Barman, 2021b; Shaver, 2010). However, there is no consistency in WBA results at the higher frequencies across the studies. Margolis et al. (1999) reported an increase in absorbance from 3000 to 8000 Hz, while the studies in young children had shown an increase in energy reflectance (decrease in absorbance) for frequencies up to 4000 to 5000 Hz (Beers et al., 2010; Hunter, Tubaugh, et al., 2008). Variations in the absorbance at the higher frequency region have been attributed to the TPP magnitude and change in the middle ear stiffness (Shaver & Sun, 2013). This was supported by the Beers et al. (2010) study where the reduction in absorbance was observed for frequencies from 630 to 2000 Hz in children with mild pressure in the middle ear, and the effect was observed up to 5000 Hz for severe middle ear pressure conditions. Further, the umbo displacement at high frequencies showed an asymmetry above 2000 Hz with either increase or no change in umbo displacement (Gan et al., 2006; Murakami et al., 1997) for negative middle-ear pressure condition that could have resulted in inconsistency findings at high frequencies.

The current WBA results in the ETD group did not significantly differ above 3000 Hz for both pressure conditions with slightly higher absorbance at 8000 Hz. Similar results of increased absorbance at 8000 Hz have been reported in the literature (Dai et al., 2008). This could be due to an increase in umbo movements compared to other frequencies, measured by laser Doppler velocimetry in human cadavers' temporal bones (Dai et al., 2008).

Furthermore, literature studies have reported a significant reduction in WBA at ambient pressure compared to the peak pressure seen below 2000 Hz in ears with negative middle ear pressure/ETD (Aithal, Aithal, Kei, Anderson, et al., 2019; Karuppannan & Barman, 2021b; Robinson et al., 2016; Shaver & Sun, 2013; Voss et al., 2012). Aithal et al. (2019) had investigated the effect of WBA in children with ETD and had demonstrated a differential absorbance pattern with significant lower WBA only at ambient pressure compared to the normal ears. Similar findings of reduced absorbance below 2000 Hz at ambient pressure than peak pressure conditions were observed in the present study. This is because the TPP plays a significant role in equalizing the pressure between the ear canal and middle ear, thereby allowing maximum energy to enter into the middle ear (Robinson et al., 2016). Thus, applying pressure to compensate for the negative middle ear pressure restores the WBA values near to the baseline values (Aithal, Aithal, Kei, Anderson, et al., 2019; Karuppannan & Barman, 2021b; Robinson et al., 2016; Shaver & Sun, 2013; Voss et al., 2012). There is a significant pressure gradient between the middle ear and ear canal at ambient pressure, resulting in an increase of the tympanic membrane's stiffness. This would have resulted in the reduction of absorbance values, especially at low frequencies. Thus, the WBA for those with negative middle ear pressure would show higher absorbance at peak pressure than the ambient pressure and reaches the absorbance values near to the Normal ear group.

Whereas the WBT_{avg} measurement was obtained only at ambient pressure condition and not at peak pressure in the current study. This may be because of the increase in the middle ear stiffness due to the decrease in middle ear pressure (Kim & Koo, 2015), which restricts the low-frequency energy transmission to the middle ear and allows only the high-frequency energy transmission virtually. This could probably lead to flat average peak absorbance values in the frequency range (375 Hz and 2000 Hz) considered to calculate WBT_{avg}. At ambient pressure, WBT_{avg} was lower for the ETD group than the Normal ear group. This could be because of the physiological changes in the middle ear structures due to negative middle ear pressure, affecting the transmission of sound to the inner ear. However, no studies have been reported to measure WBT_{avg} on ETD to support our research.

ROC analysis in the ETD group with the Normal ear group showed good sensitivity only at the ambient pressure conditions with high accuracy (> 90%) seen in the mid-frequencies (500 Hz to 1000 Hz) and WBT_{avg}. This is because there is a significant reduction in absorbance values at these frequencies due to high impedance, resulting in better differentiation of ETD from the normal ear. Whereas at peak pressure, though it has shown good specificity (>85%) from 600 Hz to 1500 Hz, the sensitivity is found to be lower. However, there are no studies that have performed a ROC analysis to support our findings. As indicated in the literature, there is a significant decrease in absorbance for frequencies below 1000 Hz, with varying negative pressure levels in the middle ear (Shaver, 2010). Thus, it could have led to a significant reduction in WBT_{avg} also.

Thus, it can be concluded that WBA is useful for distinguishing ETD from the normal ear with high accuracy at mid-frequencies (500 to 1000 Hz) and WBT_{avg} in ambient pressure conditions. Thus, **the null hypothesis stating that "There is no significant**

difference in wideband absorbance between Eustachian tube dysfunction and normal (healthy) ears" has been partially refuted.

5.7 Effect of WBA on Sensorineural hearing loss

The WBA across frequencies and WBT_{avg} were measured at peak pressure and ambient pressure conditions in the SNHL group and was compared with the Normal ear group. The present study results showed a similar WBA pattern to that of normal ears, with significantly lower absorbance at frequencies at 1250 Hz and below for SNHL in both pressure conditions. Although it shows a significant difference in the lower frequencies, the WBA values are within the normative range. At the same time, the WBT_{avg} absorbance was identical without any significant difference between the groups. Nonetheless, there are no reports of WBA measures on SNHL individuals in the literature.

Feeney et al. (2003) had evaluated WBA measures in bilateral SNHL subjects who also had a history of ETD, having negative middle-ear pressure. The authors had indicated that the ears with SNHL are indistinguishable from normal ears, indicating that damage to the cochlea does not impact the WBA measurements. In general, the middle ear's mass and stiffness properties affect the transfer of acoustic energy to the middle ear across frequencies (Kim & Koo, 2015). The low-frequency transmission function is mainly affected by the middle ear stiffness, primarily due to the middle ear cavity. In contrast, the high-frequency transfer function was affected by the middle ear's mass component largely due to middle ear bones (Kim & Koo, 2015). However, in SNHL, the lesion occurs within the cochlear structure, while the middle ear remains intact (Davis, 2015). Therefore, the middle ear stiffness and mass characteristics have little or no effect on the SNHL (Kim &

Koo, 2015) and perform as similar to the normal ear. This could be probably a reason for identical WBA values across frequencies in the SNHL and Normal ear.

Like the Normal ear group, the comparison of WBA between pressure conditions (peak, ambient) in the SNHL group showed slightly reduced absorbance at ambient pressure condition than the peak pressure with significant reduction observed below 1000 Hz. While the WBT_{avg} obtained between the pressure conditions also showed a slightly reduced average absorbance at ambient pressure than the peak pressure conditions, but it did not reach significance. At TPP, the pressure across the tympanic membrane makes it more effective to transmit sound (Onusko, 2004; Schlagintweit, 2018). Whereas at ambient pressure, there is a pressure gradient across the tympanic membrane, leading to the increased stiffness. This would have resulted in reduced absorbance values at low frequencies, similar to those observed in the normal ears.

Further, at higher frequencies, the study showed no significant difference in WBA obtained at peak and ambient pressure conditions in the SNHL group. This may be because the transmission of sound energy at high frequencies is not controlled by stiffness (Pickles, 2012). As a result, the WBA values did not change at these frequencies and have a similar WBA pattern despite the pressure difference.

ROC analysis on WBA measures showed low to moderate sensitivity (50 – 60%) and specificity (70 – 80 %) in differentiating the SNHL group from the Normal ears in the current study for both pressure conditions. Though there exist a significant reduction in absorbance values at the low and mid-frequencies (250 Hz – 1250 Hz) for the SNHL group, the difference is small, resulting in lower sensitivity. Thus, it can be concluded that WBA measures are indistinguishable between the SNHL and Normal ear group. **The null**

hypothesis stating that "There is no significant difference in wideband absorbance between the ears with sensorineural hearing loss and normal (healthy) ears." has been accepted.

5.8 Effect of between-pathological groups comparison on WBA

Research on WBA has suggested that it is significantly sensitive enough to detect middle ear pathologies (Keefe et al., 2017; Keefe & Simmons, 2003; Kim et al., 2019; Sanford & Brockett, 2014; Shahnaz, Bork, et al., 2009), specifically those disorders that are failed to detect using 226 Hz tympanometry (Shahnaz & Davies, 2006). Previous studies on WBA had shown a notable change in absorbance value across frequencies for each of the middle ear disorders, and most of them are compared only with normal ears (Hunter & Margolis, 1997; Keefe & Levi, 1996; Margolis et al., 1999; Piskorski, Keefe, Simmons, & Gorga, 1999). Similar findings were obtained in the current study with distinctive WBA patterns for each of the middle ear disorders. None of the studies had compared all the middle ear disorders and its effect of WBA across frequencies.

The current study attempted to study the differences in absorbance between middle ear disorders. However, the study did not find any common absorbance value that can differentiate between the groups in any of the frequencies at both peak and ambient pressure conditions. Interestingly, the study showed a distinct WBA pattern among middle ear disorders and are different from the normal ear group. These differences are seen at the low and mid-frequencies for all the middle ear disorders consistently. In contrast, the high frequencies were almost similar for most middle ear disorders for both the peak and ambient pressure conditions. Among the disorders groups, the MEE group demonstrated the lowered WBA pattern with the reduction in absorbance at all the frequencies, while the

SNHL group showed an increased WBA pattern near to that of the normal ear. The TmP group showed a reduction of absorbance up to 2500 Hz with three prominent maxima; and Otosclerosis had shown reduction up to 2000 Hz for both peak and ambient pressure. The only conditions that differed between the pressure conditions were the ETD group, where the WBA pattern of ETD is similar to the MEE group for ambient pressure conditions, whereas the WBA pattern was near to normal at peak pressure. Thus based on the holistic pattern, the middle ear disorders can be differentiated. The reason for having a different pattern in different pathological conditions has been elaborated under each disorder condition compared to the normal middle ear group. Voss et al. (2012) suggested that the WBA could help identify the middle ear pathology if used in conjunction with other immittance measurements. **Thus, the null hypothesis stating that "There is no significant difference in wideband absorbance across the pathological conditions" has been partially accepted.**

Chapter 6

SUMMARY AND CONCLUSIONS

The present study aimed to obtain the norms for wideband absorbance in adults with normal middle ear and normal hearing sensitivity and clinically validate with the disordered group results. To achieve this, the current study had five primary objectives: (a) to study the effect of gender on wideband absorbance tympanometry results in healthy adults having a normal middle ear; (b) To study the effect of two different pressure conditions (peak and ambient pressure) on wideband absorbance tympanometry results; (c) to obtain the norms (absorbance value) across the frequencies using wideband absorbance tympanometry in healthy adults with the normal middle ear; (d) to study the effect of various middle ear disorder (Tympanic membrane perforation, Middle ear effusion without perforation, Otosclerosis, and Eustachian tube dysfunction) on wideband absorbance tympanometry and compared it with the values obtained in normal healthy middle ears; and (e) to study the effect of SNHL on wideband absorbance tympanometry findings and compare it with the values obtained in normal healthy middle ears.

The study used a cross-sectional research study design with standard group comparison. Three groups in the age range of 22 to 50 years were considered in the study. Group I consisted of normal middle ear functioning with normal hearing sensitivity (n=1127 ears), Group II consisted of middle ear disorders [Tympanic membrane perforation (n=109), Middle ear effusion without perforation (n=122), Otosclerosis (n=140), and Eustachian tube dysfunction (n=106)], and Group III consisted of SNHL (n=140). All the participants had undergone WBA measurements across frequencies and wideband average tympanometry.

Before derivative of WBA norms, the study assessed the difference in ears (right, left), genders (male, female) and pressure conditions (peak, ambient) in the normal ear group. The study had demonstrated a significant difference in WBA between pressure conditions, showing a lower estimate of absorbance values at ambient pressure than at peak pressure in the lower frequencies (250 Hz to 1000 Hz). Analysis between the genders showed a significant difference in most frequencies with a small effect size and hence not considered a true difference between males and females. While ear differences were not observed on WBA measurements in the normal ear group. Therefore, the present study had analyzed WBA across frequencies and WBT_{avg} measurements independently for each pressure conditions and successfully established the clinical norms. The WBA was lowest at 250 Hz, increased steeply with increasing frequency to about 1250 Hz, rose to a second maximum at about 2000 Hz, and reduced to a minimum absorbance at 6000 Hz and above. The reliability measure analysis (test re-test and Inter-tester) showed a moderate to good reliability across the frequencies and WBT_{avg}, indicating a reliable tool to be used.

Further, with the established WBA norms, the study had evaluated the significant difference of different middle ear disorders across the frequencies and between pressure conditions. In general, the study had shown a reduction in WBA for all the groups, specifically at the lower frequencies. In-ear with tympanic membrane perforation, a significant reduction was observed from 250 Hz to 2500 Hz. The largest reduction was seen at mid-frequencies, while higher absorbance estimates were noticed 5000 Hz and 6000 Hz for both pressure conditions. ROC analysis had shown the highest sensitivity and specificity in the mid frequencies between 1250 Hz and 2000 Hz. In ears with middle ear effusion, a significant reduction of WBA was seen at all the frequencies up to 6000 Hz, with high sensitivity and specificity reported between 800 Hz and 3000 Hz.

Similarly, the otosclerosis ears illustrated a significant reduction of WBA up to 2000 Hz with high sensitivity and specificity obtained from 250 Hz to 1250 Hz. ETD group showed a significant reduction up to 3000 Hz with the largest reduction seen at ambient pressure condition. The WBA of the ETD group showed good sensitivity and specificity only at ambient pressure condition between 500 Hz and 1000 Hz. Though the significant difference was seen up to 1250 Hz for the SNHL group, the WBA pattern fell within the established WBA norms and had shown low sensitivity and specificity across the frequencies. The difference in absorbance obtained in the lower frequencies was attributed to the change in the middle ear's stiffness component, thereby increasing the low frequencies' impedance.

The study had also analyzed the difference in WBA obtained between the pressure conditions in each of the middle ear disorders. The study showed a significant difference in WBA across the study groups, concentrating at the lower frequencies with higher estimates of absorbance at peak pressure than ambient pressure. The frequencies at which the significant difference obtained were from 300 Hz to 1000 Hz and 2000 Hz to 2500 Hz for the Tympanic membrane perforation group; 500 Hz to 1000 Hz for the Middle ear effusion group; 250 Hz to 1500 Hz for the Otosclerosis group; 250 Hz to 2500 Hz for ETD group; and from 250 Hz to 1250 Hz for SNHL group. These difference between the pressure conditions were attributed to the pressurization in the ear canal, i.e. balancing the middle ear and ear canal's pressure to allow the sound energy into the middle ear most effectively.

With regard to wideband average absorbance tympanometry, the average absorbance values at peak pressure were obtained only for the Normal ear, Otosclerosis and SNHL group. The Otosclerosis group had a significantly lower average absorbance than the other two, while no significant difference was seen between SNHL and Normal ear. The middle ear effusion group had the lowest average absorbance value at ambient pressure conditions, followed by Eustachian tube dysfunction, Otosclerosis and Tympanic membrane perforation group. The SNHL had similar average absorbance compared to the normal ear. Interestingly, all the middle ear disordered groups had lower average absorbance values of less than 0.5, whereas the Normal ear and SNHL groups showed greater than 0.5 absorbance value. However, they exhibit a lot of overlap between the groups.

6.1 Strength and limitations of the study

- The main advantage of this study is the large sample size used to evaluate the WBA norms, and thus the outcome measures can be generalized.
- The current study validated the WBA results in a clinical population with the estimated normative. This study highlighted the importance of WBA in various clinical groups and identified each of these disorders using WBA measurements.
- However, the current study's data are pooled according to the audiometric findings and diagnosis by the Otologist. There was no surgical confirmation, especially the ossicles related disorders. Further, the study did not consider the severity of middle ear disorders, which would lead to variation in the absorbance across frequencies. For instance, an earlier study had shown the amount of reduction in absorbance depends on the quantity of fluid present in the middle ear (Voss et al., 2012), size of the tympanic membrane perforation (Voss et al., 2001b), the severity of the

otosclerosis (Shahnaz & Polka, 2002) and change in pressure in the middle ear (Robinson et al., 2016).

- The study did not find any uniformity in absorbance value across the frequencies that could differentiate middle ear disorders. However, it has shown a unique WBA pattern that can be considered signature finding in differentiating from each other.
- Further, the current study performed ROC analysis for all pathological groups and estimated sensitivity and specificity for each frequency. The best criterion to provide high sensitivity and specificity for each frequency was also estimated in the current study, which provides future research insights.
- Though there are not many studies that had analyzed the WBT_{avg} measurements in disordered populations, the present study showed good sensitivity in identifying middle ear disorders. This averaged absorbance measurement contains the single peaked tympanogram that contains wider bandwidth from 375 to 2000 Hz for easier interpretation. However, as indicated in the current findings, the low frequencies had reduced absorbance while the mid-frequencies have higher absorbance values. This might weaken the sensitivity of the WBTavg measurements, as it contains the broader frequency range from low to mid-frequencies.
- The study did not compare the single component and multi-component tympanometry findings with the WBA measurements that could have provided the difference in sensitivity with the existing middle ear analyzing tool.

6.2 Clinical implications

The present study's findings have several implications for the clinical use of WBA.

- It has been well established that WBA norms are dependent on ethnicity showing a significant difference in absorbance among the Chinese and Caucasian participants (Jaffer, 2016; Shahnaz & Bork, 2006; Shaw, 2009). Thus, the present study had developed specific WBA norms that could help identify middle ear disorders in the Indian adult population.
- The present study findings clearly indicate that WBA depends on pressure conditions, while ear and gender did not significantly affect the WBA results. Thus, it suggested establishing separate norms for pressure conditions that can be used to identify the middle ear's status more precisely. Measuring WBA at peak and ambient pressure may increase the specificity of certain pressure-related middle ear disorders, specifically the Eustachian tube dysfunction showing abnormal WBA pattern for ambient pressure and normal range at peak pressure conditions. This provides insight into the identification of middle ear pathologies if WBA is assessed at both pressure conditions.
- The estimated normative values of WBA is from a large sample size, and this norm can be considered necessary for future research. Also, the WBA measures' reliability across frequencies was consistent and can be used as a clinical diagnostic measure for identifying middle ear disorders.
- The study also considered wideband average absorbance tympanometry measurements. It is unique in this study, which has shown good sensitivity in

identifying middle ear disorders. Most studies have not demonstrated the efficacy of WBT_{avg} in clinical populations, which would add to the literature.

- The study suggests that WBA measurements in middle ear disordered conditions are distinguishable from the normal ears. The most useful frequencies were low and mid-frequency, mostly below 2000 Hz, while the high frequency is variable. Thus, the WBA measurement can be used as a diagnostic tool to assess middle ear conditions and identify middle ear disorders.

REFERENCES

AAP/AAFP Guidelines. (2004). American Academy of Pediatrics and American Academy of Family Physicians clinical Practice Guideline Subcommittee on Management of Acute Otitis Media Diagnosis and Management of Acute Otitis Media. In *Pediatrics* (Vol. 113, Issue 5). http://pediatrics.aappublications.org/content/113/5/1451.full.pdf+html

Aithal, S., Aithal, V., Kei, J., Anderson, S., & Liebenberg, S. (2019). Eustachian tube dysfunction and wideband absorbance measurements at tympanometric peak pressure and 0 daPa. *Journal of the American Academy of Audiology*, *30*(9), 781–791. https://doi.org/10.3766/jaaa.18002

Aithal, S., Aithal, V., Kei, J., & Manuel, A. (2019). Effect of negative middle ear pressure and compensated pressure on wideband absorbance and otoacoustic emissions in children. *Journal of Speech, Language, and Hearing Research*, *62*(9), 3516–3530. https://doi.org/10.1044/2019_JSLHR-H-18-0426

Aithal, S., Kei, J., Aithal, V., Manuel, A., Myers, J., Driscoll, C., & Khan, A. (2017). Normative study of wideband acoustic immittance measures in newborn infants. *Journal of Speech, Language, and Hearing Research*, *60*(5), 1417–1426. https://doi.org/10.1044/2016_JSLHR-H-16-0237

Aithal, S., Kei, J., & Driscoll, C. (2014). Wideband absorbance in young infants (0-6 months): A cross-sectional study. *Journal of the American Academy of Audiology*, *25*(5), 471–481. https://doi.org/10.3766/jaaa.25.5.6

Aithal, S., Kei, J., Driscoll, C., & Khan, A. (2013). Normative wideband reflectance measures in healthy neonates. *International Journal of Pediatric Otorhinolaryngology*, *77*(1), 29–35. https://doi.org/10.1016/j.ijporl.2012.09.024

Aithal, V., Aithal, S., Kei, J., & Manuel, A. (2019). Normative wideband acoustic immittance measurements in caucasian and aboriginal children. *American Journal of Audiology*, *28*(1), 48–61. https://doi.org/10.1044/2018_AJA-18-0065

Allen, J. B. (1986). Measurement of Eardrum Acoustic Impedance. In *Lecture Notes in Biomathematics* (pp. 44–51). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-50038-1_6

Allen, J. B., Jeng, P. S., & Levitt, H. (2005). Evaluation of human middle ear function via an acoustic power assessment. *Journal of Rehabilitation Research and Development*, *42*(4 Suppl. 2), 63–77. https://doi.org/10.1682/JRRD.2005.04.0064

Altman, D. G., & Bland, j. M. (1995). Statistics notes: The Normal distribution. *BMJ*, *310*(6975), 298. https://doi.org/10.1136/bmj.310.6975.298

ANSI. (2010). *American National Standard: Specification for audiometers. ANSI S3.6–*

2010. https://global.ihs.com/doc_detail.cfm?document_name=ANSI%2FASA S3.6&item_s_key=00009563

ANSI. (2012). *American National Standard: Specifications for instruments to measure aural acoustic impedance and admittance (Aural acoustic immittance). ANSI/ASA S3.39-1987 (R2012)*. https://webstore.ansi.org/standards/asa/ansiasas3391987r2012

ANSI. (2018). *American National Standard: Maximum permissible ambient noise levels for audiometric test rooms. ANSI S3.1-1991 (R2018)*. https://webstore.ansi.org/Standards/ASA/ANSIASAS31999R2018

ANSI. (2019). *American National Standard: Methods for Manual Pure-Tone Threshold Audiometry. S3.21-2004 (R2019)*. https://infostore.saiglobal.com/en-us/Standards/ANSI-ASA-S3-21-2004-R2019--133165_SAIG_ASA_ASA_2747350/

Baiduc, R. R., Poling, G. L., Hong, O. S., & Dhar, S. (2013). Clinical measures of auditory function: The cochlea and beyond. *Disease-a-Month*, *59*(4), 147–156. https://doi.org/10.1016/j.disamonth.2013.01.005

Beers, A. N., Shahnaz, N., Westerberg, B. D., & Kozak, F. K. (2010). Wideband reflectance in normal caucasian and chinese school-aged children and in children with otitis media with effusion. *Ear and Hearing*, *31*(2), 221–233. https://doi.org/10.1097/AUD.0b013e3181c00eae

Bell, A. C., Adair, L. S., & Popkin, B. M. (2002). Ethnic differences in the association between body mass index and hypertension. *American Journal of Epidemiology*, *155*(4), 346–353. https://doi.org/10.1093/aje/155.4.346

Bender, R., & Lange, S. (2001). Adjusting for multiple testing - When and how? *Journal of Clinical Epidemiology*, *54*(4), 343–349. https://doi.org/10.1016/S0895-4356(00)00314-0

Bianchedi, M., Croce, A., Neri, G., & Moretti, A. (1996). Multifrequency tympanometry in Meniere's disease: preliminary results. *Acta otorhinolaryngologica Italica*, *16*(1), 1–5.

Browning, G. G., & Gatehouse, S. (1992). The prevalence of middle ear disease in the adult British population. *Clinical Otolaryngology & Allied Sciences*, *17*(4), 317–321. https://doi.org/10.1111/j.1365-2273.1992.tb01004.x

Browning, G. G., Swan, I. R. C., & Gatehouse, S. (1985). The doubtful value of tympanometry in the diagnosis of otosclerosis. *The Journal of Laryngology & Otology*, *99*(6), 545–547. https://doi.org/10.1017/S002221510009722X

Brucker, M. J., Patel, J., & Sullivan, P. K. (2003). A morphometric study of the external ear: Age- and sex-related differences. *Plastic and Reconstructive Surgery*, *112*(2), 647–652. https://doi.org/10.1097/01.PRS.0000070979.20679.1F

Burdiek, L. M., & Sun, X. M. (2014). Effects of consecutive wideband tympanometry trials on energy absorbance measures of the middle ear. *Journal of Speech, Language, and Hearing Research*, *57*(5), 1997–2004. https://doi.org/10.1044/2014_JSLHR-H-13-0344

Cakir Cetin, A., Gurkan, S., Kirkim, G., & Guneri, E. A. (2019). Wide-Band tympanometry results during an acute episode of Ménière’s disease. *Audiology and Neurotology*, *24*(5), 231–236. https://doi.org/10.1159/000502768

Carhart, R., & Jerger, J. F. (1959). Preferred method for clinical determination of Pure-tone thresholds. *Journal of Speech and Hearing Disorders*, *24*(4), 330–345. https://doi.org/10.1044/jshd.2404.330

Carpenter, M. C. S., Cacace, A. T., & Mahoney, M. J. (2012). Missing links in some curious auditory phenomena: A tale from the middle ear. *Journal of the American Academy of Audiology*, *23*(2), 106–114. https://doi.org/10.3766/jaaa.23.2.5

Chan, J. C., & McPherson, B. (2001). Spontaneous and transient evoked otoacoustic emissions: A racial comparison. *Journal of Audiological Medicine*, *10*, 20–32.

