Wideband Tympanometry and High-Frequency Tympanometry in Neonates

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CERTIFICATE

This is to certify that this dissertation entitled **'Wideband Tympanometry and High-Frequency Tympanometry in Neonates'** is a bonafide work submitted in part fulfilment for degree of Master of Science (Audiology) of the student Registration Number P01II21S0055. This has been carried out under the guidance of a faculty of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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This is to certify that this dissertation entitled **'Wideband Tympanometry and High-Frequency Tympanometry in Neonates'** is the result of my own study under the guidance a faculty at All India Institute of Speech and Hearing, Mysuru, and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru
September, 2023

Registration Number: P01II21S0055

Dedicated to APPA AMMA.....,

The world's best APPA & AMMA, My amazing BROTHER & Ma fav AJJI....

Appa... The great person.....!!!

My courage

Amma... I know, thanks is not at all enough for all your sacrifices... but I promise you to be the best son and care for you until my last breath…!!!

My Brother, my fighting partner… u understands me like anything **Kaashi**.. without your presence am nothing. Thanks for existing in my life!!!

My angel sisters ... Vinutha, Pavithra, Kavitha. my guardian's, pain healers and my energy boosters…!!!

Raja and Tharun..... My caring brothers**...!!!**

Having all of you in my life makes me feel whole and complete…….

I thank ***God Almighty*** for showering all his blessings abundantly on me throughout my life…., **ಶ್ರೀ ಕಾಲಭೈರವೇಶ್ವರ..!!**

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List of Abbreviations

AABR - Automated auditory brainstem response

ABR - Auditory brainstem response

AUROC - Area under the receiver operating curve

dB - Decibels

daPa - Decapascal

DPOAE - Distortion product otoacoustic emission

EHDI - Early hearing detection and intervention

HFT - High frequency tympanometry

Hz - Hertz

KHz - Kilohertz

NHS - Newborn hearing screening

NICU - Neonatal intensive care unit

OAE - Otoacoustic emission

OM - Otitis media

ROC – Receiver operating curve

SNHL - Sensorineural hearing loss

SPL - Sound pressure level

TEOAE - Transient evoked otoacoustic emission

TPP - Tympanometric peak pressure

WAI - Wideband acoustic immittance

WBA - Wideband absorbance

WBR - Wideband reflectance

WBT – Wideband tympanometry

ABSTRACT

Aim and Objectives: The study aims to investigate the efficacy of WBT and HFT in reducing false positive rates in newborn hearing screening. Additionally, this study aims to assess middle ear function using WBT and HFT in both high-risk and non-high-risk infants, referred for detailed audiological evaluation based on initial OAE and AABR evaluations. The research also seeks to compare the incidence of middle ear issues between high-risk and non-high-risk neonates and evaluate the effectiveness of WBT and HFT in identifying middle ear function.

Methods: In a study involving 76 neonates (152 ears), with an average age of approximately 2.05 days, 44 males and 32 females were included. They were divided into two groups: Group 1 consisted of 31 neonates who passed newborn hearing screenings and had no high risk, while Group 2 comprised 45 neonates, further subdivided into Group 2a (21 neonates) with no high risk and Group 2b (24 neonates) at high risk, who were referred from newborn hearing screening. All the neonates underwent both HFT and WBT assessments.

Results and Discussion: This study compared WBA and HFT in neonates between Group 1 and Group 2a and 2b. Using descriptive and non parametric Kruskal–Wallis test, Pass criteria were established based on a control group. WBA showed that 88.09% of Group 2a and 87.5% of Group 2b neonates had middle ear pathology and reduced absorbance across all the frequency in both 2a and 2b group, possibly due to transient conditions affecting sound transmission. HFT revealed that 78.57% of Group 2a and 79.16% of Group 2b neonates had middle ear issues, likely related to fluid in the middle

ear. There was no significant difference in middle ear problem incidence between Group 2a and Group 2b. WBA was found to be more effective than HFT in identifying middle ear pathology due to its broader frequency assessment.

Conclusion: The study's results underscore the importance of including a middle ear assessment tool in newborn hearing screening to reduce false positive results. And also indicate that WBA is particularly effective in predicting the outcomes of OAE and AABR tests in newborn hearing screening. This highlights the value of comprehensive screening protocols that take middle ear function into account to improve the accuracy of identifying hearing issues in newborns and minimizing unnecessary referrals.

Keywords: Wideband absorbance tympanometry, High frequency tympanometry, High-risk neonate, non high-risk neonates, Newborn hearing screening, Otoacoustic emission, Automated auditory brainstem response.