Clark, J. G. (1981). Uses and abuses of hearing loss classification. *Asha*, *23*(7), 493–500. https://pubmed.ncbi.nlm.nih.gov/7052898/

Cohen, J. (1992). A power primer. *Psychological Bulletin*, *112*(1), 155–159. https://doi.org/10.1037/0033-2909.112.1.155

Cohen, J. (2013). *Statistical Power Analysis for the Behavioral Sciences*. United Kingdom: Elsevier Science. https://doi.org/10.4324/9780203771587

Dai, C., Wood, M. W., & Gan, R. Z. (2008). Combined effect of fluid and pressure on middle ear function. *Hearing Research*, *236*(1–2), 22–32. https://doi.org/10.1016/j.heares.2007.11.005

Danesh, A. A., Shahnaz, N., & Hall, J. W. (2018). The Audiology of Otosclerosis. *Otolaryngologic Clinics of North America*, *51*(2), 327–342. https://doi.org/10.1016/j.otc.2017.11.007

Darrouzet, V., Dulon, D., & Franco-Vidal, V. (2007). Multifrequency immittancemetry in experimentally induced stapes, round window and cochlear lesions. *Audiology and Neurotology*, *12*(2), 85–100. https://doi.org/10.1159/000097795

Davis, T. M. (2015). Acoustic immittance measures: Basic and advanced practice. *Ear and Hearing*, *37*(1), 121. https://doi.org/10.1097/AUD.0000000000000245

Demir, E., Afacan, N. N., Celiker, M., Celiker, F. B., İnecikli, M. F., Terzi, S., & Dursun, E. (2019). Can wideband tympanometry be used as a screening test for superior semicircular canal Dehiscence? *Clinical and Experimental Otorhinolaryngology*, *12*(3), 249–254. https://doi.org/10.21053/ceo.2018.01137

Demir, E., Celiker, M., Aydogan, E., Balaban, G. A., & Dursun, E. (2020). Wideband tympanometry in Meniere's disease. *Indian Journal of Otolaryngology and Head and Neck Surgery*, *72*(1), 8–13. https://doi.org/10.1007/s12070-019-01709-8

Dunn, T. J., Baguley, T., & Brunsden, V. (2014). From alpha to omega: A practical solution to the pervasive problem of internal consistency estimation. *British Journal of Psychology*, *105*(3), 399–412. https://doi.org/10.1111/bjop.12046

Durante, A. S., Santos, M., Roque, N. M. C. d. F., Gameiro, M. S., Almeida, K. de, & Sousa Neto, O. M. de. (2019). Wideband acoustic absorbance in children with Down syndrome. *Brazilian Journal of Otorhinolaryngology*, *85*(2), 193–198. https://doi.org/10.1016/j.bjorl.2017.12.006

Ellison, J. C., Gorga, M., Cohn, E., Fitzpatrick, D., Sanford, C. A., & Keefe, D. H. (2012). Wideband acoustic transfer functions predict middle-ear effusion. *Laryngoscope*, *122*(4), 887–894. https://doi.org/10.1002/lary.23182

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191. https://doi.org/10.3758/BF03193146

Feeney, M. P. (2013). Editorial: Eriksholm workshop on wideband absorbance measures of the middle ear. *Ear and Hearing*, *34 Suppl 1*, 1–2. https://doi.org/10.1097/AUD.0b013e31829c70f0

Feeney, M. P., Grant, I. L., & Marryott, L. P. (2003). Wideband energy reflectance measurements in adults with middle-ear disorders. *Journal of Speech, Language, and Hearing Research*, *46*(4), 901–911. https://doi.org/10.1044/1092-4388(2003/070)

Feeney, M. P., Grant, I. L., & Mills, D. M. (2009). Wideband energy reflectance measurements of ossicular chain discontinuity and repair in human temporal bone. *Ear and Hearing*, *30*(4), 391–400. https://doi.org/10.1097/AUD.0b013e3181a283ed

Feeney, M. P., & Keefe, D. H. (1999). Acoustic reflex detection using wide-band acoustic reflectance, admittance, and power measurements. *Journal of Speech, Language, and Hearing Research*, *42*(5), 1029–1041. https://doi.org/10.1044/jslhr.4205.1029

Feeney, M. P., Keefe, D. H., Hunter, L. L., Fitzpatrick, D. F., Garinis, A. C., Putterman, D. B., & McMillan, G. P. (2017). Normative wideband reflectance, equivalent admittance at the tympanic membrane, and acoustic stapedius reflex threshold in adults. *Ear and Hearing*, *38*(3), e142–e160. https://doi.org/10.1097/AUD.0000000000000399

Feeney, M. P., Keefe, D. H., & Sanford, C. A. (2004). Wideband reflectance measures of the ipsilateral acoustic stapedius reflex threshold. *Ear and Hearing*, *25*(5), 421–430.

https://doi.org/10.1097/01.aud.0000145110.60657.73

Feeney, M. P., & Sanford, C. A. (2004). Age effects in the human middle ear: Wideband acoustical measures. *The Journal of the Acoustical Society of America*, *116*(6), 3546–3558. https://doi.org/10.1121/1.1808221

Feeney, M. P., Stover, B., Keefe, D. H., Garinis, A. C., Day, J. E., & Seixas, N. (2014). Sources of variability in wideband energy reflectance measurements in adults. *Journal of the American Academy of Audiology*, *25*(5), 449–461. https://doi.org/10.3766/jaaa.25.5.4

Feeney, P. M., Hunter, L. L., Kei, J., Lilly, D. J., Margolis, R. H., Nakajima, H. H., Neely, S. T., Prieve, B. A., Rosowski, J. J., Sanford, C. A., Schairer, K. S., Shahnaz, N., Stenfelt, S., & Voss, S. E. (2013). Consensus statement: Eriksholm workshop on wideband absorbance measures of the middle ear. *Ear and Hearing*, *34*(SUPPL. 1), 78s-79s. https://doi.org/10.1097/AUD.0b013e31829c726b

Feise, R. J. (2002). Do multiple outcome measures require p-value adjustment? *BMC Medical Research Methodology*, *2*(1), 1–4. https://doi.org/10.1186/1471-2288-2-8

Feldman, A. S. (1977). Diagnostic application and interpretation of tympanometry and the acoustic reflex. *International Journal of Audiology*, *16*(4), 294–306. https://doi.org/10.3109/00206097709071841

Franco-Vidal, V., Legarlantezec, C., Blanchet, H., Convert, C., Torti, F., & Darrouzet, V. (2005). Multifrequency admittancemetry in Ménière's disease: A preliminary study for a new diagnostic test. *Otology and Neurotology*, *26*(4), 723–727. https://doi.org/10.1097/01.mao.0000178136.81729.7c

Gaihede, M. (1996). Tympanometric preconditioning of the tympanic membrane. *Hearing Research*, *102*(1–2), 28–34. https://doi.org/10.1016/S0378-5955(96)00146-3

Gaihede, M., & Ovesen, T. (1997). Precision of tympanometric measurements. *Journal of Speech, Language, and Hearing Research*, *40*(1), 215–222. https://doi.org/10.1044/jslhr.4001.215

Gan, R. Z., Dai, C., & Wood, M. W. (2006). Laser interferometry measurements of middle ear fluid and pressure effects on sound transmission. *The Journal of the Acoustical Society of America*, *120*(6), 3799–3810. https://doi.org/10.1121/1.2372454

Ghasemi, A., & Zahediasl, S. (2012). Normality tests for statistical analysis: A guide for non-statisticians. *International Journal of Endocrinology and Metabolism*, *10*(2), 486–489. https://doi.org/10.5812/ijem.3505

Greenhouse, S. W., & Geisser, S. (1959). On methods in the analysis of profile data. *Psychometrika*, *24*(2), 95–112. https://doi.org/10.1007/BF02289823

Hein, T. A. D., Hatzopoulos, S., Skarzynski, P. H., & Colella-Santos, M. F. (2017). Wideband Tympanometry. In *Advances in Clinical Audiology*. InTech. https://doi.org/10.5772/67155

Holte, L. (1996). Aging effects in multifrequency tympanometry. *Ear and Hearing*, *17*(1), 12–18. https://doi.org/10.1097/00003446-199602000-00002

Hougaard, D. D., Lyhne, N. M., Skals, R. K., & Kristensen, M. (2020). Study on wideband tympanometry and absorbance within a Danish cohort of normal hearing adults. *European Archives of Oto-Rhino-Laryngology*, *277*(7), 1899–1905. https://doi.org/10.1007/s00405-020-05909-9

Howarth, A., & Shone, G. R. (2006). Ageing and the auditory system. *Postgraduate Medical Journal*, *82*(965), 166–171. https://doi.org/10.1136/pgmj.2005.039388

Huang, G. T., Rosowski, J. J., & Peake, W. T. (2000). Relating middle-ear acoustic performance to body size in the cat family: Measurements and models. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, *186*(5), 447–465. https://doi.org/10.1007/s003590050444

Hunter, L. L., Bagger-Sjöbäck, D., & Lundberg, M. (2008). Wideband reflectance associated with otitis media in infants and children with cleft palate. *International Journal of Audiology*, *47*(SUPPL. 1), S57–S61. https://doi.org/10.1080/14992020802294057

Hunter, L. L., Feeney, M. P., Lapsley Miller, J. A., Jeng, P. S., & Bohning, S. (2010). Wideband reflectance in newborns: Normative regions and relationship to hearing-screening results. *Ear and Hearing*, *31*(5), 599–610. https://doi.org/10.1097/AUD.0b013e3181e40ca7

Hunter, L. L., Keefe, D. H., Feeney, M. P., Brown, D. K., Meinzen-Derr, J., Elsayed, A. M., Amann, J. M., Manickam, V., Fitzpatrick, D., & Shott, S. R. (2017). Wideband acoustic immittance in children with Down syndrome: Prediction of middle-ear dysfunction, conductive hearing loss and patent PE tubes. *International Journal of Audiology*, *56*(9), 622–634. https://doi.org/10.1080/14992027.2017.1314557

Hunter, L. L., Keefe, D. H., Feeney, M. P., Fitzpatrick, D. F., & Lin, L. (2016). Longitudinal development of wideband reflectance tympanometry in normal and at-risk infants. *Hearing Research*, *340*, 3–14. https://doi.org/10.1016/j.heares.2015.12.014

Hunter, L. L., Prieve, B. A., Kei, J., & Sanford, C. A. (2013). Pediatric applications of wideband acoustic immittance measures. *Ear and Hearing*, *34*(SUPPL. 1), 36–42. https://doi.org/10.1097/AUD.0b013e31829d5158

Hunter, L. L., & Sanford, C. A. (2014). Tympanometry and wideband acoustic immittance. In J. Katz, M. Chasin, K. English, L. J. Hood, & K. L. Tillery (Eds.), *Handbook of Clinical Audiology* (7th ed., pp. 137–163). Philadelphia, PA: Wolters

Kluver Health.

Hunter, L. L., & Shanaz, N. (2014). *Acoustic immittance measures: Basic and advanced practice*. Plural Publishing, Incorporated.

Hunter, L. L., Tubaugh, L., Jackson, A., & Propes, S. (2008). Wideband middle ear power measurement in infants and children. *Journal of the American Academy of Audiology, 19*(4), 309–324. https://doi.org/10.3766/jaaa.19.4.4

Iacovou, E., Vlastarakos, P. V., Ferekidis, E., & Nikolopoulos, T. P. (2013). Multi-Frequency Tympanometry: Clinical Applications for the Assessment of the Middle Ear Status. In *Indian Journal of Otolaryngology and Head and Neck Surgery* (Vol. 65, Issue 3, pp. 283–287). https://doi.org/10.1007/s12070-011-0470-9

Ibraheem, W. (2014). Clinical diagnosis of middle ear disorders using wideband energy reflectance in adults. *Advanced Arab Academy of Audio-Vestibulogy Journal*, *1*(2), 87. https://doi.org/10.4103/2314-8667.149017

Interacoustics. (2016). *Titan: Technical Specifications*. https://www.interacoustics.com/support/titan/288-technical-specification-titan.

Interacoustics. (2020). *Instructions for use - US Titan.* https://www.interacoustics.com/download/titan/titan-manuals/278-instructions-for-use-titan-us.

Jaffer, S. (2016). *Wideband acoustic immittance: Instrument, ethnicity, and gender specific normative data* (Issue April) [Vancouver: University of British Columbia]. https://doi.org/10.14288/1.0300134

Jayesh, R. K., Dilip, G. V, Vidya, S. K., Urvik, K. C., Pratik, T. N., Mital, P. M., Tulsibhai, S. C., Professor, A., & Year Resident, F. (2014). Osteometric Dimension of Stapes. *Journal of Research in Medical and Dental Science*, *2*(2), 30–33. https://doi.org/10.5455/jrmds.2014227

Jeng, P. S., Allen, J. B., Miller, J. A. L., & Levitt, H. (2008). Wideband power reflectance and power transmittance as tools for assessing middle-ear function. *Perspectives on Hearing and Hearing Disorders in Childhood*, *18*(2), 44–57. https://doi.org/10.1044/hhdc18.2.44

Jerger, S., & Jerger, J. (1977). Diagnostic value of crossed vs uncrossed acoustic reflexes: Eighth nerve and brain stem disorders. *Archives of Otolaryngology*, *103*(8), 445–453. https://doi.org/10.1001/archotol.1977.00780250039002

Kaf, W. A. (2011). Wideband energy reflectance findings in presence of normal tympanogram in children with Down's syndrome. *International Journal of Pediatric Otorhinolaryngology*, *75*(2), 219–226. https://doi.org/10.1016/j.ijporl.2010.11.004

Karuppannan, A., & Barman, A. (2020). Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity. *Auris Nasus Larynx*. https://doi.org/10.1016/j.anl.2020.10.019

Karuppannan, A., & Barman, A. (2021a). Wideband absorbance tympanometry: a novel method in identifying otosclerosis. *European Archives of Oto-Rhino-Laryngology*. https://doi.org/10.1007/s00405-020-06571-x

Karuppannan, A., & Barman, A. (2021b). Evaluation of wideband absorbance tympanometry in adults with abnormal positive and negative middle ear pressure. *Journal of Hearing Science*, *10*(4), 40–47. https://doi.org/10.17430/jhs.2020.10.4.5

Karzon, R. G. (1991). Validity and reliability of tympanometric measures for pediatric patients. *Journal of Speech and Hearing Research*, *34*(2), 386–390. https://doi.org/10.1044/jshr.3402.386

Kaya, Ş., Çiçek Çınar, B., Özbal Batuk, M., Özgen, B., Sennaroğlu, G., Genç, G. A., & Sennaroğlu, L. (2019). Wideband tympanometry findings in inner ear malformations. *Auris Nasus Larynx*, *S0385-8146*(19), 30627–3. https://doi.org/10.1016/j.anl.2019.09.001

Keefe, D. H., Archer, K. L., Schmid, K. K., Fitzpatrick, D. F., Feeney, M. P., & Hunter, L. L. (2017). Identifying otosclerosis with aural acoustical tests of absorbance, group delay, acoustic reflex threshold, and otoacoustic emissions. *Journal of the American Academy of Audiology*, *28*(9), 838–860. https://doi.org/10.3766/jaaa.16172

Keefe, D. H., Bulen, J. C., Arehart, K. H., & Burns, E. M. (1993). Ear-canal impedance and reflection coefficient in human infants and adults. *Journal of the Acoustical Society of America*, *94*(5), 2617–2638. https://doi.org/10.1121/1.407347

Keefe, D. H., & Feeney, M. P. (2009). Principles of acoustic immittance and acoustic transfer functions. In et al. Katz, J., Medwetsky, L., Burkard, R. (Ed.), *Handbook of Clinical Audiology.* (7th ed., pp. 125–156). Lippincott, Williams & Wilkins.

Keefe, D. H., Folsom, R. C., Gorga, M. P., Vohr, B. R., Bulen, J. C., & Norton, S. J. (2000). Identification of neonatal hearing impairment: Ear-canal measurements of acoustic admittance and reflectance in neonates. *Ear and Hearing*, *21*(5), 443–461. https://doi.org/10.1097/00003446-200010000-00009

Keefe, D. H., Hunter, L. L., Feeney, M. P., & Fitzpatrick, D. F. (2015). Procedures for ambient-pressure and tympanometric tests of aural acoustic reflectance and admittance in human infants and adults. *The Journal of the Acoustical Society of America*, *138*(6), 3625–3653. https://doi.org/10.1121/1.4936946

Keefe, D. H., & Levi, E. (1996). Maturation of the middle and external ears: Acoustic power-based responses and reflectance tympanometry. *Ear and Hearing*, *17*(5), 361–373. https://doi.org/10.1097/00003446-199610000-00002

Keefe, D. H., Sanford, C. A., Ellison, J. C., Fitzpatrick, D. F., & Gorga, M. P. (2012). Wideband aural acoustic absorbance predicts conductive hearing loss in children. *International Journal of Audiology*, *51*(12), 880–891. https://doi.org/10.3109/14992027.2012.721936

Keefe, D. H., & Simmons, J. L. (2003). Energy transmittance predicts conductive hearing loss in older children and adults. *The Journal of the Acoustical Society of America*, *114*(6), 3217–3238. https://doi.org/10.1121/1.1625931

Kelava, I., Ries, M., Valent, A., Ajduk, J., Trotić, R., Košec, A., & Bedeković, V. (2020). The usefulness of wideband absorbance in the diagnosis of otosclerosis. *International Journal of Audiology*, *59*(11), 859–865. https://doi.org/10.1080/14992027.2020.1785644

Kenny, S. P. V. N. (2011). *Clinical application of the Interacoustics REFLWIN system wideband reflectance machine in the assessment of the Eustachian tube* [University of British Columbia]. https://doi.org/10.14288/1.0072303

Kenny, S. P. V. N. (2013). Clinical Application of the Interacoustics Reflwin System Wideband Energy Reflectance Machine in the Assessment of the Eustachian Tube. *Journal of Chemical Information and Modeling*, *53*(9), 1689–1699. https://doi.org/10.1017/CBO9781107415324.004

Kim, J., & Koo, M. (2015). Mass and stiffness impact on the middle ear and the cochlear partition. *Korean Journal of Audiology*, *19*(1), 1–6. https://doi.org/10.7874/jao.2015.19.1.1

Kim, S. Y., Han, J. J., Oh, S. H., Lee, J. H., Suh, M. whan, Kim, M. H., & Park, M. K. (2019). Differentiating among conductive hearing loss conditions with wideband tympanometry. *Auris Nasus Larynx*, *46*(1), 43–49. https://doi.org/10.1016/j.anl.2018.05.013

Koo, T. K., & Li, M. Y. (2016). A guideline of selecting and reporting Intraclass Correlation Coefficients for reliability research. *Journal of Chiropractic Medicine*, *15*(2), 155–163. https://doi.org/10.1016/j.jcm.2016.02.012

Kumar, R., & Indrayan, A. (2011). Receiver operating characteristic (ROC) curve for medical researchers. *Indian Pediatrics*, *48*(4), 277–287. https://doi.org/10.1007/s13312-011-0055-4

Lantz, J., Petrak, M., & Prigge, L. (2004). Using the 1000-Hz probe tone to measure immittance in infants. *Hearing Journal*, *57*(10), 34–42. https://doi.org/10.1097/00025572-200410000-00006

Li, S. N., Shen, M., Chen, X. P., Shen, J. L., & Yang, J. (2019). [Acoustic absorbance characteristics of wideband tympanometry in patients with Meniere's disease]. *Journal of Clinical Otorhinolaryngology, Head, and Neck Surgery*, *33*(3), 224–227. https://doi.org/10.13201/j.issn.1001-1781.2019.03.010

Lilly, D. J. (1984). Multiple Frequency, Multiple Component Tympanometry. *Ear and Hearing*, *5*(5), 300–308. https://doi.org/10.1097/00003446-198409000-00007

Lilly, D. J., & Margolis, R. H. (2013). Wideband acoustic immittance measurements of the middle ear: Introduction and some historical antecedents. *Ear and Hearing*, *34*(SUPPL. 1), 4s-8s. https://doi.org/10.1097/AUD.0b013e31829db80f

Lin, F. R., Maas, P., Chien, W., Carey, J. P., Ferrucci, L., & Thorpe, R. (2012). Association of skin color, race/ethnicity, and hearing loss among adults in the USA. *JARO - Journal of the Association for Research in Otolaryngology*, *13*(1), 109–117. https://doi.org/10.1007/s10162-011-0298-8

Lin, F. R., Thorpe, R., Gordon-Salant, S., & Ferrucci, L. (2011). Hearing loss prevalence and risk factors among older adults in the United States. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *66 A*(5), 582–590. https://doi.org/10.1093/gerona/glr002

Liu, Y. W., Sanford, C. A., Ellison, J. C., Fitzpatrick, D. F., Gorga, M. P., & Keefe, D. H. (2008). Wideband absorbance tympanometry using pressure sweeps: System development and results on adults with normal hearing. *The Journal of the Acoustical Society of America*, *124*(6), 3708–3719. https://doi.org/10.1121/1.3001712

Lynch, T. J., Nedzelnitsky, V., & Peake, W. T. (1982). Input impedance of the cochlea in cata). *Journal of the Acoustical Society of America*, *72*(1), 108–130. https://doi.org/10.1121/1.387995

Margolis, R. H., & Goycoolea, H. G. (1993). Multifrequency tympanometry in normal adults. *Ear and Hearing*, *14*(6), 408–413. https://doi.org/10.1097/00003446-199312000-00006

Margolis, R. H., Paul, S., Saly, G. L., Schachern, P. A., & Keefe, D. H. (2001). Wideband reflectance tympanometry in chinchillas and humans. *The Journal of the Acoustical Society of America*, *110*(3), 1453–1464. https://doi.org/10.1121/1.1394219

Margolis, R. H., Saly, G. L., & Keefe, D. H. (1999). Wideband reflectance tympanometry in normal adults. *The Journal of the Acoustical Society of America*, *106*(1), 265–280. https://doi.org/10.1121/1.427055

Maroonroge, S., Emanuel, D. C., & Letowski, T. R. (2009). Basic anatomy of the hearing system. In E. T. Rash, Clarence E. Russo, Michael B. Letowski, Tomasz R. Schmeisser (Ed.), *Helmet-Mounted Displays: Sensation, Perception and Cognition Issues.* (1st ed.). U.S. Army Aeromedical Research Laboratory.

Martin, F. N., & Clark, J. G. (2019). *Introduction to audiology* (13th ed.). NY: Pearson.

Martin, L. (2019). Multifrequency tympanometry. In *ENT and Audiology News* (Vol. 28, Issue 1). https://www.entandaudiologynews.com/features/audiology-features/post/multifrequency-tympanometry

Mazlan, R., Kei, J., Ya, C. L., Yusof, W. N. H. M., Saim, L., & Zhao, F. (2015). Age and gender effects on wideband absorbance in Adults with normal outer and middle ear function. *Journal of Speech, Language, and Hearing Research*, *58*(4), 1377–1386. https://doi.org/10.1044/2015_JSLHR-H-14-0199

McPherson, B., Li, S. F., Shi, B. X., Tang, J. L. F., & Wong, B. Y. K. (2006). Neonatal hearing screening: Evaluation of tone-burst and click-evoked otoacoustic emission test criteria. *Ear and Hearing*, *27*(3), 256–262. https://doi.org/10.1097/01.aud.0000215971.18998.9d

Merchant, G. R., Horton, N. J., & Voss, S. E. (2010). Normative reflectance and transmittance measurements on healthy newborn and 1-month-old infants. *Ear and Hearing*, *31*(6), 746–754. https://doi.org/10.1097/AUD.0b013e3181e68e68

Mimosa Acoustics. (2002). *Power Flow Measurement System User's Manual.* http://www.mimosaacoustics.com

Mimosa Acoustics. (2014). *Middle-ear power analyzer measurement: User manual for HearlD 5.1*.

Mirza, S., & Gokhale, S. (2017). Pathophysiology of Meniere's Disease. In *Up to Date on Meniere's Disease*. InTech Open. https://doi.org/10.5772/66388

Mishra, S. K., Dinger, Z., & Renken, L. (2017). Maturation of middle ear transmission in children. *Hearing Research*, *344*, 62–67. https://doi.org/10.1016/j.heares.2016.10.029

Møller, A. R. (2014). *Hearing: Anatomy, Physiology, and Disorders of the Auditory System* (3rd ed.). San Diego, Calif: Plural. https://doi.org/10.3109/14992027.2014.996828

Muchnik, C., Hildesheimer, M., Rubinstein, M., & Gleitman, Y. (1989). Validity of tympanometry in cases of confirmed otosclerosis. *The Journal of Laryngology & Otology*, *103*(1), 36–38. https://doi.org/10.1017/S0022215100107972

Murakami, S., Gyo, K., & Goode, R. L. (1997). Effect of middle ear pressure change on middle ear mechanics. *Acta Oto-Laryngologica*, *117*(3), 390–395. https://doi.org/10.3109/00016489709113411

Nakajima, H. H., Pisano, D. V., Roosli, C., Hamade, M. A., Merchant, G. R., Mahfoud, L., Halpin, C. F., Rosowski, J. J., & Merchant, S. N. (2012). Comparison of ear-canal reflectance and umbo velocity in patients with conductive hearing loss: A preliminary study. *Ear and Hearing*, *33*(1), 35–43. https://doi.org/10.1097/AUD.0b013e31822ccba0

Nakajima, H. H., Rosowski, J. J., Shahnaz, N., & Voss, S. E. (2013). Assessment of ear disorders using power reflectance. *Ear and Hearing*, *34*(SUPPL. 1), 48S-53S. https://doi.org/10.1097/AUD.0b013e31829c964d

Neely, S. T., Stenfelt, S., & Schairer, K. S. (2013). Alternative ear-canal measures related to absorbance. *Ear and Hearing*, *34*(SUPPL. 1), 72–77. https://doi.org/10.1097/AUD.0b013e31829c7229

Neto, L. C., Cibin, B. C., Mota, R., Carvallo, M., Gabriela, S., & Sanches, G. (2014). [Medidas de reflectância acústica na otosclerose: estudo de caso] Acoustic reflectance measurements in otosclerosis: Case study. *Audiol Commun Res*, *19*(4), 421. https://doi.org/10.1590/S2317-64312014000300001420

Niemczyk, E., Lachowska, M., Tataj, E., Kurczak, K., & Niemczyk, K. (2019). Wideband tympanometry and absorbance measurements in otosclerotic ears. *Laryngoscope*, *129*(10), E365–E376. https://doi.org/10.1002/lary.27747

Norrix, L. W., Burgan, B., Ramirez, N., & Velenovsky, D. S. (2013). Interaural multiple frequency tympanometry measures: Clinical utility for unilateral conductive hearing loss. *Journal of the American Academy of Audiology*, *24*(3), 231–240. https://doi.org/10.3766/jaaa.24.3.8

Olszewski, L., Wiktor Jedrzejczak, W., Sliwa, L., Fabijanska, A., Skarzynski, H., & Jedrzejczak, W. W. (2017). Use of wideband absorbance measurement to assess large vestibular aqueduct syndrome: A case study. *Journal of Hearing Science*, *7*(1), 52–55. https://doi.org/10.17430/904707

Onusko, E. (2004). Tympanometry. *American Family Physician*, *70*(9), 1713–1720. https://doi.org/10.1097/00003446-198409000-00003

Orlikoff, R. F., Schiavetti, N., Metz, D. E. (2015). *Evaluating Research in Communication Disorders* (7th ed.). Boston, MA: Allyn & Bacon/Pearson.

Perkins, N. J., & Schisterman, E. F. (2006). The inconsistency of “optimal” cutpoints obtained using two criteria based on the receiver operating characteristic curve. *American Journal of Epidemiology*, *163*(7), 670–675. https://doi.org/10.1093/aje/kwj063

Perneger, T. V. (1998). What’s wrong with Bonferroni adjustments. *British Medical Journal*, *316*(7139), 1236–1238. https://doi.org/10.1136/bmj.316.7139.1236

Pickles, J. (2012). *An Introduction to the Physiology of Hearing* (4th ed.). Bingley, 392 UK: Brill Academic Publishers.

Piskorski, P., Keefe, D. H., Simmons, J. L., & Gorga, M. P. (1999). Prediction of conductive hearing loss based on acoustic ear-canal response using a multivariate clinical decision theory. *The Journal of the Acoustical Society of America*, *105*(3), 1749–1764. https://doi.org/10.1121/1.426713

Polat, Z., Baş, B., Hayır, D., Bulut, E., & Ataş, A. (2015). Wideband tympanometry normative data for Turkish young adult population. *The Journal of International Advanced Otology*, *11*(2), 157–162. https://doi.org/10.5152/iao.2015.809

Pope, C., & Mays, N. (1995). Reaching the parts other methods cannot reach: An introduction to qualitative methods in health and health services research. *Bmj*, *311*(6996), 42. https://doi.org/10.1136/bmj.311.6996.42

Prieve, B. A., Werff, K. R. V., Preston, J. L., & Georgantas, L. (2013). Identification of conductive hearing loss in young infants using tympanometry and wideband reflectance. *Ear and Hearing*, *34*(2), 168–178. https://doi.org/10.1097/AUD.0b013e31826fe611

Puria, S., & Allen, J. B. (1998). Measurements and model of the cat middle ear: Evidence of tympanic membrane acoustic delay. *The Journal of the Acoustical Society of America*, *104*(6), 3463–3481. https://doi.org/10.1121/1.423930

Ravicz, M. E., Rosowski, J. J., & Merchant, S. N. (2004). Mechanisms of hearing loss resulting from middle-ear fluid. *Hearing Research*, *195*(1–2), 103–130. https://doi.org/10.1016/j.heares.2004.05.010

Richard, F. D., Bond, C. F., & Stokes-Zoota, J. J. (2003). One hundred years of social psychology quantitatively described. *Review of General Psychology*, *7*(4), 331–363. https://doi.org/10.1037/1089-2680.7.4.331

Riddler, R. J. (2017). *Effects of middle ear pressure compensation on evoked otoacoustic emissions and power absorbance in adults* [Vancouver: University of British Columbia]. https://doi.org/10.14288/1.0343220

Robinson, D. O., & Allen, D. V. (1984). Racial differences in tympanometric results. *Journal of Speech and Hearing Disorders*, *49*(2), 140–144. https://doi.org/10.1044/jshd.4902.140

Robinson, D. O., Allen, D. V., & Root, L. P. (1988). Infant tympanometry: Differential results by race. *Journal of Speech and Hearing Disorders*, *53*(3), 341–346. https://doi.org/10.1044/jshd.5303.341

Robinson, S. R., Thompson, S., & Allen, J. B. (2016). Effects of negative middle ear pressure on wideband acoustic immittance in normal-hearing adults. *Ear and Hearing*, *37*(4), 452–464. https://doi.org/10.1097/AUD.0000000000000280

Rosenfeld, R. M., Shin, J. J., Schwartz, S. R., Coggins, R., Gagnon, L., Hackell, J. M., Hoelting, D., Hunter, L. L., Kummer, A. W., Payne, S. C., Poe, D. S., Veling, M., Vila, P. M., Walsh, S. A., & Corrigan, M. D. (2016). Clinical Practice Guideline. *Otolaryngology - Head and Neck Surgery (United States)*, *154*(1_suppl), S1–S41. https://doi.org/10.1177/0194599815623467

Rosowski, J. J., Nakajima, H. H., Hamade, M. A., Mahfoud, L., Merchant, G. R., Halpin,

C. F., & Merchant, S. N. (2012). Ear-canal reflectance, umbo velocity, and tympanometry in normal-hearing adults. *Ear and Hearing*, *33*(1), 19–34. https://doi.org/10.1097/AUD.0b013e31822ccb76

Rosowski, J. J., Stenfelt, S., & Lilly, D. (2013). An overview of wideband immittance measurements techniques and terminology: You say absorbance, i say reflectance. *Ear and Hearing*, *34*(SUPPL. 1), 9–16. https://doi.org/10.1097/AUD.0b013e31829d5a14

Roup, C. M., Wiley, T. L., Safady, S. H., & Stoppenbach, D. T. (1998). Tympanometric screening norms for adults. *American Journal of Audiology*, *7*(2), 55–60. https://doi.org/10.1044/1059-0889(1998/014)

Ruah, C. B., Schachern, P. A., Zelterman, D., Paparella, M. M., & Yoon, T. H. (1991). ge-related morphologic changes in the human tympanic membrane: A light and electron microscopic study. *Archives of Otolaryngology--Head and Neck Surgery*, *117*(6), 627–634. https://doi.org/10.1001/archotol.1991.01870180063013

Rusticus, S. A., & Lovato, C. Y. (2014). Impact of sample size and variability on the power and type I error rates of equivalence tests: A simulation study. *Practical Assessment, Research and Evaluation*, *19*(11), 1–10. https://doi.org/10.7275/4s9m-4e81

Salkind, N. (2012). Nonparametric Statistics for the Behavioral Sciences. In *Encyclopedia of Research Design* (2nd ed.). McGraw-Hill. https://doi.org/10.4135/9781412961288.n273

Sanford, C. A., & Brockett, J. E. (2014). Characteristics of wideband acoustic immittance in patients with middle-ear dysfunction. *Journal of the American Academy of Audiology*, *25*(5), 425–440. https://doi.org/10.3766/jaaa.25.5.2

Sanford, C. A., & Feeney, M. P. (2008). Effects of maturation on tympanometric wideband acoustic transfer functions in human infants. *The Journal of the Acoustical Society of America*, *124*(4), 2106–2122. https://doi.org/10.1121/1.2967864

Sanford, C. A., Hunter, L. L., Patrick Feeney, M., & Nakajima, H. H. (2013). Wideband acoustic immittance: Tympanometric measures. *Ear and Hearing*, *34*(SUPPL. 1), 65–71. https://doi.org/10.1097/AUD.0b013e31829c7250

Sanford, C. A., Keefe, D. H., Liu, Y. W., Fitzpatrick, D., McCreery, R. W., Lewis, D. E., & Gorga, M. P. (2009). Sound-conduction effects on distortion-product otoacoustic emission screening outcomes in newborn infants: Test performance of wideband acoustic transfer functions and 1-kHz tympanometry. *Ear and Hearing*, *30*(6), 635–652. https://doi.org/10.1097/AUD.0b013e3181b61cdc

Santos, P. P. dos, Araújo, E. S., Costa Filho, O. A., Piza, M. de T., & Alvarenga, K. de F. (2015). Wideband acoustic immittance measures using chirp and pure tone stimuli in infants with middle ear integrity. *Audiology - Communication Research*, *20*(4), 300–

304. https://doi.org/10.1590/2317-6431-2015-1602

Schlagintweit, M. (2018). *Inter-Aural Difference Norms for wideband absorbance (WBA): Potential for identifying Otosclerosis* [University of British Columbia]. https://doi.org/10.14288/1.0375802

Shahnaz, N. (2008). Wideband reflectance in neonatal intensive care units. *Journal of the American Academy of Audiology*, *19*(5), 419–429. https://doi.org/10.3766/jaaa.19.5.4

Shahnaz, N. (2010). Review: Clinical Application of WBR in infants, children and adults. *Canadian Hearing Report*, *5*(2), 23–29.

Shahnaz, N., & Bork, K. (2006). Wideband reflectance norms for Caucasian and Chinese young adults. *Ear and Hearing*, *27*(6), 774–788. https://doi.org/10.1097/01.aud.0000240568.00816.4a

Shahnaz, N., & Bork, K. (2008). Comparison of standard and multi-frequency tympanometric measures obtained with the virtual 310 system and the Grason-Stadler Tympstar. *Canadian Journal of Speech-Language Pathology and Audiology*, *32*(4), 146–157.

Shahnaz, N., Bork, K., Polka, L., Longridge, N., Bell, D., & Westerberg, B. D. (2009). Energy reflectance and tympanometry in normal and otosclerotic ears. *Ear and Hearing*, *30*(2), 219–233. https://doi.org/10.1097/AUD.0b013e3181976a14

Shahnaz, N., Cai, A., & Qi, L. (2014). Understanding the developmental course of the acoustic properties of the human outer and middle ear over the first 6 months of life by using a longitudinal analysis of power reflectance at ambient pressure. *Journal of the American Academy of Audiology*, *25*(5), 495–511. https://doi.org/10.3766/jaaa.25.5.8

Shahnaz, N., & Davies, D. (2006). Standard and multifrequency tympanometric norms for caucasian and chinese young adults. *Ear and Hearing*, *27*(1), 75–90. https://doi.org/10.1097/01.aud.0000194516.18632.d2

Shahnaz, N., Longridge, N., & Bell, D. (2009). Wideband energy reflectance patterns in preoperative and post-operative otosclerotic ears. *International Journal of Audiology*, *48*(5), 240–247. https://doi.org/10.1080/14992020802635317

Shahnaz, N., Patrick Feeney, M., & Schairer, K. S. (2013). Wideband acoustic immittance normative data: Ethnicity, gender, aging, and instrumentation. *Ear and Hearing*, *34*(SUPPL. 1), 27S-35S. https://doi.org/10.1097/AUD.0b013e31829d5328

Shahnaz, N., & Polka, L. (1997). Standard and multifrequency tympanometry in normal and otosclerotic ears. *Ear and Hearing*, *18*(4), 326–341. https://doi.org/10.1097/00003446-199708000-00007

Shahnaz, N., & Polka, L. (2002). Distinguishing healthy from otosclerotic ears: Effect of probe-tone frequency on static immittance. *Journal of the American Academy of Audiology*, *13*(7), 345–355.

Shanks, J., & Lilly, D. J. (1981). An evaluation of tympanometric estimates of ear canal volume. *Journal of Speech and Hearing Research*, *24*(4), 557–566. https://doi.org/10.1044/jshr.2404.557

Shaver, M. D. (2010). *Wideband energy reflectance measurements: Normative study and effects of negative and compensated middle ear pressures [Unpublished Doctoral thesis]*. Wichita State University.

Shaver, M. D., & Sun, X.-M. (2013). Wideband energy reflectance measurements: Effects of negative middle ear pressure and application of a pressure compensation procedure. *The Journal of the Acoustical Society of America*, *134*(1), 332–341. https://doi.org/10.1121/1.4807509

Shaw, J. (2009). Comparison of wideband energy reflectance and tympanometric measures obtained with Reflwin Interacoustics, Mimosa acoustics and GSI Tympstar systems. *Mimosa Publications*, *September,* 5953. https://doi.org/10.14288/1.0070857

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: Uses in assessing rater reliability. *Psychological Bulletin*, *86*(2), 420–428. https://doi.org/10.1037/0033-2909.86.2.420

Śliwa, L., Kochanek, K., Jedrzejczak, W. W., Mrugała, K., & Skarżyński, H. (2020). Measurement of wideband absorbance as a test for Otosclerosis. *Journal of Clinical Medicine*, *9*(6), 1908. https://doi.org/10.3390/jcm9061908

Soares, J. C., Urosas, J. G., Calarga, K. S., Pichelli, T. S., Limongi, S. C. O., Shahnaz, N., & Carvallo, R. M. M. (2016). Wideband reflectance in Down syndrome. *International Journal of Pediatric Otorhinolaryngology*, *87*, 164–171. https://doi.org/10.1016/j.ijporl.2016.06.022

Solano, F. (2014). Melanins: Skin pigments and much More—Types, structural models, biological functions, and formation routes. *New Journal of Science*, *2014*, 1–28. https://doi.org/10.1155/2014/498276

Stinson, M. R. (1990). Revision of estimates of acoustic energy reflectance at the human eardrum. *Journal of the Acoustical Society of America*, *88*(4), 1773–1778. https://doi.org/10.1121/1.400198

Sugasawa, K., Iwasaki, S., Fujimoto, C., Kinoshita, M., Inoue, A., Egami, N., Ushio, M., Chihara, Y., & Yamasoba, T. (2013). Diagnostic usefulness of multifrequency tympanometry for Ménière’s disease. *Audiology and Neurotology*, *18*(3), 152–160. https://doi.org/10.1159/000346343

Sun, D. Q., Zhou, X., Lin, F. R., Francis, H. W., Carey, J. P., & Chien, W. W. (2014). Racial difference in cochlear pigmentation is associated with hearing loss risk. *Otology and Neurotology*, *35*(9), 1509–1514. https://doi.org/10.1097/MAO.0000000000000564

Sun, X. M. (2016). Wideband acoustic immittance: Normative study and test–retest reliability of tympanometric measurements in adults. *Journal of Speech, Language, and Hearing Research*, *59*(4), 819–834. https://doi.org/10.1044/2016_JSLHR-H-14-0322

Sun, X. M., & Shaver, M. D. (2009). Effects of negative middle ear pressure on distortion product otoacoustic emissions and application of a compensation procedure in humans. *Ear and Hearing*, *30*(2), 191–202. https://doi.org/10.1097/AUD.0b013e31819769e1

Tanno, G. A. Y., Santos, M. A. de O., Sanches, M. T. D., Durante, A. S., Almeida, K. de, Gameiro, M. S., Roque, N. M. C. de F., & Sousa Neto, O. M. de. (2020). Analysis of wideband tympanometry in Ménière's disease. *Brazilian Journal of Otorhinolaryngology*. https://doi.org/10.1016/j.bjorl.2020.05.029

Terzi, S., Özgür, A., Erdivanli, Coşkun, Z., Ogurlu, M., Demirci, M., & Dursun, E. (2015). Diagnostic value of the wideband acoustic absorbance test in middle-ear effusion. *Journal of Laryngology and Otology*, *129*(11), 1078–1084. https://doi.org/10.1017/S0022215115002339

Terzi, Suat, Özgür, A., Coşkun, Z. Ö., Erdivanli, Ö. Ç., Çeliker, M., Demirci, M., & Dursun, E. (2017). Evaluation of the myringosclerotic tympanic membrane with wideband tympanometry. *Indian Journal of Otology*, *23*(2), 117–120. https://doi.org/10.4103/indianjotol.INDIANJOTOL_1_17

Vanaja, C. S. S., & Manjula, P. (2003). Middle ear resonant frquency in normal and otosclerotic ears: Effect of procedural variation. *Journal of Speech Language Pathology and Audiology*, *27*(3), 158–162.

Vander Werff, K. R., Prieve, B. A., & Georgantas, L. M. (2007). Test-retest reliability of wideband reflectance measures in infants under screening and diagnostic test conditions. *Ear and Hearing*, *28*(5), 669–681. https://doi.org/10.1097/AUD.0b013e31812f71b1

von Unge, M., Decraemer, W. F., Dirckx, J. J., & Bagger-Sjöbäck, D. (1995). Shape and displacement patterns of the gerbil tympanic membrane in experimental otitis media with effusion. *Hearing Research*, *82*(2), 184–196. https://doi.org/10.1016/0378-5955(94)00017-K

Voss, S. E., & Allen, J. B. (1994). Measurement of acoustic impedance and reflectance in the human ear canal. *Journal of the Acoustical Society of America*, *95*(1), 372–384. https://doi.org/10.1121/1.408329

Voss, S. E., Horton, N. J., Woodbury, R. R., & Sheffield, K. N. (2008). Sources of variability in reflectance measurements on normal cadaver ears. *Ear and Hearing*, *29*(4), 651–665. https://doi.org/10.1097/AUD.0b013e318174f07c

Voss, S. E., Merchant, G. R., & Horton, N. J. (2012). Effects of middle-ear disorders on power reflectance measured in cadaveric ear canals. *Ear and Hearing*, *33*(2), 195–208. https://doi.org/10.1097/AUD.0b013e31823235b5

Voss, S. E., Rosowski, J. J., Merchant, S. N., & Peake, W. T. (2001a). How do tympanic-membrane perforations affect human middle-ear sound transmission? *Acta Oto-Laryngologica*, *121*(2), 169–173. https://doi.org/10.1080/000164801300043343

Voss, S. E., Rosowski, J. J., Merchant, S. N., & Peake, W. T. (2001b). Middle-ear function with tympanic-membrane perforations. I. Measurements and mechanisms. *The Journal of the Acoustical Society of America*, *110*(3), 1432–1444. https://doi.org/10.1121/1.1394195

Voss, S. E., Rosowski, J. J., Merchant, S. N., & Peake, W. T. (2001c). Middle-ear function with tympanic-membrane perforations. II. A simple model. *The Journal of the Acoustical Society of America*, *110*(3), 1445–1452. https://doi.org/10.1121/1.1394196

Voss, S. E., Stenfelt, S., Neely, S. T., & Rosowski, J. J. (2013). Factors that introduce intrasubject variability into ear-canal absorbance measurements. *Ear and Hearing*, *34*(SUPPL. 1), 60–64. https://doi.org/10.1097/AUD.0b013e31829cfd64

Wali, H. A., & Mazlan, R. (2018). The effect of ethnicity on wideband absorbance of neonates with healthy middle ear functions in Malaysia: A preliminary study. *Journal of Audiology and Otology*, *22*(1), 20–27. https://doi.org/10.7874/jao.2017.00227

Wan, I. K. K., & Wong, L. L. N. (2002). Tympanometric norms for Chinese young adults. *Ear and Hearing*, *23*(5), 416–421. https://doi.org/10.1097/00003446-200210000-00003

Wang, S., Hao, W., Xu, C., Ni, D., Gao, Z., & Shang, Y. (2019). A Study of wideband energy reflectance in patients with Otosclerosis: Data from a Chinese population. *BioMed Research International*, *2019*, 1–8. https://doi.org/10.1155/2019/2070548

Wei De, K. C. (2020). A Preliminary analysis of tympanometric parameters in a local multiethnic population. *Audiology Research*, *10*(2), 77–82. https://doi.org/10.3390/audiolres10020013

Werner, L. A., Levi, E. C., & Keefe, D. H. (2010). Ear-canal wideband acoustic transfer functions of adults and two- to nine-month-old infants. *Ear and Hearing*, *31*(5), 587–598. https://doi.org/10.1097/AUD.0b013e3181e0381d

Werner, Y. L., & Igić, P. G. (2002). The middle ear of gekkonoid lizards: Interspecific variation of structure in relation to body size and to auditory sensitivity. *Hearing Research*, *167*(1–2), 33–45. https://doi.org/10.1016/S0378-5955(02)00331-3

Werner, Y. L., Montgomery, L. G., Safford, S. D., Igic, P. G., & Saunders, J. C. (1998). How body size affects middle-ear structure and function and auditory sensitivity in gekkonoid lizards. *Journal of Experimental Biology*, *201*(4), 487–502.

Whitehead, M. L., Kamal, N., Lonsbury-Martin, B. L., & Martin, G. K. (1993). Spontaneous otoacoustic emissions in different racial groups. *Scandinavian Audiology*, *22*(1), 3–10. https://doi.org/10.3109/01050399309046012

Wiley, T. L., Cruickshanks, K. J., Nondahl, D. M., Tweed, T. S., Klein, R., & Klein, B. E. (1996). Tympanometric measures in older adults. *Journal of the American Academy of Audiology*, *7*(4), 260–268.

Yathiraj, A., & Vijayalakshmi, C. (2005). *Phonemically balanced word list in Kannada. Developed in Department of Audiology*. All India Institute of Speech and Hearing, Mysuru.

Youden, W. J. (1950). Index for rating diagnostic tests. *Cancer*, *3*(1), 32–35. https://doi.org/10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3

ANNEXURES

Annexure 3.1

Ethical Committees' approval copy for carrying out research

All India Institute of Speech and Hearing

(An autonomous Institute under the
Ministry of Health and Family Welfare, Govt. of India)
Manasagangothri, Mysore - 570 006.

ಅಖಿಲ ಭಾರತ ವಾಕ್ ಶ್ರವಣ ಸಂಸ್ಥೆ
ಮಾನಸಗಂಗೋತ್ರಿ, ಮೈಸೂರು – 570 006.

अखिल भारतीय वाक् श्रवण संस्थान
मानसगंगोत्री, मैसूर - 570 006

ETHICS COMMITTEE APPROVAL FOR BIO-BEHAVIORAL RESEARCH PROJECTS INVOLVING HUMAN SUBJECTS AT AIISH

AIISH ETHICS COMMITTEE (AEC)

Title of the Project	: Clinical Validation of Wideband Absorbance Tympanometry in Detecting Middle Ear Disorders
Candidate	: Arunraj K.
Guide	: Dr. Animesh Barman
Proposed Duration of the Ph.D Program	: 3 years
Estimated Budget Requirements	: Not applicable
Source of Funding	: Not applicable

Reference number of the proposal	: WOF-0404/2014-15 with effect from 04.06.14
Date on which AEC meeting was held	: 12.11.2015
Clear statement of decision reached at AEC meeting (in the event of a proposal being not approved, a statement of reasons for the same must be indicated)	: **Approved**
Advice & Suggestions (If any)	: Nil

Shyamala K.C

Date: 12.11.2015

Signature & Name of Member Secretary
Dr. Shyamala K.C
Prof. of Language Pathology
Dept. of Speech-Language Pathology
All India Institute of Speech and Hearing, Mysore

Annexure 3.2

A sample copy of the informed consent from a participant

PhD Title: "Clinical validation of wide band absorbance tympanometry in detecting middle ear disorders"

Name of the Participant: [illegible]	Mother tongue: Kannada
Age/Gender: 46/F	Date of Birth: [illegible]
Address: Mysore	Contact Number: [illegible] Email:
Weight: Height:	

ABOUT THE STUDY

The purpose of the study is to obtain the norms for wideband absorbance in adults having normal middle ear and clinically validate the results with the disordered group. This study includes the basic audiological test procedures (Puretone audiometry, Immitance & OAE) and wide band absorbance tympanometry which is completely a non-invasive test procedure. It approximately takes one hour to complete the entire test procedure for the study. It will not have any anticipatory risk for your participation. Your participation is purely voluntary and any information that is obtained in connection with the study will be retained confidential.

CONSENT FORM

I have been informed about the aims, objectives and the procedure of the study. The possible risks-benefits of my participation as human subjects in the study are clearly understood by me. I understand that I have a right to refuse participation as a subject or withdraw my consent at any time without adversely affecting my/my ward's treatment at the institute. I am also aware that by subjecting to this investigation, I will have to give more time for assessments by the investigating team and that these assessments may not result in any benefits to me. I also understood that, I have the freedom to write to Chairman, AIISH Ethic Committee (AEC), in case of any violation of these provisions without the danger of my being denied any rights to secure the clinical services at this institute.

I, [illegible], the undersigned, give my consent to be a participant of this research.

[illegible]

Signature of the participant with date

[illegible]

Name & Signature of the Researcher/ witness with date

Case No: [illegible]	Data Log No.
Date of evaluation: 24/7/19	

Annexure 4. 1

Wilcoxon signed-rank test results of WBA obtained between ears (right, left) at peak and ambient pressure with effect size in Normal ear [n=325] group

Frequency (Hz)	**Peak Pressure**			**Ambient pressure**		
	Z	***p***	**r**	**Z**	***p***	**r**
250	-6.384	<0.001*	0.2	-5.645	<0.001*	0.2
300	-6.100	<0.001*	0.2	-4.056	<0.001*	0.2
400	-4.598	<0.001*	0.2	-3.596	<0.001*	0.1
500	-4.313	<0.001*	0.2	-3.026	0.002	0.1
600	-3.405	<0.001*	0.1	-3.161	0.002	0.1
800	-1.946	0.052	0.1	-1.788	0.074	0.1
1000	-1.496	0.135	0.1	-0.523	0.601	0.0
1250	-2.544	0.011	0.1	-1.049	0.294	0.0
1500	-3.039	0.002	0.1	-1.960	0.050	0.1
2000	-0.186	0.852	0.0	-0.380	0.704	0.0
2500	-2.509	0.012	0.1	-2.042	0.041	0.1
3000	-2.312	0.021	0.1	-2.490	0.013	0.1
4000	-7.381	<0.001*	0.2	-7.473	<0.001*	0.2
5000	-2.600	0.009	0.1	-2.383	0.017	0.1
6000	-6.030	<0.001*	0.2	-5.170	<0.001*	0.2
8000	-3.286	<0.001*	0.1	-3.005	0.003	0.1
WBT_{avg}	-6.384	<0.001*	0.0	-5.645	<0.001*	0.1

Note. *Corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for $WBT_{avg,}$ and also the actual *p*-value was not depicted as the initial three values after decimals were zero.

Annexure 4. 2

Mann-Whitney U test results of WBA obtained between genders (male, female) with effect size at peak and ambient pressure in Normal ear [n=1127] group

Frequency (Hz)	**Peak pressure**				**Ambient pressure**			
	U	**Z**	***p***	**r**	**U**	**Z**	***p***	**r**
250	124105	-5.959	<0.01*	0.2	135760	-3.810	<0.01*	0.1
300	123336	-6.101	<0.01*	0.2	132071.5	-4.490	<0.01*	0.1
400	122964.5	-6.169	<0.01*	0.2	129842.5	-4.901	<0.01*	0.1
500	122155.5	-6.319	<0.01*	0.2	127621.5	-5.311	<0.01*	0.2
600	124763.5	-5.838	<0.01*	0.2	131414.5	-4.611	<0.01*	0.1
800	130412.5	-4.796	<0.01*	0.1	139389	-3.141	<0.01*	0.1
1000	143063.5	-2.463	<0.01*	0.1	147990.5	-1.554	<0.01*	0.1
1250	151349.5	-0.935	0.35	0.0	152715	-0.683	0.49	0.0
1500	153750.5	-0.492	0.62	0.0	151716.5	-0.867	0.39	0.0
2000	149717	-1.236	0.22	0.0	153305.5	-0.574	0.57	0.0
2500	132811	-4.354	<0.01*	0.1	134730.5	-4.000	<0.01*	0.1
3000	133492.50	-4.228	<0.01*	0.1	134666	-4.012	<0.01*	0.1
4000	138806	-3.248	<0.01*	0.1	139236	-3.169	<0.01*	0.1
5000	145323	-2.046	0.40	0.1	145392	-2.034	0.40	0.1
6000	128597	-5.131	<0.01*	0.2	128688.5	-5.114	<0.01*	0.2
8000	112405	-8.117	<0.01*	0.2	110355	-8.495	<0.01*	0.2
WBT_{avg}	156155	-0.049	0.96	0.0	138294.50	-3.342	<0.01*	0.1

Note. *indicates significant different at $p<0.05$. The actual *p*-value was not depicted as the initial three values after decimals were zero.