CHAPTER 1

INTRODUCTION

The normal development of speech and language depends significantly on the integrity of the auditory system. The interdependence between auditory and language development makes it essential to have an effective program for early diagnosis and rehabilitation of hearing loss. Thus, newborn hearing screening is crucial to assess the functioning of the auditory system in neonates. A newborn hearing screening program aims to distinguish between a normal ear and one with a hearing problem (Sanford et al., 2009; Keefe et al., 2000). OAE and AABR are used in the universal newborn hearing screening procedure (JCIH, 2019). The main drawback of these tests is that the results of both are affected by the presence of external and middle ear issues (ear canal and/or middle ear obstruction), which could cause false positive results (Allen et al., 2005; Doyle et al., 1997; El-Refaie et al., 1996). For example, the prevalence of bilateral SNHL is one or two out of every thousand newborns (Aidan et al., 1999). The rate of conductive hearing loss in newborns is thirty times greater than sensorineural hearing loss owing to congenital middle ear effusion or more severe outer and middle ear disorders (Gorga et al., 2001).

The conventional and straightforward clinical test for evaluating middle ear disease in older children and adults, except for ossicular chain fixation, is tympanometry, with a low probe tone frequency of 220 or 226 Hz. The use of 226 Hz probe tone tympanometry in newborns has been somewhat restricted due to their mass-dominated external and middle ear systems (Hunter & Margolis, 1992). Because of this limitation with a conventional low probe tone frequency of 226 Hz, a high-frequency probe tone has been suggested in infants. Numerous studies have shown that 1000 Hz probe tone frequency was more effective than low probe tone frequency (Meyer et al.,

1997; Kei et al., 2003). Some researchers for assessing middle ear functions in neonates have also modified traditional tympanometry, known as wideband tympanometry (Aital, 2014; Hunter et al., 2010).

Wideband Acoustic Immittance (WAI) is the name given to a group of wideband measurements. These comprise transmittance, acoustic impedance (resistance and reactance), admittance, power absorption, and transmittance in decibels (conductance and susceptance). These physiological tests assess the middle and outer ear separately from the inner ear. They provide in-depth information on the acoustic-mechanical properties of the middle and outer ear, spanning the frequency range essential for speech perception (Keefe & Bulen, 1993; Keefe & Levi, 1996). The two wideband measurements that are currently employed are absorbance and reflectance. These measurements can be carried out under ambient or pressurized settings.

The most popular way to assess WAI is via wideband reflectance (WBR). It is sometimes called reflectance, energy reflectance, or power reflectance. The ratio of incident power to reflected power is known as the WBR. Its values range from 0 (complete sound transmission to the middle ear) to 1 (no transfer of sound into the middle ear) (Allen, 1994). Wideband reflection (WBR) is the counterpart of wideband absorbance (WBA), which is also known as power absorbance, energy absorption, or absorbance (Sanford et al., 2013).

Keefe et al. (2000) claimed that WBA is superior to 226 Hz-tympanometry, and WBR in several ways. It offers thorough information on the outer and middle ear condition by measuring absorbance in a range of frequencies from 250 to 8000 Hz. The test is also quick, only requiring a few seconds to obtain the result (Keefe et al., 2000). In addition, it offers clinical data in the frequency range required for speech perception and is less likely to be affected by environmental noise, unlike OAE and ABR.

1.1. Need for the study:

Firstly, it is essential to note that newborn hearing screening has a notable high false positive rate, as highlighted by previous studies (Keefe et al., 2003; Chang et al., 2015). This issue arises due to the relatively higher prevalence of conductive hearing problems in newborns than sensorineural hearing loss (SNHL), as other studies indicate (Gorga et al., 2001; Boudewyns et al., 2011). Hence, the middle ear assessment tool is needed in conjunction with the OAE and AABR.

Secondly, though JCIH, in its 2019 position statement, has recommended the use of a middle ear assessment tool in screening newborn babies for hearing, there is currently a lack of reported data regarding both the false positive rates and the prevalence of conductive hearing loss in the young infant population in India.

As a developing country, India requires this testing in paramount importance to detect conductive problems. However, we have no studies to prove the efficacy of WBA and HFT tests for day-to-day usage on high-risk and non-high-risk babies. Hence, this study necessitates the findings to support WBA and HFT in newborn hearing screening setups.