Annexure 4. 3

Wilcoxon Signed rank test results of WBA obtained between pressure conditions (peak, ambient) with effect size in Normal ear [n=1127] group

Frequency (Hz)	**Z**	***p***	**r**
250	-18.993	<0.001*	0.6
300	-19.515	<0.001*	0.6
400	-19.61	<0.001*	0.6
500	-20.729	<0.001*	0.6
600	-20.012	<0.001*	0.6
800	-20.736	<0.001*	0.6
1000	-17.025	<0.001*	0.5
1250	-0.79	0.430	0.0
1500	-8.116	<0.001*	0.2
2000	-5.733	<0.001*	0.2
2500	-6.166	<0.001*	0.2
3000	-9.191	<0.001*	0.3
4000	-10.953	<0.001*	0.3
5000	-1.432	0.152	0.0
6000	-3.752	<0.001*	0.1
8000	-2.486	0.013	0.1
WBT_{avg}	-1.747	<0.001*	0.1

Note. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for WBT_{avg}, and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 4

Wilcoxon signed-rank test results of WBA obtained between peak and ambient pressure with effect size in Normal ear [n=150] group

Frequency (Hz)	Z	*p*	r
250	-7.251	<0.001*	0.6
300	-7.147	<0.001*	0.6
400	-7.296	<0.001*	0.6
500	-7.522	<0.001*	0.6
600	-7.333	<0.001*	0.6
800	-7.149	<0.001*	0.6
1000	-6.522	<0.001*	0.5
1250	-0.428	0.669	0.0
1500	-2.735	0.006	0.2
2000	-1.278	0.201	0.1
2500	-1.032	0.302	0.1
3000	-2.253	0.024	0.2
4000	-1.872	0.004	0.2
5000	-1.028	0.304	0.1
6000	-0.694	0.488	0.1
8000	-0.307	0.759	0.0
WBT_{avg}	-2.489	0.013	0.2

Note. *corrected required significant level is p<0.003 for WBA across frequencies and p<0.05 for $WBT_{avg,}$ and also the actual p-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 5

Mann-Whitney U test results of WBA obtained between Tympanic membrane perforation group and Normal ear [n=150] group with effect size at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure				Ambient pressure			
	U	Z	*p*	r	U	Z	*p*	r
250	2275	-9.913	<0.01*	0.6	2880	-8.896	<0.01*	0.6
300	3015.5	-8.669	<0.01*	0.5	3207.5	-8.346	<0.01*	0.5
400	4365	-6.401	<0.01*	0.4	4032	-6.961	<0.01*	0.4
500	5001	-5.333	<0.01*	0.3	4632.5	-5.952	<0.01*	0.4
600	5553	-4.405	<0.01*	0.3	5218	-4.968	<0.01*	0.3
800	4789.5	-5.688	<0.01*	0.4	4635.5	-5.947	<0.01*	0.4
1000	2616.5	-9.339	<0.01*	0.6	2511	-9.516	<0.01*	0.6
1250	1603	-11.042	<0.01*	0.7	1773.5	-10.756	<0.01*	0.7
1500	1138.5	-11.822	<0.01*	0.7	1132.5	-11.833	<0.01*	0.7
2000	1992.5	-10.388	<0.01*	0.6	2193.5	-10.05	<0.01*	0.6
2500	4391	-6.358	<0.01*	0.4	4738.5	-5.774	<0.01*	0.4
3000	7236	-1.578	0.12	0.1	7459.5	-1.202	0.23	0.1
4000	7621	-0.931	0.35	0.1	7689	-0.817	0.41	0.1
5000	5139	-5.101	<0.01*	0.3	5174.5	-5.041	<0.01*	0.3
6000	3423	-7.984	<0.01*	0.5	3543.5	-7.782	<0.01*	0.5
8000	7665.5	-0.856	0.39	0.1	7870	-0.512	0.61	0.0
WBT_{avg}	--	--	--	--	2188	-10.059	<0.01*	0.6

Note. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 6

Wilcoxon signed-rank test results of WBA obtained between peak and ambient pressure with effect size in Tympanic membrane perforation group

Frequency (Hz)	**Z**	***p***	**r**
250	-1.122	0.06	0.1
300	-4.15	<0.001*	0.4
400	-6.023	<0.001*	0.6
500	-6.155	<0.001*	0.6
600	-7.341	<0.001*	0.7
800	-5.669	<0.001*	0.5
1000	-4.383	<0.001*	0.4
1250	-0.581	0.561	0.1
1500	-0.244	0.817	0.0
2000	-4.28	<0.001*	0.4
2500	-4.709	<0.001*	0.5
3000	-0.965	0.334	0.1
4000	-0.963	0.345	0.1
5000	-0.739	0.460	0.1
6000	-1.375	0.179	0.1
8000	-1.259	0.218	0.1

Note. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for WBT_{avg}, and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 7

Mann-Whitney U test results of WBA obtained between Middle ear effusion group and Normal ear [n=150] group with effect size at peak and ambient pressure conditions

Frequency	Peak pressure				Ambient pressure			
(Hz)	U	Z	*p*	r	U	Z	*p*	r
250	2890	-9.702	<0.01*	0.6	3640	-8.54	<0.01*	0.5
300	2987.5	-9.551	<0.01*	0.6	3656.5	-8.514	<0.01*	0.5
400	2907	-9.676	<0.01*	0.6	3589	-8.619	<0.01*	0.5
500	2161	-10.832	<0.01*	0.7	2593.5	-10.162	<0.01*	0.6
600	686.5	-13.117	<0.01*	0.8	939	-12.726	<0.01*	0.8
800	12	-14.163	<0.01*	0.9	28	-14.138	<0.01*	0.9
1000	49	-14.105	<0.01*	0.9	74	-14.067	<0.01*	0.9
1250	42.5	-14.115	<0.01*	0.9	41	-14.118	<0.01*	0.9
1500	40	-14.119	<0.01*	0.9	33	-14.13	<0.01*	0.9
2000	22	-14.147	<0.01*	0.9	34.5	-14.128	<0.01*	0.9
2500	219	-13.842	<0.01*	0.8	268	-13.766	<0.01*	0.8
3000	731	-13.048	<0.01*	0.8	771	-12.986	<0.01*	0.8
4000	3318.5	-9.038	<0.01*	0.5	3119.5	-9.346	<0.01*	0.6
5000	5407	-5.801	<0.01*	0.4	4590	-7.067	<0.01*	0.4
6000	5995	-4.89	<0.01*	0.3	5171.5	-6.166	<0.01*	0.4
8000	8399	-1.164	0.24	0.1	8430	-1.116	0.26	0.1
WBT_{avg}	--	--	--	--	14	-14.16	<0.01*	0.9

Note. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 8

Wilcoxon signed-rank test results of WBA obtained between peak and ambient pressure with effect size in Middle ear effusion group

Frequency (Hz)	***Z***	***p***	***r***
250	-1.658	0.097	0.2
300	-1.827	0.068	0.2
400	-2.148	0.032	0.2
500	-3.02	0.003	0.3
600	-4.341	<0.001*	0.4
800	-5.639	<0.001*	0.5
1000	-4.556	<0.001*	0.4
1250	-2.061	0.039	0.2
1500	-0.732	0.464	0.1
2000	-2.427	0.015	0.2
2500	-2.007	0.045	0.2
3000	-0.513	0.608	0.0
4000	-2.263	0.024	0.2
5000	-2.213	0.027	0.2
6000	-2.932	0.003	0.3
8000	-1.775	0.076	0.2

Note. *corrected required significant level is $p<0.003$ for WBA across frequencies and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 9

Mann-Whitney U test results of WBA obtained between Otosclerosis group and Normal ear [n=150] group with effect size at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure				Ambient pressure			
	U	***Z***	***p***	***r***	***U***	***Z***	***p***	***r***
250	1145.0	-13.109	<0.01*	0.8	1325.0	-12.857	<0.01*	0.8
300	1092.5	-13.183	<0.01*	0.8	1135.5	-13.123	<0.01*	0.8
400	925.0	-13.418	<0.01*	0.8	832.0	-13.548	<0.01*	0.8
500	892.5	-13.463	<0.01*	0.8	787.0	-13.611	<0.01*	0.8
600	750.5	-13.662	<0.01*	0.8	635.5	-13.823	<0.01*	0.8
800	602.0	-13.87	<0.01*	0.8	403.0	-14.149	<0.01*	0.8
1000	377.5	-14.185	<0.01*	0.8	403.0	-14.149	<0.01*	0.8
1250	942.0	-13.394	<0.01*	0.8	691.5	-13.745	<0.01*	0.8
1500	4022.5	-9.077	<0.01*	0.5	3424.5	-9.915	<0.01*	0.6
2000	7567.0	-4.11	<0.01*	0.3	7531.5	-4.16	<0.01*	0.3
2500	10422.5	-0.109	0.91	0.0	10448.5	-0.072	0.94	0.0
3000	9362.0	-1.595	0.11	0.1	9479.0	-1.431	0.15	0.1
4000	9153.0	-1.888	0.06	0.1	9131.0	-1.918	0.06	0.1
5000	9853.0	-0.907	0.37	0.1	9988.5	-0.717	0.47	0.0
6000	10259	-0.338	0.74	0.0	10240.0	-0.364	0.72	0.0
8000	8613.5	-2.644	0.06	0.2	8631.0	-2.619	0.08	0.2
WBT_{avg}	8996.5	-2.108	0.03	0.1	618.5	-13.847	<0.01*	0.8

Note. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 10

Wilcoxon signed-rank test results of WBA obtained between peak and ambient pressure with effect size in Otosclerosis group

Frequency (Hz)	**Z**	***p***	**r**
250	-7.125	<0.001*	0.6
300	-7.121	<0.001*	0.6
400	-7.54	<0.001*	0.6
500	-7.81	<0.001*	0.7
600	-7.856	<0.001*	0.7
800	-7.929	<0.001*	0.7
1000	-8.073	<0.001*	0.7
1250	-7.116	<0.001*	0.6
1500	-3.7	<0.001*	0.3
2000	-1.328	0.184	0.1
2500	-0.549	0.583	0.0
3000	-0.242	0.809	0.0
4000	-4.662	0.000	0.4
5000	-1.401	0.161	0.1
6000	-2.162	0.031	0.2
8000	-2.395	0.017	0.2
WBT_{avg}	-6.626	<0.001*	0.6

Note. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for WBT_{avg}, and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 11

Mann-Whitney U test results of WBA obtained between Eustachian tube dysfunction group and Normal ear [n=150] group with effect size at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure				Ambient pressure			
	U	Z	*p*	r	U	Z	*p*	r
250	4732	-5.515	<0.01*	0.3	1488	-11.074	<0.01*	0.7
300	4941	-5.157	<0.01*	0.3	1457	-11.127	<0.01*	0.7
400	5375	-4.413	<0.01*	0.3	1191	-11.583	<0.01*	0.7
500	5118.5	-4.852	<0.01*	0.3	660	-12.493	<0.01*	0.8
600	4649.5	-5.656	<0.01*	0.4	335	-13.05	<0.01*	0.8
800	4624	-5.7	<0.01*	0.4	521	-12.731	<0.01*	0.8
1000	3949	-6.856	<0.01*	0.4	693.5	-12.435	<0.01*	0.8
1250	4329.5	-6.204	<0.01*	0.4	1175.5	-11.609	<0.01*	0.7
1500	4770.5	-5.449	<0.01*	0.3	1806	-10.529	<0.01*	0.7
2000	4752	-5.48	<0.01*	0.3	2512	-9.319	<0.01*	0.6
2500	4565	-5.801	<0.01*	0.4	3622	-7.417	<0.01*	0.5
3000	4365.5	-6.143	<0.01*	0.4	4039	-6.702	<0.01*	0.4
4000	7138	-1.392	0.16	0.1	6689.5	-2.16	0.06	0.1
5000	7167	-1.342	0.18	0.1	7507.5	-0.758	0.45	0.0
6000	7555.5	-0.676	0.50	0.0	7741.5	-0.357	0.72	0.0
8000	7355.5	-1.019	0.31	0.1	7487	-0.793	0.43	0.0
WBT_{avg}	--	--	--	--	693.5	-12.435	<0.01*	0.8

Note. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 12

Wilcoxon signed-rank test results of WBA obtained between peak and ambient pressure with effect size in Eustachian tube dysfunction group

Frequency (Hz)	**Z**	***p***	**r**
250	-8.334	<0.001*	0.8
300	-8.461	<0.001*	0.8
400	-8.652	<0.001*	0.8
500	-8.739	<0.001*	0.8
600	-8.743	<0.001*	0.8
800	-8.771	<0.001*	0.9
1000	-8.649	<0.001*	0.8
1250	-8.107	<0.001*	0.8
1500	-6.841	<0.001*	0.7
2000	-5.189	<0.001*	0.5
2500	-3.088	0.002	0.3
3000	-0.424	0.672	0.0
4000	-2.818	0.005	0.2
5000	-6.204	0.027	0.1
6000	-4.48	0.004	0.2
8000	-3.768	0.076	0.4

Note. *corrected required significant level is $p<0.003$ for WBA across frequencies and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 13

Mann-Whitney U test results of WBA obtained between Sensorineural hearing loss group and Normal ear [n=150] group with effect size at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure				Ambient pressure			
	U	Z	*p*	r	U	Z	*p*	r
250	6159	-6.083	<0.01*	0.4	6350	-5.815	<0.01*	0.3
300	6375.5	-5.78	<0.01*	0.3	6277.5	-5.917	<0.01*	0.3
400	6517	-5.581	<0.01*	0.3	6395	-5.752	<0.01*	0.3
500	6857	-5.105	<0.01*	0.3	6514.5	-5.585	<0.01*	0.3
600	6708	-5.314	<0.01*	0.3	6531	-5.562	<0.01*	0.3
800	5853.5	-6.511	<0.01*	0.4	6058.5	-6.224	<0.01*	0.4
1000	5348.5	-7.219	<0.01*	0.4	5651.5	-6.794	<0.01*	0.4
1250	7207	-4.615	<0.01*	0.3	6854	-5.109	<0.01*	0.3
1500	9882.5	-0.865	0.39	0.1	9674.5	-1.157	0.25	0.1
2000	10089	-0.576	0.57	0.0	9963	-0.753	0.45	0.0
2500	9562	-1.314	0.19	0.1	9516	-1.379	0.17	0.1
3000	8907	-2.232	0.03	0.1	8938.5	-2.188	0.03	0.1
4000	8093	-3.373	<0.01*	0.2	8090	-3.377	<0.01*	0.2
5000	8009	-3.491	<0.01*	0.2	8013.5	-3.484	<0.01*	0.2
6000	7862	-3.697	<0.01*	0.2	7945.5	-3.58	<0.01*	0.2
8000	9145	-1.899	0.06	0.1	9130.5	-1.919	0.06	0.1
WBT_{avg}	10152	-0.488	0.626	0.0	6448.5	-2.678	0.07	0.1

Note. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 14

Wilcoxon signed-rank test results of WBA obtained between peak and ambient pressure with effect size in Sensorineural hearing loss group

Frequency (Hz)	**Z**	***p***	**r**
250	-7.462	<0.001*	0.6
300	-7.339	<0.001*	0.6
400	-7.541	<0.001*	0.6
500	-7.66	<0.001*	0.6
600	-7.662	<0.001*	0.6
800	-7.724	<0.001*	0.7
1000	-6.083	<0.001*	0.5
1250	-2.282	0.023	0.2
1500	-1.738	0.082	0.1
2000	-2.522	0.012	0.2
2500	-0.812	0.417	0.1
3000	-1.268	0.205	0.1
4000	-2.27	0.004	0.2
5000	-1.346	0.178	0.1
6000	-0.412	0.680	0.0
8000	-0.936	0.349	0.1
WBT_{avg}	-1.455	0.146	0.1

Note. *corrected required significant level is $p<0.003$ for WBA across frequencies and $p<0.05$ for $WBT_{avg,}$ and also the actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 15

Kruskal-Wallis test results of WBA obtained at peak and ambient pressure conditions with effect size across the groups

Frequency (Hz)	Peak pressure			Ambient pressure		
	$\chi 2$ *(df=5)*	***p***	**r**	**$\chi 2$** *(df=5)*	***p***	**r**
250	256.067	<0.01*	0.6	265.163	<0.01*	0.6
300	250.453	<0.01*	0.6	266.868	<0.01*	0.6
400	251.719	<0.01*	0.6	277.342	<0.01*	0.6
500	270.737	<0.01*	0.6	312.568	<0.01*	0.6
600	315.166	<0.01*	0.6	368.493	<0.01*	0.7
800	380.424	<0.01*	0.7	436.842	<0.01*	0.8
1000	436.860	<0.01*	0.8	478.814	<0.01*	0.8
1250	438.540	<0.01*	0.8	474.813	<0.01*	0.8
1500	415.302	<0.01*	0.7	439.044	<0.01*	0.8
2000	370.470	<0.01*	0.7	397.406	<0.01*	0.7
2500	308.710	<0.01*	0.6	322.603	<0.01*	0.6
3000	277.252	<0.01*	0.6	288.455	<0.01*	0.6
4000	134.941	<0.01*	0.4	141.923	<0.01*	0.4
5000	117.568	<0.01*	0.3	128.885	<0.01*	0.4
6000	159.534	<0.01*	0.4	167.669	<0.01*	0.4
8000	14.123	0.02	0.0	15.141	0.01	0.0
WBT_{avg}	5.012 *(df = 2)*	0.08	0.0	472.741	<0.01*	0.6

Note. $\chi 2$– Chi-Square value. *The actual *p*-value was not depicted as the initial three values after decimals were zero. The shaded region indicates a significant difference with medium to large effect size.

Annexure 4. 16

Mann-Whitney U Post hoc test results of WBA obtained between the pathological groups at peak and ambient pressure conditions

Frequency (Hz)	Peak pressure				Groups	Ambient pressure			
	TmP	MEE	Otosc-lerosis	ETD		TmP	MEE	Otosc-lerosis	ETD
250	NS				MEE	NS			
	**	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	NS	
	**	**	**	NS	SNHL	**	**	**	**
300	NS				MEE	NS			
	**	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	NS	
	**	**	**	NS	SNHL	**	**	**	**
400	**				MEE	NS			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	**	**	NS	
	0.02	**	**	NS	SNHL	**	**	**	**
500	**				MEE	**			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	**	NS	NS	
	NS	**	**	NS	SNHL	NS	**	**	**
600	**				MEE	**			
	**	NS			Otosclerosis	**	NS		
	NS	**	**		ETD	**	NS	NS	
	NS	**	**	NS	SNHL	NS	**	**	**
800	**				MEE	**			
	**	NS			Otosclerosis	**	NS		
	NS	**	**		ETD	**	NS	NS	
	NS	**	**	NS	SNHL	NS	**	**	**
1000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	**	**	**	
	**	**	**	**	SNHL	**	**	**	**
1250	**				MEE	**			
	NS	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	**	
	**	**	**	**	SNHL	**	**	**	**
1500	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	NS	**	**	

	**	**	**	**	SNHL	**	**	**	**
2000	0.00				MEE	**			
	0.00	0.00			Otosclerosis	**	**		
	0.00	0.00	NS		ETD	**	**	**	
	0.00	0.00	0.00	0.00	SNHL	**	**	**	**
2500	**				MEE	**			
	**	**			Otosclerosis	**	**		
	NS	**	**		ETD	NS	**	**	
	**	**	NS	**	SNHL	**	**	NS	**
3000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	**		ETD	**	**	**	
	NS	**	**	**	SNHL	NS	**	**	**
4000	**				MEE	**			
	NS	**			Otosclerosis	NS	**		
	NS	**	**		ETD	NS	**	**	
	**	**	**	NS	SNHL	**	**	**	NS
5000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	**	**	NS	
	**	NS	**	**	SNHL	**	NS	**	NS
6000	**				MEE	**			
	**	**			Otosclerosis	**	**		
	**	**	NS		ETD	**	**	NS	
	**	NS	NS	NS	SNHL	**	NS	NS	NS
8000	NS				MEE	NS			
	NS	NS			Otosclerosis	NS	NS		
	NS	NS	NS		ETD	NS	NS	**	
	NS	NS	NS	NS	SNHL	NS	NS	NS	NS
WBT_{avg}	--				MEE	**			
	--	--			Otosclerosis	**	**		
	--	--	--		ETD	**	**	NS	
	--	--	**	--	SNHL	**	**	**	**

Note. ** indicates a significant different at $p \leq 0.05$. NS indicate no significant different at $p > 0.05$.

PUBLICATIONS

1. Karuppannan, A., & Barman, A. (2020). Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity. *Auris Nasus Larynx*. https://doi.org/10.1016/j.anl.2020.10.019

2. Karuppannan, A., & Barman, A. (2021a). Wideband absorbance tympanometry: a novel method in identifying otosclerosis. *European Archives of Oto-Rhino-Laryngology*. https://doi.org/10.1007/s00405-020-06571-x

3. Karuppannan, A., & Barman, A. (2021b). Evaluation of wideband absorbance tympanometry in adults with abnormal positive and negative middle ear pressure. *Journal of Hearing Science*, 10(4), 40–47. https://doi.org/10.17430/jhs.2020.10.4.5

Auris Nasus Larynx xxx (xxxx) xxx

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Original Article

Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity

Arunraj Karuppannan[a,*], Animesh Barman[b]

[a] Department of Audiology/Prevention of Communication Disorders, All India Institute of Speech and Hearing, Manasagangothri, Mysuru, Karnataka, India
[b] Department of Audiology, All India Institute of Speech and Hearing, Manasagangothri, Mysuru, Karnataka, India

ARTICLE INFO

Article history:
Received 27 June 2020
Accepted 30 October 2020
Available online xxx

Keywords:
Wideband absorbance tympanometry
Middle ear disorders
Otosclerosis
Ossicular chain discontinuity
Resonance frequency

ABSTRACT

Objectives: Evidence from previous literature had shown that the use of a single frequency probe tone is not sensitive enough to detect middle ear pathologies, especially related to the ossicles, which hinders accurate diagnosis. The goal of the present study was to compare the outcome of wideband absorbance (WBA) tympanometry and to determine the difference in WBA pattern in adults with otosclerosis and ossicular chain discontinuity.

Materials and methods: Estimated adult cases of otosclerosis (10 ears) and ossicular chain discontinuity (06 ears) along with healthy individuals (10 ears) in the age range of 24 to 48 years (mean age: 38.6 years) were considered for the study. WBA was measured at peak and ambient pressure along with resonance frequency and compared with the data obtained from the healthy individuals to determine the WBA pattern.

Results: Data analysis revealed a distinct WBA pattern showing high absorbance at 750 Hz for ossicular chain discontinuity compared to healthy individuals, whereas the otosclerosis group showed reduced absorbance ($p < 0.05$) at low frequencies (250 Hz to 1500 Hz). WBA measured at the peak and ambient pressure did not elicit any significant difference across the frequencies. Also, the average WBA tympanogram measured between 375 Hz and 2000 Hz showed a significant difference in ambient pressure only in the otosclerosis group. In comparison to healthy individuals (901 Hz), ossicular chain discontinuity showed a significant reduction in resonance frequency (674 Hz), whereas in cases with otosclerosis had higher resonance frequency (1445 Hz).

Conclusions and significance: The present study showed different WBA patterns between the groups and the absorbance values were significantly different at the low frequencies. This suggests that WBA has the potential to differentiate ossicles related pathologies from normal and also between the ear with otosclerosis and ossicular chain discontinuity.

1. Introduction

The middle ear is a complex mechanical system that collects sound energy from the external ear canal, converts the signal into vibration, and passes to the inner ear. The primary functions of the middle ear are to match the impedance between the ear canal and the fluid-filled cochlea,

Abbreviations: WBA, Wideband Absorbance; OCD, Ossicular Chain Discontinuity; TPP, Tympanometric Peak Pressure; dB HL, Decibel Hearing Level; dB peSPL, Decibel Peak Sound Pressure Level; daPa, Decapascals.

* Corresponding author.
E-mail address: nahularun@gmail.com (A. Karuppannan).

https://doi.org/10.1016/j.anl.2020.10.019
0385-8146/

Please cite this article as: A. Karuppannan and A. Barman, Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity, Auris Nasus Larynx, https://doi.org/10.1016/j.anl.2020.10.019

thereby smooth transferring of sound energy into the cochlea. However, ease of flow of sound energy is highly frequency-dependent. It is influenced by the mass and stiffness component generated by the movements of the tympanic membrane, ossicular chain, and supporting ligaments [1]. Usually, the presence of fluid in the middle ear, perforation in the eardrum, ossification, or separation of ossicles would alter the stiffness and mass component, leading to hearing loss [2]. Although pure tone audiometry and tympanometry are performed to identify and confirm most middle ear disorders, they lack in providing a precise diagnosis of the middle ear abnormalities, especially the ossicular fixation or discontinuity [3].

The occurrence of conductive hearing loss with a large air-bone gap in the audiogram, having intact eardrum and aerated middle ear, could be because of the presence of ossicular chain abnormalities, i.e., either fixation or discontinuity, or third window lesion of the inner ear [4]. Further, additional information on the type of lesion can be obtained from the static admittance measurements of 226 Hz tympanometry and establishing resonance frequency. The fixation of ossicles leads to the enhancement of the stiffness component, affecting low-frequency sound transmission [5,6], and discontinuity enhances the mass component affecting mainly high-frequency sound transmission [1]. Thus, the frequency-specific information on the transmission of sound would be missed out with the use of single-frequency tympanometry and cannot reliably differentiate the nature of ossicular chain abnormalities [7]. Also, there is an overlap of admittance values and resonance frequency with that of normal-hearing individuals [3,8].

Wideband absorbance (WBA) tympanometry is a recent development that provides promising results in differentiating middle ear disorders [3,5,9]. WBA tympanometry uses either a click or chirp stimulus, and it measures the amount of absorption of sound energy entering the middle ear or the reflection of sound energy back to the ear canal across the broad frequency range (226 to 8000 Hz) and pressure. WBA overcomes the limitations of conventional 226 Hz tympanometry and has the potential to predict the cause of conductive hearing loss [10]. It would also provide insight into the management options, including surgical intervention and decision making [11].

Researchers have explored the characteristics of wideband absorbance in the population with ossicles related disorders [9,12]. Though limited studies are available, there seems to be a typical consistent pattern caused by middle ear disorders. Otosclerotic ear showed decreased absorbance till 1000 Hz [7,13] whereas ossicular chain discontinuity (OCD) showed increased absorbance of sharp peak around 500 and 800 Hz [3,8], compared to the normal individuals. This suggests the importance of WBA in identifying the population with ossicles related disorders. However, clinical data on WBA remains limited. Also, most of the studies reporting WBA in OCD were performed in the human cadaver temporal bones [14].

Further, studies showed that pressure variations would influence the absorbance measurements. Wideband absorbance can be measured either at the peak pressure or at ambient pressure [15]. Researchers had shown that WBA estimated at peak pressure would provide a reliable measure in differentiating the middle ear disorders rather than at ambient pressure [15,16]. Given such differences reported on WBA and also, there are not enough studies that have compared WBA on individuals with ossicles related disorders and determined the typical pattern.

Thus, the present study aimed at investigating the differences in WBA obtained across the frequencies at peak and ambient pressure and also to determine the WBA patterns in adults with estimated otosclerosis and OCD.

2. Materials and methods

The present study considered two groups of subjects from the Indian population with the age range of 24 to 48 years. The pathological group composed of ossicular disorders, viz. Otosclerosis (10 patients with a mean age of 37.5 ± 6.9 years) and OCD (06 patients with a mean age: 40.8 ± 7.8 years). These patients were diagnosed as having Otosclerosis or OCD and had recommended for exploratory middle ear surgery for confirmation by the experienced Otolaryngologist. All the patients had undergone pre-operative audiological evaluation (Pure-tone audiometry and tympanometry) along with otoscopic examination and results were correlated well with the diagnosis made by Otolaryngologist. Patients with otosclerosis included in the study had a history of progressive conductive hearing loss of raising pattern with a 2000 Hz Carhart notch and presence of 'A_s' type tympanogram with an absent acoustic reflex threshold [17]. Those patients with suspected OCD had flat or high-frequency conductive hearing loss with 'A_d' type tympanogram and absent acoustic reflex threshold [18,19]. The average pure tone threshold in hearing level (HL) calculated using four frequency average (500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz) was 36.5±13.8 dB for the Otosclerosis group and 39.3 ± 12.4 dB for the OCD group. All the patients had an intact tympanic membrane with the absence of confounding pathologies, such as middle ear effusion, tympanic membrane lesions, and retro-cochlear pathology.

The control group comprised 10 subjects (mean age: 39.3 ± 6.18 years) who had normal hearing thresholds (<15 dB HL at octave frequencies), 'A' type tympanogram with the presence of acoustic reflexes thresholds, and an intact tympanic membrane with no history of any middle ear abnormalities in the ear structure. In those subjects who had normal hearing in both the ears, only one ear was considered randomly to include in the control group [20]. In individuals with pathological groups, we considered the ears where the surgery was recommended.

The institutional ethical committee for Bio-behavioural research involving human subjects reviewed and approved the study. All the subjects had given informed consent before the administration of WBA test procedures. Interacoustics Titan Suite IMP440/WBT440 equipment (Interacoustics A/S, Middelfart, Denmark) used for WBA measurements was calibrated as recommended by the manufacturer [10]. The sub-

Please cite this article as: A. Karuppannan and A. Barman, Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity, Auris Nasus Larynx, https://doi.org/10.1016/j.anl.2020.10.019

jects were instructed to sit quietly during the WBA tympanometry, and the probe tip was inserted gently into the ear canal, ensuring proper hermetic seal. The pressure was swept using a descending sweep method from +200 to −400 daPa at a rate of 200 daPa/s. A click stimulus at an intensity level of 100 dB peSPL was presented to the ear canal at the click rate of 21.5 Hz. The absorbance values were measured at peak pressure (pressure at which maximum admittance occurred) and ambient pressure (0 daPa) as a function of frequency that ranges from 226 to 8000 Hz. Static admittance of 226 Hz probe tone tympanometry, resonance frequency, and the average WBA tympanometry (375–2000 Hz), along with frequency-specific WBA measurements at the octave and inter-octave frequencies were extracted. The obtained data was automatically transferred to an excel sheet [Microsoft Excel 2016 (Microsoft; Redmond, Washington, USA)] using MATLAB software version 9.7 (MATLAB R2019b, The MathWorks, Inc., Natick, US) and further into IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 21.0 (IBM Corp., Armonk, NY, USA) for statistical analysis.