To date, no studies have been conducted on middle ear assessment using both 1000 Hz HFT and wideband absorbance tympanometry in neonates with and without high risk for those referred from newborn hearing screening. Aithal et al. (2015) stated that although otitis media is common in children, research on assessing their outer and middle ear function at birth is still lacking.

The prevalence of conductive hearing loss is high among infants referred from newborn hearing screening (Aithal, 2013). Based on the available data of the children who failed OAE and AABR-based screening and were referred for detailed audiological evaluation, only 14% had SNHL (NBS Report, AIISH 2021). The remaining 86% might

have some conductive component at the time of screening, which was not ruled out, leading to an unnecessary increase in diagnostic caseload and a high false positive rate. Hence, there is a need to evaluate and increase the efficacy of the NBHS test battery by adding middle ear examination to the protocol and to differentiate between congenital (permanent) hearing loss from middle ear effusion using wideband absorbance (WBA) and high-frequency tympanometry (HFT) in non-high-risk as well as high-risk babies.

1.2. Aim of the study

The present study aimed to investigate the efficacy of WBT and/or HFT in reducing false positive rates in newborn hearing screening.

1.3. Objectives

1.3.1. To examine middle ear function using WBT in non-high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.

1.3.2. To examine the middle ear function using WBT in high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.

1.3.3. To examine the middle ear function using HFT in non-high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.

1.3.4. To examine the middle ear function using HFT in high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.

1.3.5. To compare the middle ear problems in high-risk and non-high-risk neonates.

1.3.6. To compare the output of WBT and HFT in identifying the middle ear function.

CHAPTER 2

REVIEW OF LITERATURE

Auditory assessment evaluates an individual's hearing abilities and identifies any hearing impairments or disorders. Hearing assessment is required for individuals of all age groups who feel that their hearing sensitivity is reducing or they cannot hear or understand speech. In very young children, auditory assessment is essential, as they cannot tell whether they have reduced hearing or difficulties listening to speech. Auditory assessment in infants and neonates is also crucial for several other reasons. Firstly, early detection of hearing loss is essential for timely intervention and rehabilitation. The first few months are critical for language and cognitive development (Lenneberg, 1967). Unattended hearing loss can lead to delayed speech, language, and communication skills, potentially affecting a child's social and academic progress (Tong et al., 1980; Northern & Downs, 1978). Secondly, auditory assessment can uncover congenital or acquired hearing disorders indicative of underlying medical conditions, such as infections or genetic syndromes, warranting further medical attention (Downs, 1977). Thirdly, identifying hearing issues in newborns can provide emotional and psychological support to families, helping them to understand their child's needs and connect with appropriate resources and support networks (Meadow-Orlans & Steinberg, 1993)

Early identification of hearing loss is challenging and has evolved over a period. In the late 1980s, U.S. government agencies like the Health Resources and Services Administration, the National Institute on Deafness and Other Communication Disorders, and the National Institutes of Health recognized new technology for testing newborns' hearing. They saw that universal newborn hearing screening (UNHS) could help identify hearing problems early in infants who were deaf or hard of hearing. In the

1990s, some states started programs to screen newborns for hearing issues. Since the 2000s, this has become the standard practice in the U.S. and many other countries worldwide. In the beginning, screening was carried out only for high-risk babies. However, relying solely on screening high-risk infants may miss approximately 50% of newborns with hearing impairment (JCIH, 1990. Mauk et al., 1991). Thus, comprehensive auditory assessment of high-risk and non-high-risk neonates is essential to ensure early detection and appropriate intervention.

High-risk neonates are at risk of developing hearing loss due to certain physiological conditions. Rubella, also known as German measles, is a viral infection that can cause hearing loss if contracted by a pregnant woman during the first trimester. Murhekar et al. (2020) estimated the prevalence of congenital rubella syndrome from 2016-2018 among 645 children born to mothers who contracted rubella during pregnancy. The prevalence of hearing loss was 38.6% in these children. Cytomegalovirus (CMV) can cause hearing loss, particularly in infants and young children. It is a common virus of the herpes virus group. Congenital sensorineural hearing loss is seen in 15% of the children born with cytomegalovirus infection (Fowler, 2013). Meningitis can cause hearing loss if the infection spreads to the inner ear. It is an inflammation of the membranes surrounding the brain and spinal cord. The reported prevalence of hearing loss in children with meningitis is around 30.6% (Kutz et al., 2006). Head and neck anomalies, like congenital malformations and acquired conditions such as tumors, can also lead to hearing loss. The prevalence of hearing loss is high in such individuals and may exceed up to 70% (Juliano & Moonis, 2015). Pinna et al. (2012) reported that unilateral hearing loss was seen in 90% of the 865 cases with vestibular schwannomas, a type of head and neck tumor.

Neonatal jaundice or hyperbilirubinemia is also one of the causes of sensorineural hearing loss in infants (Boskabadi et al., 2018). Bilirubin levels exceeding 95% for a long duration in newborns may lead to kernicterus. Teixeira et al. (2020) reviewed and found that kernicterus is maximally associated with hearing loss among all the other complications of neonatal jaundice. Among a group of newborns with severe hyperbilirubinemia, the prevalence of hearing loss was approximately 5% (Hardani et al., 2020).

Chemical exposure can cause hearing loss by damaging the hair cells in the inner ear (Campo et al., 2013). Some chemicals linked to hearing loss include certain solvents, pesticides, and heavy metals (Choi & Kim, 2014). The most significant hearing loss was reported among workers exposed to the highest levels of toluene (Chang et al., 2006). Guven et al. (2019) found that prenatal noise exposure, which refers to exposure to loud noises in utero, can cause hearing loss in neonates. Among newborns whose mothers were exposed to occupational noise during pregnancy, the prevalence of hearing loss is approximately 1.82% (Selander et al., 2016).

Fifty percent of congenital sensorineural hearing loss cases in developing countries are caused by nutritional deficiencies, specifically vitamin A (Emmett & West, 2014) and folic acid (Silva et al., 2017). Attias et al. (2012) reported that around 27.3 to 45.4% of infants with thiamine deficiency develop hearing loss. Olusanya (2010) said that in developing countries, approximately 37.9% of children develop hearing loss due to malnutrition.

Hearing loss is also associated with syndromic conditions. The prevalence of syndromic hearing loss can vary depending on the particular syndrome and the studied population. Madden (2003) found that among a group of children with Waardenburg syndrome, a genetic disorder, the prevalence of hearing loss was approximately 78%.

High-frequency hearing loss is common in children with Down's syndrome (De Schrijver et al., 2019). Usher syndrome, a genetic disorder that affects both hearing and vision, the prevalence of hearing loss was approximately 11.3% (Kimberling et al., 2010).

Shearer et al. (1993) stated that around 80% of congenital hearing loss is genetic. Mutations in autosomal recessive genes cause the most common form of hereditary hearing loss. It affects males and females equally and occurs in approximately 1 in 20,000 newborns. Some examples of autosomal recessive genes that can cause congenital hearing loss include GJB2, SLC26A4, and DFNB1 (Mahdieh et al., 2010). Mutations in autosomal dominant genes cause a less common form of congenital hearing loss. Some examples of autosomal dominant genes that can cause congenital hearing loss include MYH9, MYO15A, and DFNA9 (Venkatesh et al., 2015). Congenital hearing loss caused by mutations in X-linked genes is rare, but it affects males primarily (Shearer et al., 1993). X-linked is a chromosomal abnormality that can lead to hearing loss. It is a genetic hearing loss because of gene mutations on the X chromosome. They also inherit in an X-linked manner. Liu et al. (1993) found that DFN3, DFN8, and DFNX1 are some of the X-linked genes related to congenital hearing loss. Mitochondrial hearing loss, another type of genetic hearing loss, is due to mutations in the mitochondrial DNA. It is maternally inherited (Usami & Nishio, 1993). Mutations in mitochondrial DNA causing congenital hearing loss are rare. However, if it happens, it affects both genders equally. Of the cases with hearing loss due to mitochondrial inheritance, Alemi and Lustig (2013) reported that around 75-80% are due to autosomal recessive genes, whereas 20% are due to autosomal dominant conditions. Sheffield and Smith (2019) found that X-linked hearing loss accounts for

less than 2%, whereas mitochondrial hearing loss accounts for 1% of total genetic hearing loss.

Testing children with high-risk factors is vital as the prevalence of hearing loss is two to three times more likely in high-risk individuals than in non-high-risk individuals. Although it never meant that hearing loss cannot happen in non-high-risk children, it is just that the likelihood is higher in high-risk children. The newborn hearing screening program covers both high-risk and non-high-risk children. The program involves a series of tests and procedures to evaluate the hearing sensitivity of neonates. It involves two main types of test procedures. One set of procedures relies on behavioral responses, whereas others are more objective and involve tests such as evoked response audiometry and acoustic immittance (Gravel, 1994).