We performed descriptive and inferential statistics to compare the group differences for the WBA at peak and ambient pressure across the frequencies, average WBA tympanometry, and resonance frequency. Kruskal–Wallis test was used to compare the control group with the corresponding pathological groups (Otosclerosis and OCD). The pair-wise comparisons using the Mann-Whitney U test without Bonferroni alpha correction [21] were performed to test for significant differences between the groups. The pathological groups were compared with not only the control group but also with each other. The significance level of $p < 0.05$ was used in all statistical analyses.

3. Results

At 226 Hz, the mean admittance value for the control group was 0.59±0.32 cc with the mean tympanometric peak pressure (TPP) of −7.45±16.60 daPa. The otosclerosis group had a mean admittance value of 0.34±0.12 cc with TPP of −10.84±21.28 daPa, whereas the OCD group had mean admittance of 2.06±0.55 cc with TPP of −17.33±33.60 daPa. Fig. 1 shows the WBA across the frequencies (226 Hz to 8000 Hz) of both control and pathological groups. In the control group, the WBA increases with an increase in frequency with a maximum absorbance occurred at 2000 Hz and decreases further to a minimum for higher frequencies. Whereas in the otosclerosis group, a drastic shift in the absorbance pattern was seen towards the higher frequency region with the maximum absorbance occurred at 3000 Hz.

In contrast, the OCD group showed increased absorbance at low frequencies, reaching a maximum at 750 Hz and reduced beyond that. However, there exists no significant difference of WBA between peak and ambient pressure within the groups. The Kruskal-Wallis test indicated that WBA across the frequencies was significantly different between the groups ($p < 0.05$) for both peak pressure and ambient pressure. The post-hoc Mann-Whitney U test revealed that the otosclerosis group had a significant reduction of mean and median WBA values from 250 Hz to 1500 Hz and slightly increased absorbance from 4000 to 8000 Hz, compared to the control group. However, no significant difference was observed at mid frequencies between 2000 Hz and 3000 Hz. In contrast, the mean and median values of the OCD group were significantly higher at 750 Hz and 1000 Hz, with a slight reduction at 1500 Hz to 3000 Hz, and absorbance values reduced insignificantly beyond 3000 Hz.

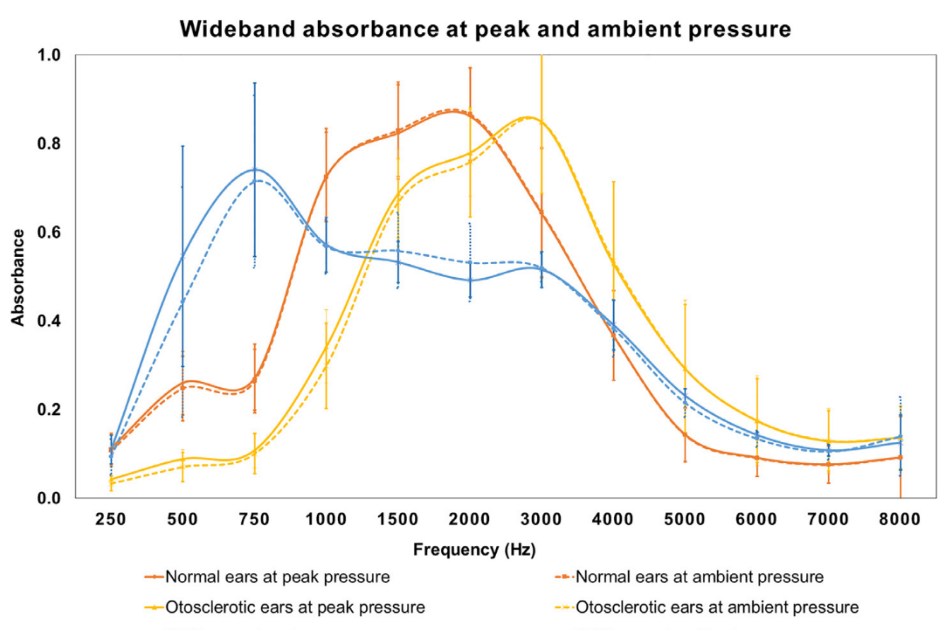


Fig. 1. Wideband absorbance pattern across frequencies at peak and ambient pressure of the control group (Normal ears), Otosclerosis and Ossicular chain discontinuity.

Please cite this article as: A. Karuppannan and A. Barman, Wideband absorbance pattern in adults with otosclerosis and ossicular chain discontinuity, Auris Nasus Larynx, https://doi.org/10.1016/j.anl.2020.10.019

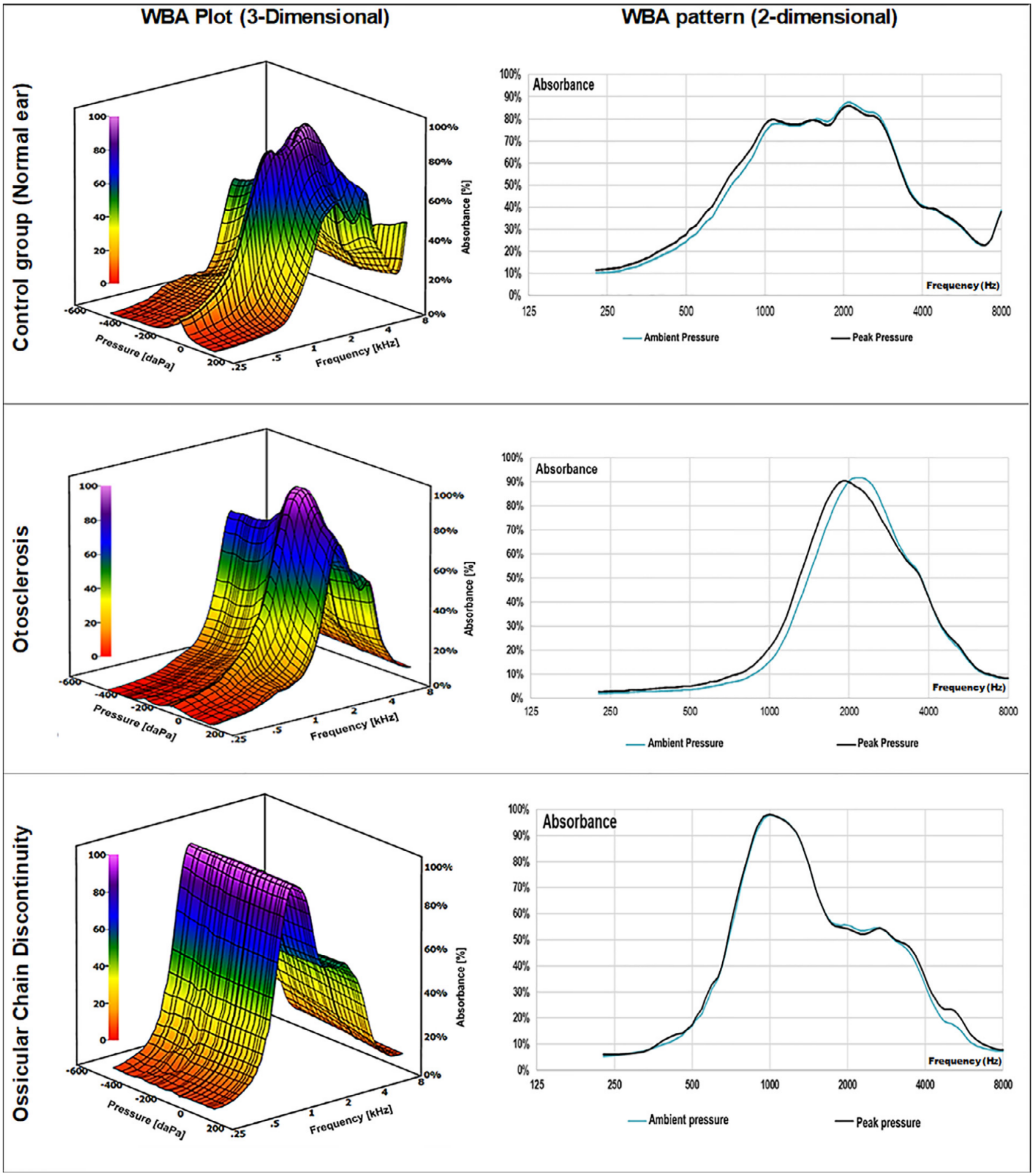


Fig. 2. Wideband absorbance pattern (3- and 2-dimesional plot) of one of the participants in each group (Control, Otoscleorsis and Ossicular chain discontinuity).

The most striking observation was that low frequencies showed a distinct pattern of differentiation between pathological groups. Below 1000 Hz, the otosclerosis group had reduced absorbance, whereas the OCD group had high absorbance significantly, and negligible WBA difference was observed above that. Fig. 2 reflects the 3-dimensional and 2-dimensional WBA plot of one of the subjects from each group. A noticeable absorbance pattern was seen between the groups in the lower frequency region.

Further, the study also analysed the average WBA tympanogram (375–2000 Hz) for peak and ambient pressure, and the resonance frequency obtained from the control and pathological groups, and the descriptive statistics (mean, median, and SD) are shown in Table 1. The mean average WBA was similar across the groups at peak pressure. In contrast, the mean average WBA value at ambient pressure was lower for the otosclerosis group compared to the control and OCD group. The average WBA tympanogram analysis using the Kruskal-Wallis test showed a significant difference at ambient pressure, $\chi^2(2) = 17.849$, $p = 0.00$, but not at peak pressure $\chi 2(2) = 4.519$, $p = 0.10$. Post hoc Mann-Whitney U test revealed a significant reduction in mean and median

Table 1
Mean, Median and Standard deviation (SD) of average wideband absorbance tympanogram at peak and ambient pressure and the resonance frequency in ears with normal hearing (Control group), otosclerosis and OCD.

Parameters	Control group ($N = 10$)		Otosclerosis ($N = 10$)		OCD ($N = 6$)	
	Mean (Median)	SD	Mean (Median)	SD	Mean (Median)	SD
Average absorbance at peak pressure	0.63 (0.65)	0.06	0.58 (0.63)	0.13	0.58 (0.58)	0.03
Average absorbance at ambient pressure	0.59 (0.59)	0.06	0.37 (0.37)*	0.06	0.60 (0.61)	0.02
Resonance Frequency (Hz)	901.20 (918.50)**	110.47	1445 (1434.5)**	167.79	673.67 (654)**	109.67

* Otosclerosis group is significantly different from other two groups at $p < 0.05$.
** All three groups are significantly different from each other at $p < 0.05$.

average WBA values in the otosclerosis group compared to the control group ($U = 0.00$, $z = -3.787$, $p = 0.00$) and OCD group ($U = 0.00$, $z = -3.266$, $p = 0.00$). In contrast, no difference was seen between the OCD and control group ($U = 27.50$, $z = -0.272$, $p = 0.792$).

Similarly, the analysis of the resonance frequency in the otosclerosis group showed increased resonance (Mean: 1445±168 Hz; Range: 1216–1731 Hz) compared to the control group (Mean: 901±110 Hz; Range: 753–1130 Hz) whereas the OCD group showed lower resonance frequency (Mean: 674±110 Hz; Range: 548–848 Hz), as indicated in Table 1. A Kruskal-Wallis test showed a statistically significant difference in resonance frequency across the groups, $\chi^2(2) = 20.866$, $p = 0.00$, with a mean rank value of 11.10 for the control group, 21.50 for the otosclerosis group, and 4.17 for the OCD group. The pair-wise analysis showed a significant difference between the groups indicating each group having a distinct feature (Control vs. Otosclerosis: $U = 0.00$, $z = -3.781$, $p = 0.00$; Control vs. OCD: $U = 4.00$, $z = -2.820$, $p = 0.00$; Otosclerosis vs. OCD: $U = 0.00$, $z = -3.256$, $p = 0.00$).

4. Discussion

In the control group, the maximum WBA occurred at mid frequencies at 1000 Hz and 2000 Hz and reduced WBA in the extreme low and high frequencies. The findings of our study are in agreement with the previous results reported in the literature [22,23]. A complex interaction of stiffness and mass reactance that occurs in the mid-frequency region gets cancels each other, allowing a maximum flow of sound energy into the middle ear in those frequencies. In contrast, the eardrum resistance is small compared to its reactance in the low and high-frequency region and thus reflecting most of the energy into the ear canal [24].

Previous literature on WBA results suggests that absorbance measure is a potential tool to detect the middle ear pathologies [8,25]. In our study, the WBA results obtained from otosclerosis and OCD were opposite to each other, indicating a clear boundary between the two, compared to the control group. In the otosclerosis group, a sharp decrease in absorbance was observed at low frequencies up to 1500 Hz whereas, an abrupt increase in absorbance around 750 Hz was seen in the OCD group. Our study supports the previous research, showing a distinctive WBA pattern at lower frequencies for individuals with otosclerosis [3,8,23,24] and OCD [3,14,26].

Sound transmission through the middle ear is affected by both stiffness and mass component of the middle ear [1]. Reduction in absorbance below 1000 Hz in the otosclerotic ear could be because of the increase in stiffness of the middle ear caused by the fixation of stapes footplate. This generates huge impedance at the level of the tympanic membrane, thereby reflecting most of the low-frequency energy into the ear canal [24,27]. Conversely, OCD makes the middle ear system as a mass-dominating system by reducing the stiffness component due to the discontinuity of the ossicles. Thus, the increased contribution of the mass component decreases the transmission of high frequencies and allows the low-frequency energy to the middle ear [1]. This could be the reason for an increase in absorbance around 750 Hz in-ears with OCD [14,26].

There are very limited studies on average WBA tympanogram in ears with otosclerosis and OCD. A recent study on WBA averaged over 600 Hz to 1000 Hz along with the air-bone gap averaged between 1000 Hz and 4000 Hz, showed a good accuracy in identifying otosclerosis and OCD disorders [3]. However, our data showed no significant difference in wideband average absorbance values for all the groups except for ears with otosclerosis at ambient pressure. This could be because of the inclusion of a wide frequency range in obtaining average absorbance values calculated from 375 Hz to 2000 Hz, which could have probably led to no significant difference. However, the reason for the difference in average WBA for the otosclerosis group obtained only at ambient pressure needs to be further explored.

Studies have shown that changes in characteristics of the middle ear alter the tympanometric findings obtained with higher probe frequencies, shifting the resonant frequency of the middle ear [6,14,28]. As earlier mentioned, increased stiffness due to otosclerosis would increase the resonance frequency of the middle ear [6,29] beyond the normal range of 800 Hz to 1200 Hz [30], as seen in our present study. In contrast, disarticulation or erosion of ossicular joint decreases the stiffness of the middle ear system, i.e. an increase in the mass component. It thus shifts the resonance frequency towards the lower frequencies [29,31].

Earlier studies had shown that resonance frequency is a better sensitive tool compared to 226 Hz tympanometry [6,28,32], but less sensitive than WBA [5], to detect even the slight changes in the transmission characteristics of the middle ear system. There were reports of extensive overlap in static admittance and resonance frequency measures with the normal hearing individuals and thus limits the diagnostic value [6]. Studies had shown that the admittance value of 226 Hz/678 Hz probe tone frequency was mostly within the normal range in individuals with otosclerosis [6,32,33]. Thus, standard 226-Hz tympanometry is typically inadequate for distinguishing middle ear disorders. However, the inclusion of higher frequencies would improve the sensitivity of the results, as observed in the resonance frequency and WBA measurements.

In summary, the findings of the present study had shown a difference in WBA, especially in the lower frequencies, to differentiate otosclerosis and OCD from the normal ear. Besides, it is interesting to note that pathologies related to ossicles have a unique WBA pattern, which makes it easy to identify the disorders related to ossicles. However, the diagnosis of having otosclerosis and OCD were made based on the criteria given by Nadol [17] and Farahmand et al. [18] and the experienced Otolaryngologist also made a similar diagnosis. But the diagnosis was not confirmed through the surgical operation in the present study. Also, the study groups were globally matched without consideration of gender due to its limited sample size.

5. Conclusion

In the present study, the WBA pattern and the absorbance values obtained at different frequencies showed that it has the potential to differentiate different ossicular chain related pathologies from normal hearing individuals and also differentiating otosclerosis from OCD. However, the outcome of WBA values should be used in caution until it is validated with the large sample size of confirmed cases.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

The authors declare that the study does not have any funding sources and support.

Acknowledgements

The authors express thanks to Interacoustics, Denmark, for providing a Research module of TITAN WBT software for conducting the study.

Author's Contribution

Arunraj Karuppannan's contribution: Involved in the conception and design of the study, data collection, statistical analysis and interpretation of data, and drafted the manuscript.

Animesh Barman's contribution: Involved in the conception and design of the study, analysis and interpretation of data. Provided input on the protocol used, statistical analysis, edited, and approved the manuscript.

Ethical Approval

Approval was obtained from the institutional ethical review committee of the All India Institute of Speech and Hearing (AIISH), and it is based on the ethical guidelines of AIISH for bio-behavioral research involving the human subject. The procedure used in this study adhere to the tenants of the 1964 declaration of Helsinki.

Consent to Participate

Informed consent was taken from all the participants, who had enrolled for the study

References

[1] Kim J, Koo M. Mass and stiffness impact on the middle ear and the cochlear partition. Korean J Audiol 2015;19:1–6 Korean Audiological Society.
[2] Merchant SN, Ravicz ME, Voss SE, Peake WT, Rosowski JJ. Middle ear mechanics in normal, diseased and reconstructed ears. J Laryngol Otol 1998;112(8):715–31.
[3] Nakajima HH, Pisano DV, Roosli C, Hamade MA, Merchant GR, Mahfoud L, et al. comparison of ear-canal reflectance and umbo velocity in patients with conductive hearing loss: a preliminary study. Ear Hear 2012;33(1):35–43.
[4] Merchant SN, Rosowski J. Acoustics and mechanics of the middle ear-Glasscock-Shambaug surgery of the ear. In: Minor L, Glasscock M, Poe D, editors. Glasscock-Shambaugh surgery of the ear. Shelton, CT: People's Medical Publishing House-USA; 2010. p. 49–72.
[5] Shahnaz N, Bork K, Polka L, Longridge N, Bell D, Westerberg BD. Energy reflectance and tympanometry in normal and otosclerotic ears. Ear Hear 2009;30(2):219–33.
[6] Shahnaz N, Polka L. Standard and multifrequency tympanometry in normal and otosclerotic ears. Ear Hear 1997;18(4):326–41.
[7] Shahnaz N, Longridge N, Bell D. Wideband energy reflectance patterns in pre-operative and post-operative otosclerotic ears. Int J Audiol 2009;48(5):240–7.
[8] Feeney MP, Grant IL, Marryott LP. Wideband energy reflectance measurements in adults with middle-ear disorders. J Speech Lang Hear Res 2003;46(4):901–11.
[9] Nakajima HH, Rosowski JJ, Shahnaz N, Voss SE. Assessment of ear disorders using power reflectance. Ear Hear 2013;34(SUPPL. 1) 48S–53S.
[10] Keefe DH, Simmons JL. Energy transmittance predicts conductive hearing loss in older children and adults. J Acoust Soc Am 2003;114(6):3217–38.
[11] Li PMMC, Bergeron C, Monfared A, Agrawal S, Blevins NH. Superior semicircular canal dehiscence diagnosed after failed stapedotomy for conductive hearing loss. Am J Otolaryngol - Head Neck Med Surg 2011;32(5):441–4.
[12] Kim SY, Han JJ, Oh SH, Lee JH, whan SuhM, Kim MH, et al. Differentiating among conductive hearing loss conditions with wideband tympanometry. Auris Nasus Larynx 2019;46(1):43–9 [cited 2020 Sep 18]Available from https://pubmed.ncbi.nlm.nih.gov/29885747/.

[13] Wang S, Hao W, Xu C, Ni D, Gao Z, Shang Y. A study of wideband energy reflectance in patients with otosclerosis: data from a Chinese population. Biomed Res Int 2019;2019:1–8.
[14] Feeney MP, Grant IL, Mills DM. Wideband energy reflectance measurements of ossicular chain discontinuity and repair in human temporal bone. Ear Hear 2009;30(4):391–400.
[15] Shahnaz N, Patrick Feeney M, Schairer KS. Wideband acoustic immittance normative data: ethnicity, gender, aging, and instrumentation. Ear Hear 2013;34(SUPPL. 1) 27S–35S.
[16] Feeney MP, Stover B, Keefe DH, Garinis AC, Day JE, Seixas N. Sources of variability in wideband energy reflectance measurements in adults. J Am Acad Audiol 2014;25(5):449–61 [cited 2020 Apr 6]Available from http://www.ncbi.nlm.nih.gov/pubmed/25257719.
[17] Nadol JB. Otosclerosis: clinical aspects and management. Audio-Digest Otolaryngol 1998;31:2.
[18] Farahmand RB, Merchant GR, Lookabaugh SA, Röösli C, Ulku CH, McKenna MJ, et al. The audiometric and mechanical effects of partial ossicular discontinuity. Ear Hear 2016;37(2):206–15 [cited 2020 Jun 3]Available from: /pmc/articles/PMC4767640/?report=abstract.
[19] Campbell E, Tan NC. Ossicular-Chain dislocation, StatPearls: StatPearls Publishing; 2020. [cited 2020 Sep 17]. Available from http://www.ncbi.nlm.nih.gov/pubmed/32809456.
[20] Whittemore KR, Merchant SN, Poon BB, Rosowski JJ. A normative study of tympanic membrane motion in humans using a laser Doppler vibrometer (LDV). Hear Res 2004;187(1–2):85–104 [cited 2020 Sep 17]Available from https://pubmed.ncbi.nlm.nih.gov/14698090/.
[21] Feise RJ. Do multiple outcome measures require p-value adjustment? BMC Med Res Methodol 2002;2(1):1–4.
[22] Margolis RH, Saly GL, Keefe DH. Wideband reflectance tympanometry in normal adults. J Acoust Soc Am 1999;106(1):265–80.
[23] Shahnaz N, Bork K. Wideband reflectance norms for Caucasian and Chinese young adults. Ear Hear 2006;27(6):774–88.
[24] Allen JB, Jeng PS, Levitt H. Evaluation of human middle ear function via an acoustic power assessment. J Rehabil Res Dev 2005;42(4 Suppl. 2):63–77.
[25] Prieve BA, Patrick Feeney M, Stenfelt S, Shahnaz N. Prediction of conductive hearing loss using wideband acoustic immittance. Ear Hear 2013;34(SUPPL. 1) 54S–59S.
[26] Feeney MP, Grant IL. Ossicular chain reconstruction in a temporal bone model using ionomeric cement. In: Wideband energy reflectance measurements in adults with middle-ear disorders; 2003. p. 901–11.
[27] Feeney MP, Keefe DH. Acoustic reflex detection using wideband acoustic reflectance, admittance, and power measurements. J Speech Lang Hear Res 1999;42(5):1029–41.
[28] André KD, Sanches SGG, Carvallo RMM. Middle ear resonance in infants: age effects. Int Arch Otorhinolaryngol 2012;16(3):353–7.
[29] Levina IV, Ivanets IV. Diagnostic implications of examination of the middle ear resonance frequency. Vestn Otorinolaringol 2002(2):11–13 Available from http://www.ncbi.nlm.nih.gov/pubmed/12056151.
[30] Shanks JE. Tympanometry. Ear Hear 1984;5(5):268–80 [cited 2020 Jun 13]Available from http://journals.lww.com/00003446-198409000-00003.
[31] Zhao F, Wada H, Koike T, Stephens D. The influence of middle ear disorders on otoacoustic emissions. Clinical Otolaryngology and Allied Sciences. Clin Otolaryngol Allied Sci 2000;25:3–8.
[32] Margolis RH, Shanks I. Tympanometry: basic principles and clinical applications. In: Rintelman WF, editor. Hearing assessment. Austin, TX: Pro-ed; 1991. p. 179–245.
[33] Muchnik C, Hildesheimer M, Rubinstein M, Gleitman Y. Validity of tympanometry in cases of confirmed otosclerosis. J Laryngol Otol 1989;103(1):36–8.

European Archives of Oto-Rhino-Laryngology
https://doi.org/10.1007/s00405-020-06571-x

OTOLOGY

Wideband absorbance tympanometry: a novel method in identifying otosclerosis

Arunraj Karuppannan[1] · **Animesh Barman**[1]

Received: 10 June 2020 / Accepted: 12 December 2020

Abstract

Purpose The purpose of the study was to know whether the wideband absorbance measurements can be a useful tool to identify ears with otosclerosis. The present study analyzed WBA measurements and highlighted its effectiveness in identifying ears with otosclerosis and differentiating from healthy normal ears.

Methods The study included 42 ears with otosclerosis which were compared with an equal sample size of healthy normal ears. WBA across frequencies and wideband average absorbance (375–2000 Hz) at the peak and ambient pressure, and resonance frequency were measured and analyzed.

Results Results showed that WBA levels increased with an increase in frequencies up to 2000 Hz and decreased thereafter, both in the otosclerosis and healthy normal ears. The mean WBA in the otosclerosis group was significantly lower in the 250–2000 Hz frequency range than in the healthy normal ear group. The WBA values at ambient pressure reduced significantly up to 500 Hz for the healthy normal ear group and 1500 Hz for otosclerosis group, compared with peak pressure. Further, the analysis of wideband average absorbance at ambient pressure showed reduced absorbance (0.35) and higher resonance frequency (1350.33 Hz) in the otosclerosis group compared with the healthy normal ear group (0.60 and 930.14 Hz, respectively). ROC analysis indicated that WBA is suitable for identifying otosclerotic ears and also in differentiating from healthy normal ears based on WBA values from 250 to 1500 Hz. High diagnostic values of WBA (> 90% sensitivity and specificity) were observed at a frequency of 1000 Hz.

Conclusions The inclusion of WBA into clinical routine test procedures could be a useful tool for detecting otosclerosis. Further research is required to validate its clinical use in combination with other middle ear measures.

Keywords Wideband absorbance · Resonance frequency · Tympanometry · Otosclerosis

Introduction

Otosclerosis is an ontological condition that occurs when sclerotic bone growth involves the oval window and the stapedial footplate, often called stapedial otosclerosis. Individuals with otosclerosis present with progressive conductive hearing loss of mild low frequency at initial stages to a flat, severe conductive/mixed hearing loss in the late stages of the disease [1]. This disease influences the ossicular mass, stiffness of the tympanic membrane, and supporting structures in the middle ear. Usually, it occurs in early adulthood between 15 and 40 years of age, affecting women twice as compared with men [2]. Although the etiology of otosclerosis remains a matter of debate, family history is present in more than 50% of cases [1].