Behavioural Observation Audiometry is a standard method of hearing assessment. A series of sounds, varying in intensity and frequency, were presented to the child, and their behavioral responses in terms of the startle reflex, change in sucking behavior or other behavioral patterns, or change in respiratory or heart rate were observed. However, Hirsch (1991) has found that this test needs reliability. Therefore, it is advisable to use physiological tests in newborn hearing screening.

UNHS presently recommends the use of otoacoustic emission (OAE) and automated auditory brainstem response (AABR) for newborn hearing screening (JCIH, 2019). OAE testing measures the sounds produced by the inner ear in response to a sound stimulus, evaluating the functionality of the cochlea's outer hair cells. If an infant has normal hearing, OAEs will be present. OAE testing is quick, non-invasive, and can be performed while the neonate is asleep. It helps identify infants with mild to moderate hearing loss (Kemp, 1978).

Raghuwanshi et al. (2019) revealed that OAE has 66.7% sensitivity and 98.8% specificity for identifying neonatal hearing impairment, with 33.3% positive and 99.7% negative predictive values. Additionally, AABR testing measures the electrical activity of the auditory nerve and brainstem in response to sound stimuli, assessing the auditory pathway's integrity from the ear to the brainstem. AABR uses small electrodes on the baby's head and earphones to deliver sound stimuli. It can detect even mild hearing loss that OAE testing alone might miss. AABR is particularly useful for identifying infants with neural hearing loss or those at risk for auditory neuropathy spectrum disorder (Van Straaten et al., 1999). Mason et al. (1998) found a sensitivity of 90% and specificity of 93% for AABR, while OAE screening in a similar group had a sensitivity of 80% and specificity of 92%. Heidari, Manesh, and Rajabi (2015) reviewed 57 articles on the sensitivity and specificity of OAEs and AABR. The meta-analysis results revealed that the OAE has a pooled sensitivity of 77% and specificity of 93%. AABR has a pooled sensitivity of 93% and a specificity of 97%. The combined OAE and AABR have a sensitivity of 96% and a specificity of 98%.

If the child fails OAE and AABR screening, further confirmation may be done using Auditory Brainstem Response (ABR). ABR testing also measures electrical activity in response to sound stimuli, but it is typically performed manually using more extensive electrode placements on the child's head. ABR evaluates neural responses along the auditory pathway to higher brain centers that process sound information. It provides detailed information about specific levels of hearing loss and can differentiate between conductive and sensorineural components of hearing loss (Hood, 1998; Necker et al., 2020).

Despite the high sensitivity and specificity of OAE and AABR in the universal newborn hearing screening procedure (JCIH, 2019), the main objective of a newborn hearing screening program is to distinguish between a normal ear and one with a hearing problem (Sanford et al., 2009; Keefe et al., 2000). The main drawback of these tests is that the results of both are affected by the presence of external and middle ear issues (ear canal and/or middle ear obstruction), which could cause false positive results (Allen et al., 2005; Doyle et al., 1997; El-Refaie et al., 1996). For example, the prevalence of bilateral SNHL is one or two out of every thousand newborns (Aidan et al., 1999). The rate of conductive hearing loss in newborns is thirty times greater than sensorineural hearing loss owing to congenital middle ear effusion or more severe outer and middle ear disorders (Gorga et al., 2001). Hence, there is a need to include a middle ear assessment tool in newborn hearing screening. Wideband Tympanometry (WBT) and High-Frequency Tympanometry (HFT) are two non-invasive methods used to assess the function of the middle ear. Each method has its principles and procedures.

Wideband Tympanometry (WBT) is a tool to evaluate the functioning of the middle ear. In contrast to conventional tympanometry, which relies on a single-frequency probe tone, WBT adopts a multifrequency approach, often covering a broad spectrum from 226 Hz to 8000 Hz. The principle of WBT involves the measurement of both reflectance (the sound energy that bounces back from the middle ear) and absorbance (the sound energy absorbed by the middle ear) across the range of frequencies. This comprehensive assessment provides a more detailed understanding of middle ear function. The procedure begins with the patient seated in a comfortable position. An audiologist or a skilled healthcare practitioner inserts a tiny probe into the ear canal. This probe emits a series of acoustic signals covering a variety of frequencies, usually ranging from 226 Hz to 2000 Hz or even higher. Simultaneously, while emitting

these signals, the probe records the ear's response by capturing the sound energy reflected from the middle ear (Katz, 2015).