Clinical diagnosis of otosclerosis is based on the combination of medical history, audiological test (pure-tone audiometry and tympanometry) results, and imaging findings [3]. Individuals with otosclerosis present with an audiogram showing air–bone gap greater than 10 dB HL with increased air conduction thresholds, especially at the lower frequencies and presence of 2 kHz notch in the bone conduction thresholds [4]. This could be because of fixation of the stapes footplate, enhancing the stiffness causing the diminished continuation of the sound wave entering the cochlea [5]. Along with pure tone audiometry, single-component low-frequency tympanometry with 226 Hz probe tone has been commonly

✉ Arunraj Karuppannan
nahularun@gmail.com

Animesh Barman
barmananimesh197@gmail.com

1 Department of Audiology, All India Institute of Speech and Hearing, Manasagangothri, Mysuru, Karnataka, India

Published online: 03 January 2021

used for the diagnosis of patients with otosclerosis [5, 6]. Clinical findings of single-component tympanometry with 226 Hz probe tone show 'A/As' type tympanogram with absent/abnormal middle ear muscle reflexes. However, these tympanogram patterns are not unique to otosclerosis and often mimic the findings of normal hearing individuals [5]. Many researchers have suggested that a conventional 226 Hz tympanogram is less sensitive in differentiating otosclerosis ears from other middle ear disorders [5, 7].

The normal middle ear is primarily dominated by the stiffness of low-frequency sounds (226 Hz) and is sensitive in the identification of various middle ear disorders [5, 8]. However, any change in the stiffness or the mass component of the middle ear alters the tympanogram obtained with the higher probe frequencies and influences the resonant frequency of the middle ear transmission system [8, 9]. In ears with otosclerosis, there is an increase in the stiffness of the middle ear that shifts the resonance frequency to the higher value compared with the normal middle ear system [5, 10]. Thus, this would result in decreased admittance for low frequencies. Studies have shown that the multi-frequency tympanometry is more appropriate than the traditional single-frequency tympanometry to detect small changes in the middle ear system and has high sensitivity in diagnosing the ossicular chain disorders [5, 7, 10–12]. However, it has received little attention in clinical practice and is not included in the audiological testing battery for testing patients with suspected otosclerosis. The possible reasons are complex tympanogram obtained at higher frequencies compared with traditional 226 Hz tympanogram, leading to difficulty in interpretation [11]. Also, there are reports of the large overlap in the results of normal ears with other middle ear-related disorders such as ossicular chain discontinuity with fibrosis [5, 7, 11, 13]. Apart from these tests, high-resolution computed tomography (HRCT) has been the preferred method for diagnosing otosclerosis [3], despite its low sensitivity and high cost [14]. A non-invasive, less expensive clinical tool to identify otosclerosis from other ontological conditions would be beneficial for otolaryngologists and also patients.

Wideband absorbance tympanometry (WBT) is being introduced recently to overcome these drawbacks and to assess middle ear functioning. Recent research has revealed WBT to have good sensitivity compared with 226 Hz and multi-frequency tympanometry [15, 16]. Instead of using a single probe tone (226 Hz) or the multi-frequency probe tone to measure the admittance at a particular frequency, the WBT uses a transient wideband stimulus with either click or chirp and measures the absorbance from 226 to 8000 Hz range in small increments [17]. With a single pressure sweep, WBT performs a comprehensive analysis of middle ear status over a frequency range at a shorter time. Besides this, tympanometric peak pressure and resonance frequency are also measured, resulting in an increase of WBT's sensitivity in identifying middle ear disorders [5, 15]. In addition, WBT measurements are less susceptible to myogenic noise due to the presence of multiple frequencies in the transient stimuli [18]. However, this procedure is still in the research phase, with only limited studies revealing distinct wideband absorbance (WBA) patterns for middle ear disorders [19–21].

A few researchers have explored the characteristics of wideband absorbance in the population with otosclerosis. Energy reflectance measured in patients with otosclerosis has shown increased reflectance at low frequencies (< 1000 Hz) compared with normal individuals [22]. Following this, a few other investigators have made similar observations [23–25]. Recent investigation on the otosclerosis group showed reduced absorbance at ambient pressure for 4 kHz [20]. Further, these investigators have suggested that the difference in absorbance at low frequency plays a critical role in identifying the population with otosclerosis. However, clinical data on WBA related to otosclerosis remain limited. To date, there are no studies that have investigated wideband average absorbance measurements in ears with otosclerosis and have not attempted to set criteria in detecting otosclerosis using WBA measurements.

Hence, the current study aimed at obtaining absorbance measurements across the frequencies, wideband average absorbance, and resonance frequency in ears with otosclerosis and compared those with the healthy normal ear. Furthermore, the present study also assessed the sensitivity and specificity of WBA and delineated a criterion to identify otosclerosis.

Materials and methods

The study was conducted in an institutional setup and followed the non-experimental standard group comparison design. The procedure followed in the study adheres to the tenet of the 2013 Declaration of Helsinki [26] with prior approval from the institutional ethical committee. In addition, informed consent was obtained from all the participants before the testing.

Participants

The otosclerosis group consisted of 42 ears from 26 adults with a mean age of 36 years (range 22–50 years) and diagnosed with otosclerosis by an experienced otorhinolaryngologist. The diagnosis was confirmed based on the clinically accepted criteria [27], i.e. the presence of a history of progressive hearing loss, negative Rinne's test, conductive hearing loss of raising pattern with a 2 kHz Carhart notch in the pure tone audiometry, and presence of A/A_s type tympanogram with an absence of acoustic reflex. The audiogram results showed mild-to-moderately severe conductive hearing loss with the pure tone average thresholds ranging from 26 to 70 dB, indicating the severity of otosclerosis. All the participants had an intact tympanic membrane with no other associated middle ear pathologies that were confirmed by an experienced otorhinolaryngologist.

The otosclerosis group was compared with 42 healthy normal ears from 24 adults with a mean age of 38 years (range 22–50 years). The healthy ears had normal pure tone threshold of < 15 dB HL at octave frequencies with no air–bone gap, normal otoscopic findings, intact middle ear functions showing A-type tympanogram with the presence of acoustic reflexes, and the presence of transient evoked otoacoustic emission (≥ 3 dB SNR).

Test procedure

All participants had undergone audiological evaluation that included pure-tone audiometry, immittance measurements using 226 Hz probe tone, and otoacoustic emission in a sound-treated room. The entire testing for the otosclerosis group was performed before the initiation of their medical treatment. Interacoustics Titan Suite IMP440/WBT440 version 3.3.1 advanced research module (Interacoustics A/S, Middelfart, Denmark) was used to measure wideband absorbance. Calibration of the Titan WBT equipment was performed using the couplers before performing the WBA measurements [28].

The participants were made to sit comfortably and quietly to measure WBA. With the help of a suitable eartip, the probe was placed in the ear canal to obtain an airtight seal. A wideband click stimulus at 100 dB peSPL was presented to the ear canal at a click rate of 21.5 Hz. The pressure was swept from + 200 to − 400 daPa at a rate of 200 daPa/s [22, 28]. WBA was measured across a wide frequency range of 226–8000 Hz, and the results were plotted on a three-dimensional graph (Fig. 1). The value of the measured absorbance lies between 0 and 1, where ‘0’ indicates all acoustic energy reflected on the ear canal, and ‘1’ indicates all acoustic energy being absorbed by the middle ear [28]. WBA that is measured at peak pressure and ambient pressure was automatically stored in MATLAB format (.m-file extension). The WBA values at 1/24th octave frequencies (121 frequencies) were extracted into an Excel spreadsheet using the MATLAB version 9.7 software (MATLAB R2019b, The MathWorks, Inc., Natick, USA). However, to reduce the number of samples for analysis, absorbent values at octave and inter-octave frequencies from 250 to 8000 Hz (12 frequencies) were considered. Apart from WBA values, resonance frequency (RF), and wideband average absorbance (375–2000 Hz) at peak pressure ($AvgWBA_{PP}$), and

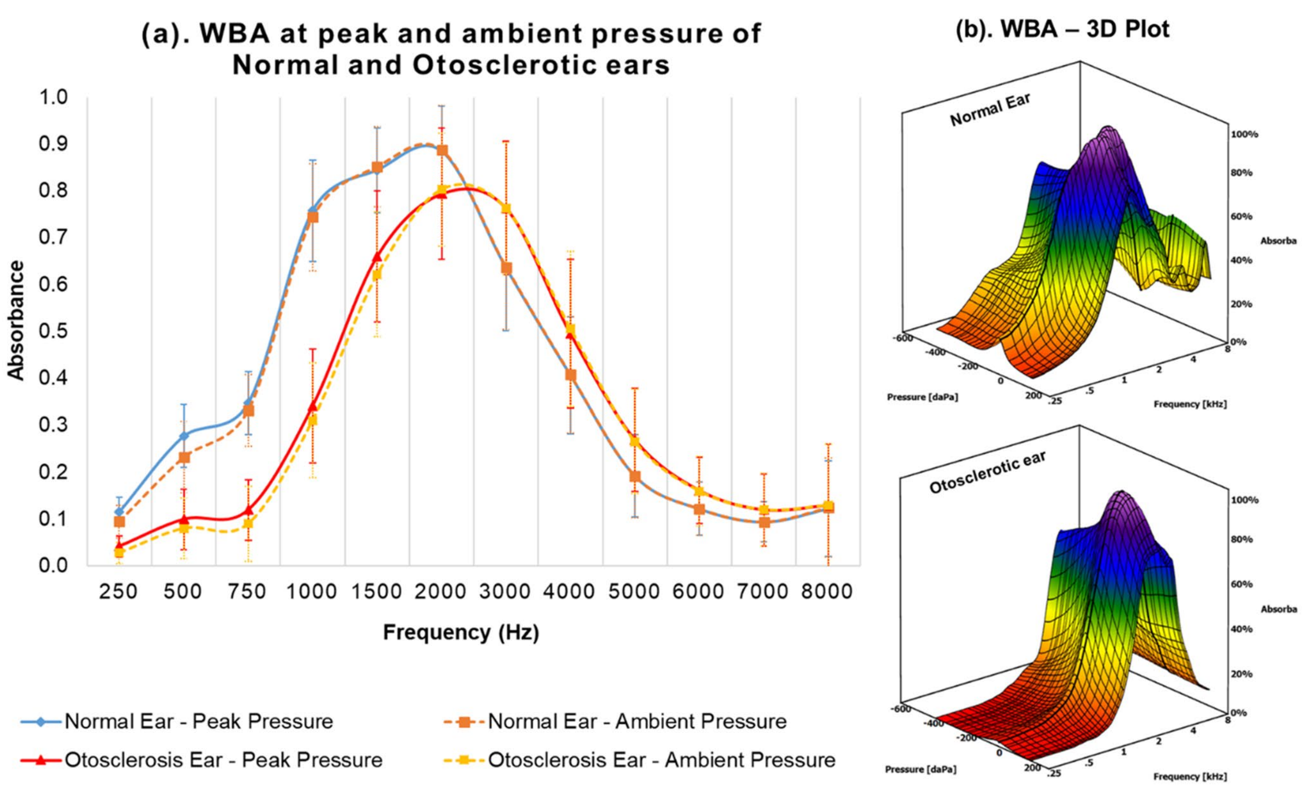


Fig. 1 **a** WBA pattern at peak pressure and ambient pressure for normal and otosclerosis ears. **b** WBA 3D plot of normal ear and otosclerotic ear

ambient pressure ($AvgWBA_0$) provided by the equipment were also considered for the analysis.

Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Shapiro–Wilk test was used to check the normality of WBA values at each of the frequencies from 250 to 8000 Hz, resonance frequency, $AvgWBA_{PP}$, and $AvgWBA_0$. However, the test results revealed that the data were not normally distributed ($p < 0.05$). Hence, the non-parametric Mann–Whitney *U* test without any alpha corrections [29] was used to check for the significant difference in WBA between the otosclerotic and healthy normal ears. Wilcoxon's signed-rank test was used to compare WBA values measured at peak pressure and ambient pressure within the groups. Receiver operating characteristic (ROC) curves were obtained to estimate the sensitivity and specificity for each of the parameters. The area under the ROC (AUROC) value was estimated to quantify the accuracy of the test with a statistically significant value of $p < 0.05$.

Results

Wideband absorbance across frequencies

The WBA was measured at peak and ambient pressure at 12 different octaves and inter-octave frequencies from 250 to 8000 Hz. The WBA pattern for otosclerosis and healthy normal ear group was found to be similar regardless of the pressure conditions, as shown in Fig. 1. The WBA values tend to increase with increasing frequency from 250 Hz, reaching its maximum at 2000 Hz and decreased thereafter, reaching a minimum value at 5000 Hz and beyond. Although the pattern was similar, there was a significant difference in WBA values between the groups at all frequencies except at 8000 Hz. The WBA values for the otosclerosis group were significantly lesser up to 2000 Hz, above which, an increase in WBA values was observed compared with the healthy normal ear group. This can be observed for both peak pressure and ambient pressure measurements. Similarly, the difference in WBA values obtained at peak and ambient pressure within the group revealed significant reductions in WBA values for ambient pressure. This reduction is seen at low and mid-frequencies compared with absorbance values at peak pressure. In the healthy normal ear group, the WBA values at ambient pressure were reduced up to 500 Hz, whereas the reduction was seen up to 1500 Hz for the otosclerosis group significantly, compared with peak pressure.

Further, ROC analysis with AUROC was computed to determine the efficiency of WBT to differentiate the otosclerosis from healthy normal ears at each octave and inter-octave frequency based on the WBA values. Figure 2 indicates that ROC at low and mid-frequencies from 250 to 1500 Hz lie close to the left and top border of the ROC space above the diagonal line for both peak and ambient pressure. AUROC value obtained was 0.7 and above, which is statistically significant ($p < 0.05$). It shows the accuracy of the WBA for detecting the otosclerosis from the healthy normal ears at low and mid-frequency regions up to 1500 Hz. As the frequencies increase beyond 2000 Hz, the ROC curve flattens and falls below the diagonal line. The AUROC value for higher frequencies falls below the chance level (< 0.5), indicating poor efficiency in detecting otosclerosis. The cut-off criterion was estimated based on the results of the ROC analysis along with sensitivity and specificity, and the values are summarized in Table 1. ROC analysis showed that WBA is suitable for differentiating between otosclerotic and healthy normal ears at low and mid-frequency regions, i.e. up to 1500 Hz. High diagnostic value of WBA with sensitivity > 83% and specificity > 95% has been observed for frequency up to 1000 Hz, having a maximum sensitivity (92.86%) and specificity (100%) at 1000 Hz with an absorbance value of 0.55. Similar cut-off criteria were observed for WBA measured at both peak and ambient pressure conditions.

Wideband average absorbance

Descriptive statistics for wideband average absorbance at peak pressure and ambient pressure were computed. The mean wideband average absorbance value obtained at peak pressure for the otosclerosis group is 0.58 with a standard deviation (SD) of 0.10, and for the healthy normal ear group is 0.61 with an SD of 0.07 and are not statistically different ($U = 703.50$, $p = 0.11$). At ambient pressure, the mean wideband average absorbance of otosclerotic group is lesser (mean: 0.35 ± 0.08 SD) compared with the healthy normal ear group (mean: 0.60 ± 0.07 SD), and the values were found to be significantly different ($U = 35.50$, $p < 0.01$).

The ROC curve for wideband average absorbance at ambient pressure showed higher accuracy in detecting otosclerosis (Fig. 3a) with an AUROC value of 0.98 ($p < 0.05$). However, the ROC curve for wideband average absorbance at peak pressure is relatively flattened and low with an AUROC value of 0.60 and failed to reach a significant level ($p > 0.05$). A dot plot graph was drawn to understand the distribution of values for each of the groups with a cut-off criterion (Fig. 3c, d). A clear demarcation in wideband average absorbance values at ambient pressure was observed between the otosclerosis and healthy normal ear group. The otosclerosis group had an average absorbance value ranging below 0.45 at ambient pressure compared with the healthy normal ear group that ranged

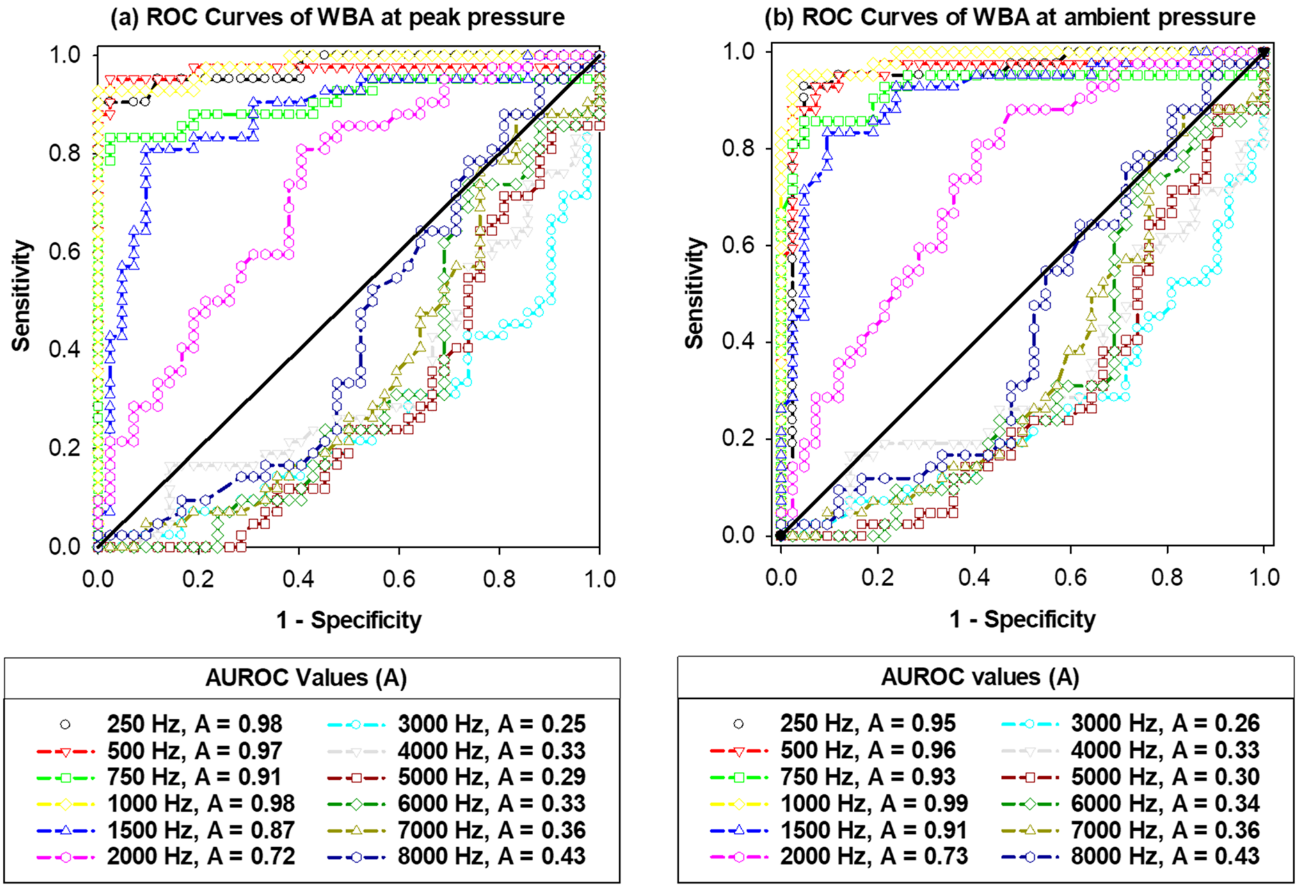


Fig. 2 ROC curve analysis for WBA as a function of frequencies at (**a**) peak pressure and (**b**) ambient pressure along with AUROC values

Table 1 AUROC values, cut-off value, sensitivity and specificity of WBA at peak pressure and ambient pressure

Frequencies	AUROC value	Cut-off value	Sensitivity (%)	Specificity (%)
WBA at peak pressure				
250 Hz	0.976	≤0.06	90.48	100.00
500 Hz	0.970	≤0.17	95.24	97.62
750 Hz	0.908	≤0.17	83.33	97.62
1000 Hz	0.981	≤0.55	92.86	100.00
1500 Hz	0.874	≤0.78	80.95	90.48
2000 Hz	0.723	≤0.90	80.95	59.52
WBA at ambient pressure				
250 Hz	0.951	≤0.06	92.86	95.24
500 Hz	0.958	≤0.13	88.10	97.62
750 Hz	0.927	≤0.16	85.71	95.24
1000 Hz	0.987	≤0.52	95.24	97.62
1500 Hz	0.907	≤0.75	83.33	90.48
2000 Hz	0.732	≤0.91	80.95	59.52

above 0.45, with a sensitivity and specificity of 92.2% and 97%, respectively. In contrast, the distribution of average absorbance was similar for both groups at peak pressure.

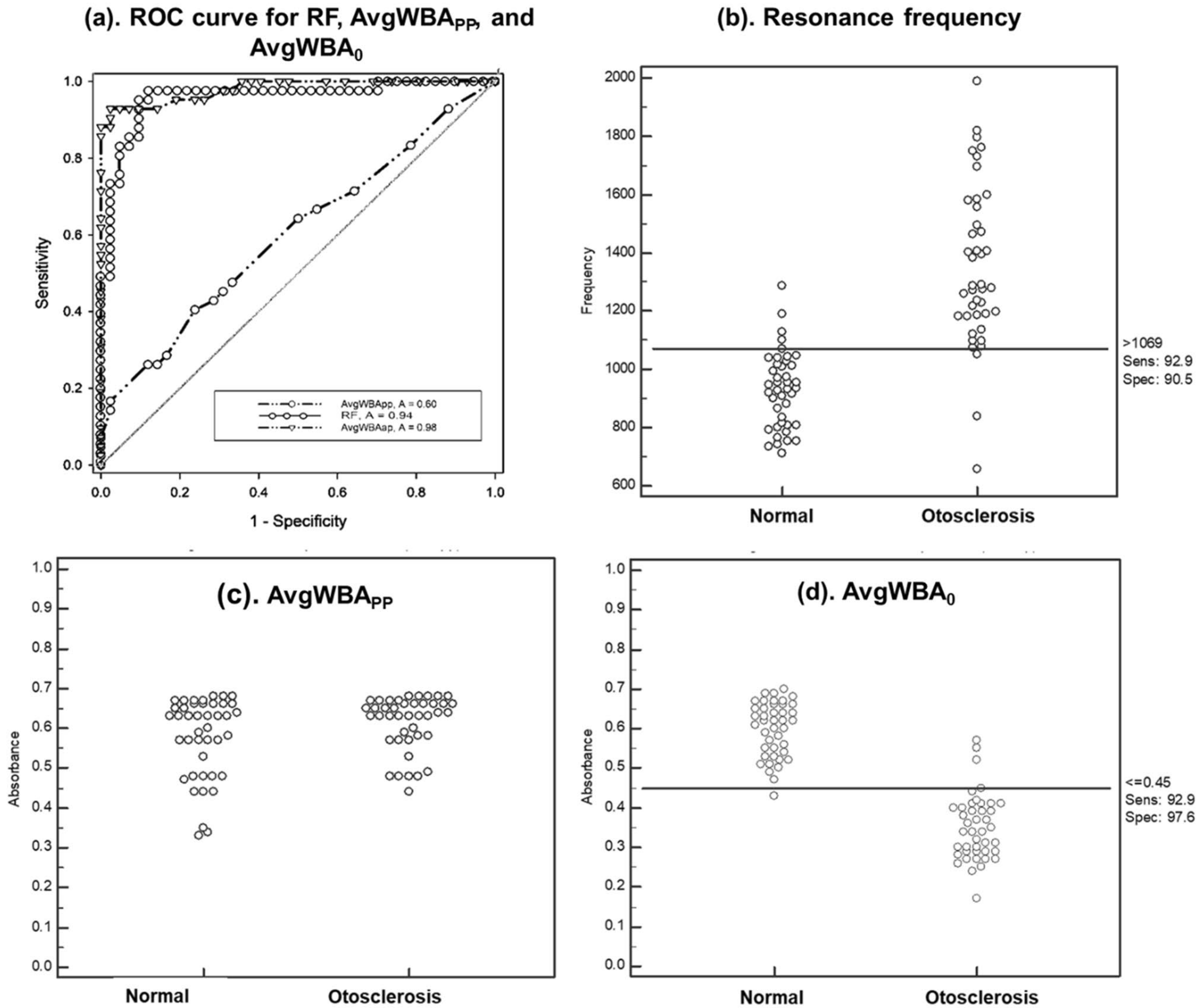


Fig. 3 **a** ROC curve of RF, average absorbance at peak pressure, and ambient pressure; dot plot graph of (**b**) RF, (**c**) average absorbance at peak pressure, and (**d**) ambient pressure

Resonance frequency

The mean RF of the healthy normal ear group was 930.14 Hz with an SD of 130.78 Hz, whereas higher mean RF of 1350.33 Hz with an SD of 275.20 Hz was obtained in ears with otosclerosis. Statistical results using Mann–Whitney U test on RF showed significant differences between the groups ($U = 113.00$, $p < 0.01$). The AUROC value of RF was found to be 0.94 ($p < 0.05$), with the ROC curve lying close to the left and top border of ROC space (Fig. 3a). High sensitivity (92.9%) and specificity (90.5%) were obtained at a cut-off value of 1069 Hz, where a healthy normal ear had an RF of less than 1069 Hz and individuals in the otosclerosis group had a higher RF (Fig. 3b).

Discussion

The present study measured the WBA across the frequencies, wideband average absorbance, and resonance frequency in ears with otosclerosis and compared with those with the healthy normal ear. The results indicated a significant difference in WBA and RF measurements between the otosclerosis and healthy normal ear groups. The study findings are discussed under each of the parameters considered for analysis.

WBA across frequencies

WBA measured at peak and ambient pressure showed reduced absorbance at low frequencies (250–1500 Hz) and slightly increased absorbance at high frequencies above 2000 Hz in otosclerotic ears. This finding was consistent with the previously reported WBT studies where low absorbance values were observed between 400 and 1000 Hz. [11, 22, 23]. This reduction depends on the severity of the otosclerosis and can be seen even beyond 1000 Hz [11]. The decline of WBA at low frequencies could be because of the stiffening of annular ligament generating large impedance, reflecting most of the energy below 2000 Hz on the ear canal [23, 30]. In contrast, the transmission is not stiffness controlled at higher frequencies, i.e., above 2000 Hz, and therefore, has less impact on WBA values [31]. However, the absorbance values at high frequencies are highly variable [23]. Few studies have shown reduced absorbance at 1260 Hz, 2520 Hz, and 4000 Hz in ears with otosclerosis [13, 22]. In contrast, an increase in absorbance from 5000 to 8000 Hz has also been reported [13], similar to the results of this study.

ROC analysis of WBA at peak pressure and ambient pressure showed high accuracy, especially in the detection of otosclerosis at low and mid-frequencies. To our knowledge, few researchers have used ROC analysis to estimate the accuracy of distinguishing between otosclerotic and healthy normal ears. Studies on the effectiveness of WBA have shown high AUROC values at 315 Hz and 500 Hz with 82% sensitivity in otosclerotic ears [11]. Similarly, the results of this study confirmed high sensitivity of over 83% and 95% specificity at frequencies up to 1000 Hz. At 1000 Hz, maximum sensitivity (92.86%) and specificity (100%) were observed at 0.55 absorbance value. Though the current finding warrants for verification with other middle ear pathologies, it can be considered valid if a person has 'A' or 'As' type of tympanogram for 226 Hz probe tone, as this type of tympanogram is uncommon in other middle ear pathologies. Thus, it can be concluded that low and mid-frequencies are efficient in differentiating otosclerosis ears from normal ears.

Studies on changes in pressure in the middle ear have shown significant effects on absorbance patterns [13]. Several studies have reported lower WBA in ambient pressure compared with peak pressure [28, 32]. The WBA measured in Caucasian and Chinese subjects showed low absorbance at ambient pressure for frequencies from 250 to 2500 Hz, while higher WBA for Caucasians at 4000 and 5000 Hz [33]. In the present study, reduced absorbance at ambient pressure was found up to 2000 Hz for both the groups compared with peak pressure. However, a significant difference was obtained between the pressure conditions at 250 Hz and 500 Hz in the healthy normal ears and up to 1500 Hz for ears with otosclerosis. This is because the increase in stiffness of the middle ear due to pressurization, i.e. at peak pressure, improves the transmission of sound energy to the middle ear [34]. However, measuring WBA at ambient pressure causes a negative or positive pressure relative to the middle ear pressure in the ear canal, resulting in a slight change in the stiffness of the tympanic membrane.

Thus, there exists a minimal amount of pressure gradient between the ear canal and middle ear at ambient conditions which would influence the WBA measurements [33]. However, there are no studies that have compared the peak pressure with ambient pressure in the pathological group. In our study, the WBA values at ambient pressure were lesser compared with peak pressure for frequencies from 250 to 1500 Hz in ears with otosclerosis.

Wideband average absorbance

Wideband average absorbance (average of absorbance values obtained between 375 and 2000 Hz) was measured at peak pressure and ambient pressure. The present study compared the average absorbance of otosclerosis with that of the healthy normal ear group at peak and ambient pressure. The study revealed a significantly reduced average absorbance at ambient pressure in the otosclerosis group compared with the healthy normal ear group, and no differences were observed between the groups at the peak pressure. However, only limited research has focused on this area. The result of one of the studies indicated that the average absorbance measured at peak pressure ranged from 0.64 to 0.92 [35]. In another study, results showed reduced overall absorbance rate at peak pressure ($<30\%$) in both the ears for individuals with otosclerosis, compared with normal hearing individuals [36]. However, the average absorbance of the current study at peak pressure ranged between 0.33 and 0.68 in ears with otosclerosis. Currently, there are no studies that compare the average absorbance measured at ambient pressure in the pathological groups.

A WBA study, averaged over 600 Hz and 1000 Hz at peak pressure, in combination with an air–bone gap averaged over 1000–4000 Hz, showed 86% sensitivity and 100% specificity to differentiate otosclerosis [25]. The present study on the wideband averaged absorbance between 375 and 2000 Hz at ambient pressure showed a sensitivity of 92.2% and a specificity of 97% at a cut-off point of 0.45 absorbance value.

Resonance frequency

Analysis of resonance frequency of the middle ear showed increased resonance frequency for the otosclerosis group. Several studies have shown an increase in resonance frequency, as one of the indicators for identifying otosclerosis [5, 13]. Typically, the mass component limits high-frequency transmission, while the stiffness component limits low-frequency transmission [8]. In ears with otosclerosis, there is an increase in the stiffness of the middle ear, which limits the low frequencies and enhances the high-frequency transmissions [8]. However, in the present study, a significant overlap of the resonance frequency was observed between otosclerosis (659–1989 Hz) and the normal healthy ear group (710–1288 Hz), which limits the effectiveness of the diagnosis of otosclerosis [5, 13]. ROC curve analysis for the resonance frequency showed a high sensitivity (92.9%) and specificity (90.5%) at the cut-off frequency of 1069 Hz. Earlier, such an investigation has shown similar results with high sensitivity and specificity of 80% and 82%, respectively, at a cut-off value of 1025 Hz [37]. In contrary, a few studies have reported similar resonance frequency findings between the two groups [5, 13], and this similarity has been attributed to the severity of otosclerosis that changes the stiffness of the middle ear [5, 11, 22]. Studies are also indicating high resonance frequency in other pathological cases such as tympanosclerosis or Meniere's disease [37]. Thus, the use of resonance frequency as an independent test tool is critical and hence should be used cautiously while diagnosing otosclerosis.

The results of the current study suggest high sensitivity and specificity of > 90% for all the parameters analyzed, with resonance frequency showing the least sensitivity and specificity and WBA measured at 1000 Hz showing the highest sensitivity and specificity. Thus, the results of the current study indicate that WBA measurement is probably the best tool to identify ears with otosclerosis.

Limitations of the study

The current study has shown differences in WBA and RF measurements in otosclerosis with healthy, normal ears, but with some limitations. First, the number of samples recruited for this study was relatively small. Thus, the power of the statistical analysis could have reduced. Second, the criteria for enrollment of participants in the otosclerosis group were based solely on clinically accepted criteria [27] and confirmation from an otolaryngologist. However, administration of computed tomography (CT) would have objectively confirmed the diagnosis of all participants in the otosclerosis group. Third, the present study had shown that 1000 Hz is the most sensitive to identify otosclerotic ears, which needs to be evaluated with other middle ear disorders to know whether it is unique to ears with otosclerosis or can be seen in other conditions also.

Conclusion

The present study showed a high sensitivity and specificity of WBA measurements in differentiating ears with otosclerosis from the healthy normal ears. This also suggests that the WBA pattern obtained at frequencies below 1500 Hz could effectively identify ears with otosclerosis from healthy normal ears, especially the absorbance value obtained at 1000 Hz. Wideband average absorbance obtained at ambient pressure could also be used to identify ears with otosclerosis. However, it should always be supplemented by other audiological test findings suggesting otosclerosis. Though resonance frequency for individuals with otosclerosis was higher compared with the healthy normal ear, it cannot be utilized as an independent tool due to a wider overlap of resonance frequency with that of healthy normal ears. The current study results suggest that WBA findings can effectively improve identification of otosclerosis along with the existing middle ear measurements. However, further research is required to standardize the test and incorporate it into routine clinical practice.

Acknowledgements We thank Dr. Vasanthalakshmi M.S., Biostatistician, All India Institute of Speech and Hearing, and Dr. Vijay Kumar Narne, Research Fellowship for their assistance in statistics and data extraction from MATLAB for the study. Special thanks to Interacoustics, Denmark, for providing Research module of TITAN software, and the All India Institute of Speech and Hearing, Mysuru affliated to the University of Mysore for providing infrastructure to carry forward the research.

Author contributions Arunraj Karuppannan's contribution: involved in the conception and design of the study, data collection, statistical analysis and interpretation of data, and drafted the manuscript. Animesh Barman's contribution: involved in the conception and design of the study, analysis, and interpretation of data. Provided input on the protocol used, statistical analysis, and edited and approved the manuscript.

Funding The authors declare that the study does not have any funding sources and support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Approval was obtained from the institutional ethical review committee of the All India Institute of Speech and Hearing (AIISH), and it is based on the ethical guidelines of AIISH for biobehavioral research involving the human subject. The procedure used in this study adheres to the tenets of the 2013 Declaration of Helsinki.

Consent to participate Informed consent was taken from all the participants, who had enrolled for the study.

Consent for publication Not applicable.

Availability of data and material The data used in this study can be availed from the corresponding author [Arunraj Karuppannan] upon reasonable request.

Code availability Not applicable.

References

1. Markou K, Goudakos J (2009) An overview of the etiology of otosclerosis. Eur Arch Oto-Rhino-Laryngol 266:25–35. https://doi.org/10.1007/s00405-008-0790-x
2. Schrauwen I, Khalfallah A, Ealy M et al (2012) COL1A1 association and otosclerosis: a meta-analysis. Am J Med Genet Part A 158:1066–1070. https://doi.org/10.1002/ajmg.a.35276
3. Kanzara T, Virk JS (2017) Diagnostic performance of high resolution computed tomography in otosclerosis. World J Clin Cases 5:286. https://doi.org/10.12998/wjcc.v5.i7.286
4. Danesh AA, Shahnaz N, Hall JW (2018) The audiology of otosclerosis. Otolaryngol Clin North Am 51:327–342. https://doi.org/10.1016/j.otc.2017.11.007
5. Shahnaz N, Polka L (1997) Standard and multifrequency tympanometry in normal and otosclerotic ears. Ear Hear 18:326–341. https://doi.org/10.1097/00003446-199708000-00007
6. Browning GG, Swan IRC, Gatehouse S (1985) The doubtful value of tympanometry in the diagnosis of otosclerosis. J Laryngol Otol 99:545–547. https://doi.org/10.1017/S002221510009722X
7. Shahnaz N, Polka L (2002) Distinguishing healthy from otosclerotic ears: effect of probe-tone frequency on static immittance. J Am Acad Audiol 13:345–355
8. Kim J, Koo M (2015) Mass and stiffness impact on the middle ear and the cochlear partition. Korean J Audiol 19:1–6
9. André KD, Sanches SGG, Carvallo RMM (2012) Middle ear resonance in infants: age effects. Int Arch Otorhinolaryngol 16:353–357. https://doi.org/10.7162/S1809-97772012000300009
10. Miani C, Bergamin AM, Barotti A, Isola M (2000) Multifrequency multicomponent tympanometry in normal and otosclerotic ears. Scand Audiol 29:225–237. https://doi.org/10.1080/010503900750022853
11. Shahnaz N, Bork K, Polka L et al (2009) Energy reflectance and tympanometry in normal and otosclerotic ears. Ear Hear 30:219–233. https://doi.org/10.1097/AUD.0b013e3181976a14
12. Holte L (1996) Aging effects in multifrequency tympanometry. Ear Hear 17:12–18. https://doi.org/10.1097/00003446-199602000-00002
13. Wang S, Hao W, Xu C et al (2019) A study of wideband energy reflectance in patients with otosclerosis: data from a Chinese population. Biomed Res Int 2019:1–8. https://doi.org/10.1155/2019/2070548
14. Naumann IC, Porcellini B, Fisch U (2005) Otosclerosis: incidence of positive findings on high-resolution computed tomography and their correlation to audiological test data. Ann Otol Rhinol Laryngol 114:709–716. https://doi.org/10.1177/000348940511400910
15. Aithal S, Kei J, Driscoll C et al (2015) Wideband absorbance outcomes in newborns: a comparison with high-frequency tympanometry, automated brainstem response, and transient evoked and distortion product otoacoustic emissions. Ear Hear 36:e237–e250. https://doi.org/10.1097/AUD.0000000000000175
16. Feeney MP, Keefe DH, Marryott LP (2003) Contralateral acoustic reflex thresholds for tonal activators using wideband energy reflectance and admittance. J Speech, Lang Hear Res 46:128–136. https://doi.org/10.1044/1092-4388(2003/010)
17. Interacoustics (2016) Titan: Technical Specifications. https://www.interacoustics.com/support/titan/288-technical-specification-titan. Accessed 31 Mar 2020
18. Prieve BA, Werff KRV, Preston JL, Georgantas L (2013) Identification of conductive hearing loss in young infants using tympanometry and wideband reflectance. Ear Hear 34:168–178. https://doi.org/10.1097/AUD.0b013e31826fe611
19. Nakajima HH, Rosowski JJ, Shahnaz N, Voss SE (2013) Assessment of ear disorders using power reflectance. Ear Hear 34:48S-53S. https://doi.org/10.1097/AUD.0b013e31829c964d
20. Keefe DH, Archer KL, Schmid KK et al (2017) Identifying otosclerosis with aural acoustical tests of absorbance, group delay, acoustic reflex threshold, and otoacoustic emissions. J Am Acad Audiol 28:838–860. https://doi.org/10.3766/jaaa.16172
21. Voss S, Merchant GR, Horton NJ (2012) Effects of middle-ear disorders on power reflectance measured in cadaveric ear canals. Ear Hear 33:195–208. https://doi.org/10.1097/AUD.0b013e31823235b5
22. Feeney MP, Grant IL, Marryott LP (2003) Wideband energy reflectance measurements in adults with middle-ear disorders. J Speech Lang Hear Res 46:901–911. https://doi.org/10.1044/1092-4388(2003/070)
23. Allen JB, Jeng PS, Levitt H (2005) Evaluation of human middle ear function via an acoustic power assessment. J Rehabil Res Dev 42:63–77. https://doi.org/10.1682/JRRD.2005.04.0064
24. Shahnaz N, Longridge N, Bell D (2009) Wideband energy reflectance patterns in preoperative and post-operative otosclerotic ears. Int J Audiol 48:240–247. https://doi.org/10.1080/14992020802635317
25. Nakajima HH, Pisano DV, Roosli C et al (2012) Comparison of ear-canal reflectance and umbo velocity in patients with conductive hearing loss: a preliminary study. Ear Hear 33:35–43. https://doi.org/10.1097/AUD.0b013e31822ccba0
26. World Medical Association (2013) World Medical Association declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA - J Am Med Assoc 310:2191–2194. https://doi.org/10.1001/jama.2013.281053
27. Nadol JB (1998) Otosclerosis: clinical aspects and management. Audio Digest Otolaryngol 31:2
28. Liu Y-W, Sanford CA, Ellison JC et al (2008) Wideband absorbance tympanometry using pressure sweeps: system development and results on adults with normal hearing. J Acoust Soc Am 124:3708–3719. https://doi.org/10.1121/1.3001712
29. Feise RJ (2002) Do multiple outcome measures require *p*-value adjustment? BMC Med Res Methodol 2:1–4. https://doi.org/10.1186/1471-2288-2-8
30. Feeney MP, Keefe DH (1999) Acoustic reflex detection using wide-band acoustic reflectance, admittance, and power measurements. J Speech Lang Hear Res 42:1029–1041. https://doi.org/10.1044/jslhr.4205.1029
31. Pickles J (2012) An introduction to the physiology of hearing, 4th edn. Brill Academic Publishers, UK
32. Shahnaz N, Bork K (2006) Wideband reflectance norms for Caucasian and Chinese young adults. Ear Hear 27:774–788. https://doi.org/10.1097/01.aud.0000240568.00816.4a
33. Kenny SPVN (2013) Clinical application of the interacoustics REFLWIN system wideband energy reflectance machine in the assessment of the eustachian tube. J Chem Inf Model 53:1689–1699. https://doi.org/10.1017/CBO9781107415324.004
34. Shahnaz N, Patrick Feeney M, Schairer KS (2013) Wideband acoustic immittance normative data: Ethnicity, gender, aging, and instrumentation. Ear Hear 34:27S-35S. https://doi.org/10.1097/AUD.0b013e31829d5328
35. Niemczyk E, Lachowska M, Tataj E et al (2019) Wideband tympanometry and absorbance measurements in otosclerotic ears. Laryngoscope 129:E365–E376. https://doi.org/10.1002/lary.27747
36. Neto LC, Cibin BC, Mota R et al (2014) Case Report Acoustic reflectance measurements in otosclerosis: case study Medidas de reflectância acústica na otosclerose: estudo de caso. Audiol Commun Res 19:421. https://doi.org/10.1590/S2317-64312014000300001420
37. Ogut F, Serbetcioglu B, Kirazli T et al (2008) Results of multiple-frequency tympanometry measures in normal and otosclerotic middle ears. Int J Audiol 47:615–620. https://doi.org/10.1080/14992020802178656

J Hear Sci, 2020; 10(4): 40–47
DOI: 10.17430/JHS.2020.10.4.5

ISSN: 2083-389X,
eISSN: 2084-3127

EVALUATION OF WIDEBAND ABSORBANCE TYMPANOMETRY IN ADULTS WITH ABNORMAL POSITIVE AND NEGATIVE MIDDLE EAR PRESSURE

Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

Arunraj Karuppannan[1,2,A-F], Animesh Barman[3,A,C-E]

[1] Department of Audiology/Prevention of Communication Disorders, All India Institute of Speech and Hearing, India
[2] Department of Audiology, All India Institute of Speech and Hearing, India

Corresponding author: Arunraj Karuppannan; Department of Audiology/Prevention of Communication Disorders, All India Institute of Speech and Hearing, Manasagangothri, 570006, Mysuru, India; email: nahularun@gmail.com

Abstract

Background: Middle ear pressure plays a vital role in the transmission of sound to the inner ear. However, limited research data exists to understand the effect of abnormal middle ear pressure on wideband absorbance (WBA) tympanometry. The purpose of the study was to evaluate WBA at peak pressure and ambient pressure in adults with abnormal positive and negative middle ear pressure and compare them with normal adult ears having normal middle ear pressure.

Material and methods: Three groups of adults – normal middle ear pressure group (56 ears), negative middle ear pressure group (30 ears), and positive middle ear pressure group (15 ears) – in the age range 22 to 50 years were considered. WBA was measured at peak and ambient pressures across the frequencies from 250 to 8000 Hz.

Results: WBA at peak pressure was observed to be higher than at ambient pressure in all the groups, with the difference seen mostly at low and mid-frequencies up to 2000 Hz. The negative middle ear pressure group showed the most considerable difference in mean WBA, seen between 600 Hz and 1000 Hz, followed by the positive middle ear pressure group, with a negligible difference for the normal middle ear pressure group.

Conclusions: The study highlighted the importance of measuring WBA at peak pressure and ambient pressure. The results suggest that the comparison of WBA at peak and ambient pressures, especially from lower to mid-frequencies up to 2000 Hz, would help in differentiating abnormal negative/positive pressure from normal middle ear pressure and also between ears having negative and positive pressure.

Key words: absorbance • peak pressure • middle ear pressure • ambient pressure

OCENA ABSORBANCJI SZEROKOPASMOWEJ U DOROSŁYCH Z PODCIŚNIENIEM I NADCIŚNIENIEM W UCHU ŚRODKOWYM

Streszczenie

Wstęp: Ciśnienie w uchu środkowym odgrywa istotną rolę w przekazywaniu dźwięku do ucha wewnętrznego. Jednak istnieją ograniczone dane badawcze pozwalające zrozumieć wpływ nieprawidłowego ciśnienia w uchu środkowym na tympanometrię absorbancji szerokopasmowej (WBA). Celem pracy była ocena WBA przy ciśnieniu szczytowym i ciśnieniu otoczenia u dorosłych z podciśnieniem i nadciśnieniem w uchu środkowym i porównanie ich z wynikami u dorosłych z prawidłowym ciśnieniem w uchu środkowym.

Materiał i metody: W badaniu udział wzięły trzy grupy dorosłych w wieku od 22 do 50 lat: grupa z normalnym ciśnieniem w uchu środkowym (56 uszu), grupa z podciśnieniem w uchu środkowym (30 uszu) i grupa z nadciśnieniem w uchu środkowym (15 uszu). WBA mierzono przy ciśnieniu szczytowym i ciśnieniu otoczenia w zakresie częstotliwości od 250 Hz do 8000 Hz.

Wyniki: Zaobserwowano, że WBA przy ciśnieniu szczytowym jest wyższe niż przy ciśnieniu otoczenia we wszystkich grupach, z różnicą obserwowaną głównie przy częstotliwościach niskich i średnich do 2000 Hz. Grupa z podciśnieniem w uchu środkowym wykazała największą różnicę w średnim WBA, obserwowaną między 600 Hz a 1000 Hz. Następna była grupa z nadciśnieniem w uchu środkowym, natomiast w grupie z normalnym ciśnieniem w uchu środkowym różnica była nieistotna.

Wnioski: Badanie wskazuje na znaczenie oceny WBA przy ciśnieniu szczytowym i ciśnieniu otoczenia. Wyniki sugerują, że porównanie WBA przy ciśnieniu szczytowym i ciśnieniu otoczenia, zwłaszcza w przedziale od niskich do średnich częstotliwości do 2000 Hz, pomogłoby w odróżnieniu podciśnienia/nadciśnienia od normalnego ciśnienia w uchu środkowym, a także rozróżnieniu podciśnienia i nadciśnienia pomiędzy obojgiem uszu.

Słowa kluczowe: absorbancja • ciśnienie szczytowe • ciśnienie w uchu środkowym • ciśnienie otoczenia

Introduction

Conventional tympanometry is carried out using either a single probe tone frequency or multiple frequencies by varying air pressure in the ear canal to measure the admittance of the middle ear. The transmission of sound energy is usually maximum at the level where the pressure is equal on both sides of the tympanic membrane [1].

In individuals with a healthy middle ear, the maximum energy flows into the middle ear at a pressure closest to that of the atmosphere [1]. Any deviation of middle ear pressure (MEP) from atmospheric pressure is likely to hinder the effective transmission of sound to the inner ear. Abnormal MEP is indicative of some abnormality in the middle ear, perhaps leading to middle ear disorders. Thus, air pressure plays a significant role in the transmission of sound to the middle ear.

Recent studies have indicated that wideband absorbance (WBA) is a sensitive tool in assessing middle ear function compared to conventional tympanometry [2] and in the differential diagnosis of middle ear disorders [3,4]. WBA is a non-invasive middle ear analysis technique that measures either the absorbance or reflectance of sound energy across a wide range of frequencies between 200 to 8000 Hz [5]. Studies have shown a distinct pattern of WBA for various pathological conditions such as otosclerosis, ossicular chain discontinuity, tympanic membrane perforation, and fluid in the middle ear cavity [2,3,6].

The most common occurring middle ear pathology that affects the transmission of sound to the inner ear is abnormal negative or positive MEP [7]. In the condition of Eustachian tube dysfunction (ETD), maximum energy flows at a negative pressure; on the other hand a positive pressure range is usually seen in the early stages of acute otitis media without effusion, where there is a bulging tympanic membrane [1,8]. Such abnormal pressure characteristics can be effectively studied by measuring middle ear absorbance across frequency, and also at different pressures, improving the accuracy with which middle ear disorders can be diagnosed.

Few efforts have been made to study the effect of abnormal MEP on WBA measurements [7,9–11], and most of these studies were based on simulated conditions in healthy individuals or cadavers. Studies have shown that any alteration in MEP increases energy reflectance (decreases absorbance) below 3 to 4 kHz and decreases reflectance (increases absorbance) at frequencies beyond 4 kHz [12]. On the other hand, studies on young children who had tympanometric peak pressure (TPP) more negative than −100 daPa have shown increased energy reflectance (decreased absorbance) for frequencies up to 4 to 5 kHz [9,10]. Thus, there is an apparent inconsistency in the findings of WBA under altered MEP conditions, especially at higher frequencies.

Further, the effect of pressure variations (ambient and peak) on WBA tympanometry readings has indicated a reduction in absorbance below 2 kHz under ambient pressure conditions compared to WBA at peak pressure [4,7,13]. To date, only one study has evaluated the difference in pressure variation on children with ETD and compared the result in children with normal ears [11]. Interestingly, the study showed a differential absorbance pattern with significant lower WBA only at ambient pressure for children with ETD, compared to normal ears [11]. On the other hand, no attempts have been made to use WBA tympanometry to evaluate the effect of positive MEP.

It is well known that a variation in MEP induces physiological changes in middle ear structures and thereby affects transmission of sound to the inner ear. Thus, it is crucial to understand the effect of MEP on WBA across frequency and to quantify the effects of positive and negative MEP. Also, to the best of our knowledge, there has been no previous report of WBA findings on an adult population who have abnormal positive or negative MEP. Thus, the purpose of this study was to examine WBA tympanometry findings on adults who had abnormal negative or positive MEP (as indicated by conventional tympanometry). The study also sought to determine the differences in WBA between peak and ambient pressures in those abnormal MEP subjects compared with the results from subjects with normal MEP.

Materials and methods

A total of three groups of adult subjects in the age range 22 to 50 years (mean age 36.0 ± 10.1 years) were considered for the study. The control group (normal MEP group) consisted of 37 healthy individuals (56 ears) having normal MEP of +50 to −100 daPa (mean −10.89 ± 12.28 daPa; range −32 to 12 daPa) as given by Jerger [14]. This middle ear pressure was obtained using conventional 226 Hz probe tone tympanometry by inserting a probe into the ear canal. The ear canal pressure at which the peak of the tympanogram occurred was considered as the TPP/MEP [28]. All participants in this group had normal hearing thresholds of less than 15 dB HL at all octave frequencies, static admittance of <1.6 mmho (mean 0.73 ± 0.34; range 0.22 to 1.54) with the presence of normal acoustic reflex thresholds, and the presence of transient evoked otoacoustic emission (≥3 dB SNR).

The clinical study group consisted of two sub-groups that included adults having 'negative middle ear pressure' more negative than −100 daPa (MEP_N) and a 'positive middle ear pressure' group having pressure greater than 50 daPa (MEP_P), measured using conventional 226 Hz probe tone tympanometry [14]. The MEP_N group included 25 participants (30 ears) with a mean static admittance of 0.70 mmho ± 0.41 SD (range 0.16 to 1.87) and mean TPP of −207.0 daPa ± 99.9 SD (range −353 to −108 daPa). MEP_P consisted of 14 participants (15 ears) who had mean TPP of 156.9 daPa ±43.6 SD (range 75 to 168 daPa) and mean static admittance of 0.58 mmho ± 0.24 SD (range 0.15 to 1.17 daPa). Both study groups had hearing thresholds that ranged between minimal to mild conductive hearing loss, with elevated or absent acoustic reflex thresholds. However, all groups had an intact tympanic membrane without any perforation or active ear discharge. The institutional Ethics committee of bio-behavioural research involving human subjects reviewed and approved this study (No. WOF-0404/2014-15 with effect from 04.06.14). Informed consent was obtained from each of the subjects before enrolling in the present study.

Wideband absorbance measurement was performed using the Interacoustics Titan IMP/WBT 440 equipment and it measured absorbance values under two conditions: at peak pressure (WBA_{PP}) and ambient (0 daPa) pressure (WBA_0). The peak pressure is the ear canal pressure that was obtained using the wideband average tympanometry (i.e. average of WBA from 375 to 2000 Hz) that was automatically generated during WBA measurement in the Titan equipment.

However, the mean TPP obtained from WBA measurement and the conventional 226 Hz probe tympanometry were identical. The probe was inserted into the ear canal, and a click stimulus of 100 dB peSPL (approx. 65 dBnHL) at a rate of 21.5 Hz was delivered. WBA was measured across frequencies (250–8000 Hz) while ear canal pressure was swept from +200 to −400 daPa at a rate of 200 daPa/sec. Before administrating the test, daily calibration of the WBA equipment was performed using four couplers as recommended by the manufacturer. The study extracted WBA values at 1/3rd octave frequencies (16 frequencies) at peak pressure and ambient pressure which were placed in an Excel spreadsheet and transferred to SPSS version 21.0 for statistical analysis.

Statistical analysis

Data were analysed using descriptive and inferential statistics. Parametric analysis was performed as the absorbance data followed a normal distribution as indicted by a Shapiro–Wilk normality test ($p>0.05$). A mixed-model ANOVA was carried out to analyse WBA between the groups (normal MEP, MEP_N, and MEP_P) and pressure conditions (peak, ambient) across 16 frequencies (250 to 8000 Hz). A Greenhouse–Geisser approach [15] was used to compensate for violation of compound symmetricity and sphericity. A post hoc Tukey's Honestly Significant Difference (HSD) was administered for multiple pair-wise comparisons with a *p*-value of 0.05 as the level of significance. A paired-sample *T*-test was performed within the subject groups to compare peak and ambient pressure across the frequencies.

Results

Descriptive statistical results (mean and standard error) for WBA_{PP} and WBA_0 measured across frequencies for the three groups are illustrated in Figure 1. It can be observed that in the normal MEP group, at both peak and ambient pressure, WBA increases gradually as the frequency increases from 250 Hz, reaches a maximum at 2000 Hz, and reduces thereon to a minimum at 6000 Hz. At peak pressure, the MEP_P group showed a biphasic pattern, i.e., an increase then plateau at mid-frequencies, followed by a rise to a maximum at 2500 Hz, and then a further decline. At peak pressure the MEP_N group showed a similar pattern to the normal MEP group with a lower absorbance from frequencies between 250 Hz and 3000 Hz. However, at ambient pressure, all three groups showed a similar pattern, though the absorbance for the clinical groups was significantly lower than the normal group. On the other hand, at and above 4000 Hz there was no noticeable difference in mean absorbance across the groups for either WBA_{PP} or WBA_0.

A mixed ANOVA model was used to analyse the WBA, with pressure conditions (WBA_{PP} and WBA_0) and frequency (250 to 8000 Hz) as within-subject factors, and the study groups (normal MEP, MEP_N, and MEP_P) as between-subject factors. The main effects of pressure conditions ($F = 1.775$, $p = 0.00$, $\eta_p^2 = 0.634$), groups ($F = 49.850$, $p = 0.00$, $\eta_p^2 = 0.507$), and frequency ($F = 281.32$, $p = 0.00$, $\eta_p^2 = 0.744$) were significant. The interaction effect between groups and pressure conditions ($F = 112.42$, $p = 0.00$, $\eta_p^2 = 0.699$), groups and frequency ($F = 15.60$, $p = 0.00$, $\eta_p^2 = 0.243$), and pressure conditions and frequency ($F = 38.16$, $p = 0.00$, $\eta_p^2 = 0.282$) were also significant. Further, MANOVA was performed to investigate the frequencies at which the group differences occurred under the two different pressure conditions (peak, ambient). The result revealed a significant difference between the groups at low and mid-frequencies until 3000 Hz ($p<0.05$) for both pressure conditions, whereas high frequencies (4000–8000 Hz) did not show any significant difference ($p>0.05$).

To further investigate the interaction between groups and frequency across the pressure conditions, a post hoc Tukey's HSD test was performed. In comparison to the normal MEP group, a significant decrease in absorbance at WBA_{PP} was observed from 250 to 3000 Hz for the MEP_P group, and from 600 to 3000 Hz for the MEP_N group. Whereas between the MEP_N and MEP_P groups, a significantly lower absorbance was seen between 1000 and 2000 Hz for MEP_P compared to MEP_N. Compared to the normal MEP group, MEP_P had the most significant reduction in absorbance up to 3000 Hz,

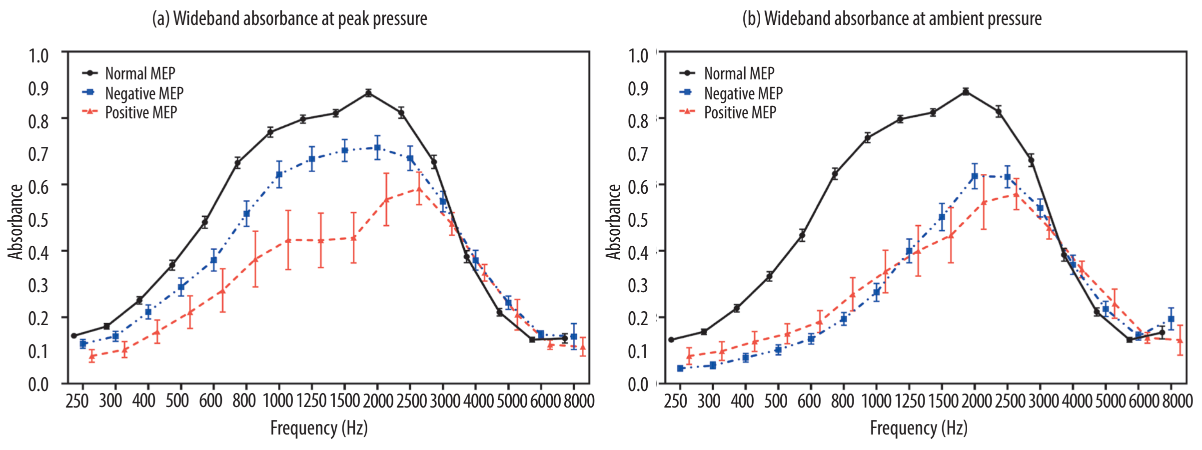


Figure 1. Comparison of mean absorbance and standard error (whiskers) measured at (a) peak pressure (WBA_{PP}) and (b) ambient pressure (WBA_0) for each of the three groups

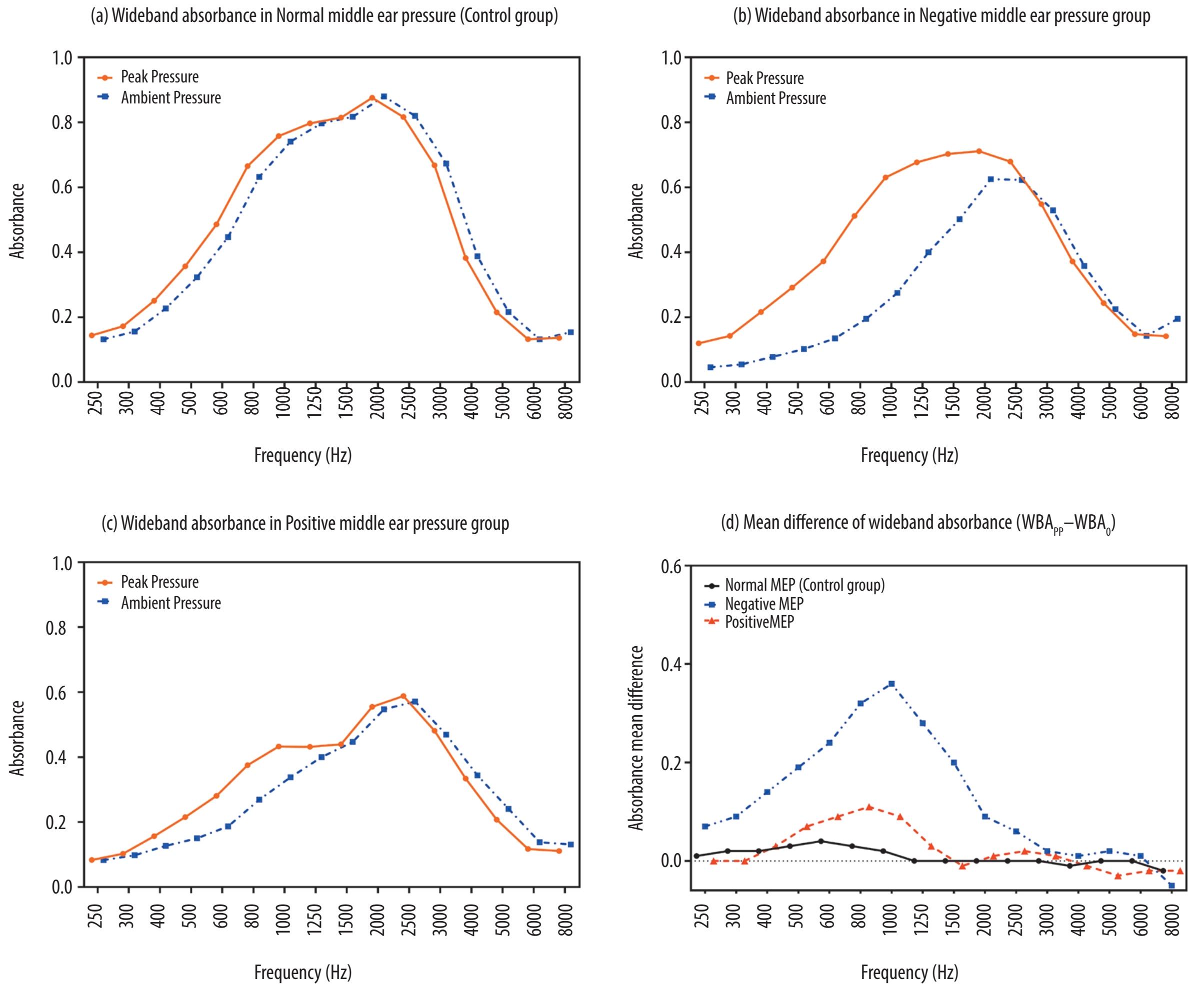


Figure 2. Comparison of WBA measured at peak pressure and ambient pressure in (a) Normal MEP group; (b) Negative MEP group; (c) Positive MEP group; and (d) Mean difference in WBA between pressure conditions (WBA_{pp} – WBA_0)

followed by MEP_N (as seen in Figure 1a). In examining WBA_0 (Figure 1b), it was noted that both MEP_N and MEP_P showed a significant difference (p<0.05) in comparison to the normal MEP group, indicating a decrease in absorbance for frequencies between 250 and 3000 Hz. However, no significant difference was found between the MEP_N and MEP_P group, showing similar absorbance values across frequencies.

In comparing WBA_{PP} and WBA_0 between the groups, the normal MEP group had similar mean absorbance values across the frequencies for both peak and ambient pressures (Figure 2a). In contrast, lower absorbance was observed for ambient pressure up to 1250 Hz for the MEP_P group (Figure 2c) and up to 2500 Hz for the MEP_N group (Figure 2b). The results of a paired T-test performed between peak and ambient pressure within groups showed a significant difference at low and mid-frequencies between any two groups. Though little difference in absorbance values was observed for the normal MEP group, there was a small but significant difference between 250 and 1000 Hz (Figure 2a). Also, there was a significant difference for frequencies between 500 and 1000 Hz for the MEP_P group, and from 250 to 2500 Hz for the MEP_N group.

The most marked observation that emerged from the data comparison, as seen in Figure 2d, was the mean difference in absorbance between peak and ambient pressure. For the normal MEP group, the difference in WBA across frequencies are hardly distinguishable, ranging from 0.02 to 0.04 absorbance units between 300 and 1000 Hz. Whereas, for the MEP_P group there was a marginal increase in mean difference between 500 and 1000 Hz (0.07 to 0.11 units). Of most interest, the mean difference was most significant for the MEP_N group, which increased from 0.07 absorbance at 250 Hz to a maximum of 0.36 absorbance at 1000 Hz and decreased beyond. At frequencies above 2500 Hz, there was a negligible mean difference for all the study groups.

Table 1 summarises the mean difference of WBA_{PP}–WBA_0 for each of the groups and the significance level obtained between the groups. Overall, the findings show a significant mean absorbance difference across the groups [$F(32,166) = 9.17$, p <0.000; Wilk's $\Lambda = 0.131$, $\eta_p^2 = 0.639$]. Further analysis across frequencies revealed a significant difference for all frequencies up to 2500 Hz (p<0.05), with the effect size (η_p^2) increasing from 0.31 at 250 Hz to 0.64 up to 1000 Hz and reducing to <0.3 above 1500 Hz. Additionally, post hoc Tukey's HSD analysis revealed significant differences between the normal MEP group and MEP_N up to 2500 Hz, and MEP_N and MEP_P up to 2000 Hz, whereas a significant difference was obtained only at 1000 Hz between the normal MEP group and the MEP_P group. On the whole, WBA at 1000 Hz showed a significant difference in all the groups, pointing to it being a distinctive indicator that could be considered for differentiating the abnormal pressure group from the normal MEP group.

To summarise, WBA at peak pressure was higher than at ambient pressure, especially at low and mid frequencies, irrespective of the study group. However, the difference in magnitude was more significant for the MEP_N group, negligible for the normal MEP group, and intermediate for the MEP_P group. The most notable mean difference between WBA_{PP} and WBA_0 was seen between 600 and 1000 Hz, with a significant difference at 1000 Hz for all the groups and this could be a distinctive indicator for differentiation. Also, in the MEP_N group the difference in absorbance between the two pressure conditions was extended across a wider frequency range. In contrast, for the MEP_P group it is restricted to a smaller frequency range. The findings of the study confirm the usefulness of measuring WBA_{PP} and WBA_0. Further, the difference in absorbance values between pressure conditions seems to be most sensitive across the mid-frequency range.

Discussion

The purpose of this study was to compare WBA at peak pressure and ambient pressure in ears with an abnormal positive and negative pressure with that of normal individuals having normal middle ear pressure. The WBA was least at 250 Hz, increased gradually as the frequency increased and reached a maximum at 2000 Hz in ears with normal hearing. At higher frequencies, the absorbance reduced gradually to a minimum at 6000 Hz and beyond. These findings are very similar to what has been found previously and have an identical absorbance pattern. However, the reported frequencies of maximum absorbance are not exactly consistent across studies and are reported to be anywhere between 2000 and 4000 Hz [16–18]. A few studies have shown other maxima in the region of 1000 Hz to 1500 Hz [16,19], whereas the present study found maximum absorbance at 2000 Hz. This variation in absorbance values might be attributed to racial differences, since studies have reported differences in absorbance in different ethnic populations [17,20].

The outcomes of the present study indicate that, especially in the negative MEP group, there is a significant difference in WBA at low and mid-frequencies, with lower absorbance at ambient pressure compared to peak pressure. In contrast, a negligible difference in WBA occurs for adults with normal MEP. Limited studies have reported a change in WBA measured at abnormal MEP, and these have been performed mostly in young children [9–11,21]. Few efforts have been made to examine the effect of negative MEP on WBA in adults. Earlier studies on negative MEP indicated a decrease in absorbance for frequencies below 3 to 4 kHz and a small increase in absorbance at higher frequencies [4,7,12]. Our findings appear to well support the earlier studies. The present study showed decreased absorbance at low and mid

Table 1. Mean difference of WBA_{pp}–WBA_0 for three groups and its significance level

Frequency (Hz)	Mean difference of WBA_{pp}–WBA_0			Significance level (*p*-value)			Effect size (ηp^2)
	CG	MEP_P	MEP_N	CG vs. MEP_P	CG vs. MEP_N	MEP_N vs. MEP_P	
250	0.01	0.00	0.07	0.66	0.00*	0.00*	0.31
300	0.02	0.00	0.09	0.68	0.00*	0.00*	0.34
400	0.02	0.03	0.14	0.94	0.00*	0.00*	0.37
500	0.03	0.07	0.19	0.43	0.00*	0.00*	0.38
600	0.04	0.09	0.24	0.18	0.00*	0.00*	0.41
800	0.03	0.11	0.32	0.09	0.00*	0.00*	0.53
1000	0.02	0.09	0.36	0.05*	0.00*	0.00*	0.64
1250	0.00	0.03	0.28	0.65	0.00*	0.00*	0.52
1500	0.00	–0.01	0.20	0.99	0.00*	0.00*	0.33
2000	0.00	0.01	0.09	0.91	0.00*	0.04*	0.14
2500	0.00	0.02	0.06	0.50	0.00*	0.14	0.15
3000	0.00	0.01	0.02	0.48	0.09	0.89	0.05
4000	–0.01	–0.01	0.01	0.88	0.08	0.07	0.07
5000	0.00	–0.03	0.02	0.95	0.12	0.06	0.32
6000	0.00	–0.02	0.01	0.74	0.31	0.56	0.28
8000	–0.02	–0.02	–0.05	0.95	0.69	0.66	0.01

*Significance level $p<0.05$
CG - Control group (Normal MEP); MEP_N - Negative middle ear pressure group; MEP_P - Positive middle ear pressure group

frequencies up to 2500 Hz, with increased absorbance at high frequencies and a noticeable change of WBA between 1.0 to 1.5 kHz. Also, at ambient pressure, studies have reported a decrease in absorbance up to 4000 Hz for children with mild negative MEP of < –100 daPa [10]. Similar findings of reduced WBA from 0.5 to 1.5 kHz with self-induced negative MEP varying from –40 to –125 daPa were reported in adults, compared to baseline ambient pressure measurements [22].

In ears with positive MEP, the present study shows a decrease in WBA up to 3000 Hz, with the most significant reduction seen around 1000 to 2000 Hz for both the peak and ambient pressure. These results agree well with another study that has reported WBA in ears with positive MEP compared in normal MEP, a decrease in WBA of around 0.2 absorbance units at 1000 Hz, with a decrease to 0.11 absorbance units at 2850 Hz [23]. However, studies of the effect of positive MEP on WBA have rarely been reported.

Similarly, studies in the literature have reported a significant reduction in WBA at ambient pressure compared to the peak pressure, irrespective of the middle ear pressure conditions [7,11,13]. In the present study, WBA at peak pressure was higher than the ambient pressure conditions for both the normal MEP group and the clinical group. In the normal MEP group, the mean difference calculated between peak and ambient pressure was small, having a maximum mean difference of about 0.03 at 800 Hz. In the clinical group, the mean difference was most considerable for the negative MEP group, which had a mean difference ranging from 0.90 to 0.36 between 300 and 2000 Hz and reaching a maximum at 1000 Hz. On the other hand, positive MEP elicited a maximum difference of 0.11 at 800 Hz. Similar findings have also been reported in a recent study that showed the highest mean difference of 0.12 to 0.42 between 250 and 2000 Hz in an ETD group, i.e., individuals with negative MEP and a minimum difference for the normal MEP group of 0.06–0.09 from 600 to 1500 Hz [11].

The amount of reduction in absorbance at low and mid frequencies could be due to increased stiffness of the middle ear due to the presence of negative or positive MEP [13]. This generates a larger impedance at the level of the TM by increasing its stiffness, thereby reflecting more energy back to the ear canal [6,13,24]. Further, at higher frequencies the study indicated no significant difference in WBA regardless of a change in MEP, and the WBA values were similar across the study groups. This could be because the transmission of sound energy at high frequencies is not stiffness controlled [25]. Hence, the WBA values at those frequencies are not affected and have a similar pattern despite pressure variations.

However, for those with negative MEP, the WBA was significantly higher at peak pressure compared to ambient pressure, and reached absorbance values similar to those with normal MEP. This is because the TPP plays a significant role in equalising the pressure between the ear canal and middle ear, thereby allowing maximum energy to enter the middle ear [13]. Thus, applying ear canal pressure to compensate for the negative MEP helps restore the WBA values nearer to baseline values [7,11,13]. By way of contrast, in the positive MEP group the WBA did not improve much with compensated pressure, i.e. at TPP. Though there is no research focused on positive MEP, similar findings of reduced WBA at low and mid-frequencies with a minimum difference between peak and ambient pressures were reported in the early stages of otitis media [11].

In an apparent contradiction, there are studies which report similar WBA findings irrespective of the MEP, i.e. positive or negative. This might be attributed to a situation in which the position of TM and the direction of umbo displacement are generally similar, generating stiffness at the level of the TM for both conditions [12,23]. As indicated earlier, the stiffness of the TM significantly alters the transmission of low-frequency sounds more than the transmission of high-frequency sounds [6,13,24]. This is probably the reason why significantly lower absorbance values are measured at low frequencies. However, the reason for having different absorbance values at TPP between negative pressure and positive pressure groups is not clear. This difference suggests that, in addition to altered TM stiffness which is the major contributing factor, there may be different mechanisms that affect the transmission of sound. To the best of the authors' knowledge there have so far been no explanations that can support these differences.

However, there are two different mechanisms that could be postulated to explain the difference, both of which consider physiological changes due to MEP.

First, despite maintaining the ear canal pressure which is equal to MEP, the fact of the matter is that the MEP remains either positive or negative or might make it more positive or negative due to a change in position of the TM. Generally, positive pressure tends to push the mucous layer against the bony wall of the middle ear, leading to a more rigid surface that tends to reflect more sound. Whereas negative pressure tends to push the mucous layer against the wall making it more flaccid, and it tends to absorb sound [26]. The TM moves to and fro with the sound wave, which results in movement of the air particles present in the middle ear in a similar fashion having the same frequency. In the case of positive pressure in the middle ear, which increases the rigidity of the middle ear wall, would reflect the sound waves [26]. Thus, it might restrict the movement of TM, leading to a reduction in transmission of sound, which is more likely to affect the lower frequencies compared to high frequencies because of its wavelength properties.

Another possible reason could be that positive pressure in the middle ear would push the round window inside the scala tympani, leading to a reduction in volume. This would probably increase the pressure and restrict the movement of the stapes footplate, leading to reduced transmission of sounds [27]. However, an opposite action can be expected in case of negative MEP.

These two reasons could have altered the WBA; however, it has to be experimentally verified in vivo or in vitro.

As shown in our current results and also in support of the earlier literature [4,7,9,13], the mid-frequency region between 600 and 1000 Hz can be considered as a way to identify individuals with abnormal positive or negative pressure. Any difference in mean WBA between peak and ambient pressure of more than 0.19, observed from 600 to 1000 Hz, indicates negative pressure; a difference of around 0.03 to 0.11 indicates positive pressure; and no change or negligible change of less than 0.03 can be considered as an indicator of a healthy middle ear with normal MEP. Also, concerning the WBA pattern, one can expect a lesser change in absorbance between the peak and ambient pressure in individuals with normal MEP and normal hearing. Similarly, a more significant change in WBA observed only at ambient pressure is an indication of abnormal negative pressure, whereas individuals with abnormal positive pressure will show a more considerable change in WBA observed at both peak and ambient pressure.

Clinical implications

An important implication of these findings is the importance of the difference in WBA value obtained between peak and ambient pressure in ears with positive and negative MEP. The difference in WBA patterns obtained at TPP and ambient pressure could indicate the type of pressure within the middle ear. The deterioration of WBA at peak pressure to the level of ambient pressure without any difference between the two, suggests the presence of positive MEP, and thus the possibility of having an early stage of acute otitis media without effusion. Therefore, understanding the effects of MEP on WBA can aid in improving the diagnostic accuracy and differential diagnoses of middle ear pathologies. Although the study showed interesting results on the effect of MEP on WBA, it suffers from some limitations due to small sample size and the participants being selected based on the outcome of the conventional 226 Hz probe tone tympanometry.

Conclusion

The present study showed a difference in WBA measured between the peak and ambient pressure at low and mid-frequencies with a noticeable change seen between 600 and 1000 Hz. The study suggests that the differential criteria – the mean difference between WBA_{PP} and WBA_0 – along with the WBA pattern, could be used to differentiate abnormal MEP from the MEP of healthy ears. Also, one can expect to see a larger difference in absorbance values between the two pressure conditions in the limited mid-frequency range for MEP_P individuals, with a wider frequency range for MEP_N individuals. Thus, the inclusion of WBA measured at peak and ambient pressure could be a supplementary tool for early identification of middle ear pathologies, in particular abnormal MEP due to middle ear effusion or ETD, and thereby could promote effective treatment.

Funding. The authors declare that the study does not have any funding sources or support.

Conflict of interests. The authors declare that they have no conflict of interest.

Acknowledgments: The authors express thanks to Interacoustics, Denmark, for providing the research module of the Titan WBT software. The authors also acknowledge the All India Institute of Speech and Hearing, Mysuru, affiliated to the University of Mysore for infrastructure support.

References

1. Player B. Earache. In: Kliegman R, Lye SP, Bordini JB, Toth H, Basel D, editors. Nelson Pediatric Symptom-Based Diagnosis. 20th ed. Philadelphia PA: Elsevier; 2018. p. 61–74.e1.
2. Shahnaz N, Polka L. Standard and multifrequency tympanometry in normal and otosclerotic ears. Ear Hear, 1997; 18: 326–41.
3. Nakajima HH, Pisano DV, Roosli C, et al. Comparison of ear-canal reflectance and umbo velocity in patients with conductive hearing loss: a preliminary study. Ear Hear, 2012 Jan; 33: 35–43.
4. Voss SE, Merchant GR, Horton NJ. Effects of middle-ear disorders on power reflectance measured in cadaveric ear canals. Ear Hear, 2012;33:195–208.
5. Keefe DH, Levi E. Maturation of the middle and external ears: acoustic power-based responses and reflectance tympanometry. Ear Hear, 1996; 17: 361–73.
6. Allen JB, Jeng PS, Levitt H. Evaluation of human middle ear function via an acoustic power assessment. J Rehabil Res Dev, 2005; 42: 63–77.
7. Shaver MD, Sun X-M. Wideband energy reflectance measurements: effects of negative middle ear pressure and application of a pressure compensation procedure. J Acoust Soc Am, 2013; 134: 332–41.
8. Onusko E. Tympanometry. Am Fam Physician, 2004 Nov; 70: 1713–20.
9. Beers AN, Shahnaz N, Westerberg BD, Kozak FK. Wideband reflectance in normal Caucasian and Chinese school-aged children and in children with otitis media with effusion. Ear Hear, 2010; 31: 221–33.
10. Hunter LL, Tubaugh L, Jackson A, Propes S. Wideband middle ear power measurement in infants and children. J Am Acad Audiol, 2008; 19: 309–24.
11. Aithal S, Aithal V, Kei J, Anderson S, Liebenberg S. Eustachian tube dysfunction and wideband absorbance measurements at tympanometric peak pressure and 0 daPa. J Am Acad Audiol, 2019; 30: 781–91.
12. Margolis RH, Saly GL, Keefe DH. Wideband reflectance tympanometry in normal adults. J Acoust Soc Am, 1999; 106: 265–80.
13. Robinson SR, Thompson S, Allen JB. Effects of negative middle ear pressure on wideband acoustic immittance in normal-hearing adults. Ear Hear, 2016 Jul 1; 37: 452–64.
14. Jerger J. Clinical experience with impedance audiometry. Arch Otolaryngol, 1970; 92: 311–24.
15. Greenhouse SW, Geisser S. On methods in the analysis of profile data. Psychometrika, 1959 Jun; 24: 95–112.
16. Śliwa L, Kochanek K, Jedrzejczak WW, Mrugała K, Skarżyński H. Measurement of wideband absorbance as a test for otosclerosis. J Clin Med, 2020 Jun 18; 9: 1908.
17. Shahnaz N, Bork K. Wideband reflectance norms for Caucasian and Chinese young adults. Ear Hear, 2006 Dec; 27: 774–88.
18. Feeney MP, Sanford CA. Age effects in the human middle ear: wideband acoustical measures. J Acoust Soc Am, 2004; 116: 3546–58.
19. Wang S, Hao W, Xu C, Ni D, Gao Z, Shang Y. A study of wideband energy reflectance in patients with otosclerosis: data from a Chinese population. Biomed Res Int, 2019; 2019: 1–8.

20. Shaw J. Comparison of Wideband Energy Reflectance and Tympanometric Measures Obtained With Reflwin Interacoustics, Mimosa Acoustics and GSI Tympstar Systems. MSc thesis, Uni British Columbia, 2009.
21. Sanford CA, Brockett JE. Characteristics of wideband acoustic immittance in patients with middle-ear dysfunction. J Am Acad Audiol, 2014; 25: 425–40.
22. Shaver MD. Wideband energy reflectance measurements: normative study and effects of negative and compensated middle ear pressures [Unpublished doctoral thesis]. Wichita State University, 2004.
23. Sanford CA, Feeney MP. Effects of maturation on tympanometric wideband acoustic transfer functions in human infants. J Acoust Soc Am, 2008; 124: 2106–22.
24. Feeney MP, Keefe DH. Acoustic reflex detection using wideband acoustic reflectance, admittance, and power measurements. J Speech Lang Hear Res, 1999; 42: 1029–41.
25. Pickles J. An Introduction to the Physiology of Hearing. 4th ed. Bingley, UK. Brill Academic Publishers; 2012.
26. Gea SLR, Decraemer WF, Funnell RWJ, Dirckx JJJ, Maier H. Tympanic membrane boundary deformations derived from static displacements observed with computerised tomography in human and gerbil. JARO, 2010; 11: 1–17.
27. Jang CH, Park H, Choi CH, Cho YB, Park IY. The effect of increased inner ear pressure on tympanic membrane vibration. Int J Pediatr Otorhinolaryngol, 2009 Mar; 73: 371–5.
28. Margolis RH, Hunter L. Acoustic immittance measurements. In: Roeser RJ, Valente M, Hosford-Dunn H, editors. Audiology Diagnosis. New York: Thieme Medical Publishers, 2000, pp. 381–423.