High-frequency tympanometry (HFT) or 1000Hz tympanometry measures the compliance or stiffness of the eardrum and middle ear system at this specific frequency, providing valuable information about mobility and pressure within the middle ear. The procedure for high-frequency tympanometry involves the insertion of a probe into the ear canal. The probe emits a high-frequency tone and measures the sound pressure within the ear canal while the pressure varies, usually using a small pump. As the pressure changes, the instrument records the impedance or compliance of the middle ear system at a specific high frequency. The resulting data is typically plotted on a graph known as a tympanogram (Katz, 2015).

WBT and HFT objectively measure middle ear status, particularly useful in neonates who cannot provide subjective feedback. The test is quick and non-invasive, making it suitable for neonates who may be sensitive or easily disturbed by invasive procedures. WBT and HFT have been shown to have higher sensitivity than traditional tympanometry in detecting OME and other middle ear pathologies, especially in neonates.

In addition to this, Park (2017) found that WBT can detect subtle abnormalities in the middle ear, such as small perforations or ossicular discontinuity, which may not be easily identified through HFT. WBT also provides frequency-specific information about the middle ear, allowing for a more detailed assessment of middle ear function than HFT.

According to the research conducted by White et al. (1993), they found that among well-babies, 17 out of 1000 had conductive hearing loss. In comparison, among babies in the newborn intensive care unit (NICU), the prevalence was higher, with 36

out of 1000 experiencing conductive hearing loss. Otitis media with effusion (OME) is a frequently encountered condition leading to false positive results in newborn hearing screenings (approximately 64.5% prevalence). This observation was made during a study by Boone et al. (2005).

According to a study conducted by Boudewyns et al. (2011), the researchers found that out of 152 infants referred due to unilateral or bilateral failure on auditory brainstem response (AABR) screening, 53.5% were diagnosed with middle ear effusion. Chang et al. (2015) conducted a study involving 41 full-term neonates. Researchers conducted initial OAE (otoacoustic emissions) examinations and a second OAE test after cleaning ear canals with debris. Another investigator, unaware of the initial results, also examined the ears otoscopically. The pre-cleaned OAE pass rate was 76%, which improved to 91% after debris removal. It highlights the significance of examining and cleaning the external ear canal during neonatal screening to improve outcomes.

The studies above prove that middle ear conditions, such as otitis media with effusion and middle ear effusion, are more commonly observed in neonates than sensorineural conditions. These middle ear issues contribute to a higher incidence of false positive results in newborn hearing screenings and are more prevalent among infants referred for further evaluation in hearing assessments. However, it is essential to note that sensorineural conditions can also occur in neonates. However, their occurrence appears to be comparatively less frequent when compared to middle ear conditions in the studies mentioned.

Engel et al. (1999) conducted a prospective-longitudinal study to analyze otitis media with effusion's (OME) prevalence rates in 150 healthy-born and 100 high-risk-born infants aged 0-2 years. OME prevalence increased rapidly in both groups initially,

with no significant difference. After six months, the high-risk group had a significantly higher prevalence (59% vs. 49%) lasting up to 24 months.

Keefe et al. (2003) conducted a study showing that adding the WAI test to UNHS programs reduced false-positive rates from 5% to 1%. It indicates that information about middle ear status enhances the accuracy of referring neonates for diagnostic hearing assessments and predicting hearing status. Consequently, the WAI is recommended as an additional tool in newborn hearing screening programs.

During birth, the middle ear cavity is often filled with various substances such as amniotic fluid, mesenchyme, meconium, exudates, blood, desquamated epithelial cells, hair, keratinized cells, inflammatory cells, mucosal infiltrate, and reactive polyps (Palva et al., 1999). Aeration of the middle ear typically occurs within the first 48 hours after birth, as Piza et al. (1989) documented.

In high-risk neonates, the higher prevalence of middle ear effusion can be attributed to factors like an immature immune system, increased exposure to risk factors, higher incidence of respiratory infections, structural abnormalities, and increased use of medical interventions. These conditions contribute to a greater susceptibility to infections and impaired fluid drainage from the middle ear, leading to effusion accumulation (Engel et al., (2001).

Kei et al. (2003) used 226 Hz and 1000 Hz probe tones on healthy neonates with typical TEOAE outcomes. They found three admittance tympanogram forms at 1000 Hz for TEOAE-passing infants: 92.2% were type I (single peak), 5.7% were type II (flat), and 1.2% were type III (double peak). Interestingly, using 226 Hz, 47.5% displayed double-peaked tympanograms. They concluded that a type I tympanogram indicates healthy middle ear function.

Swanepoel et al. (2007) examined 1000 Hz tympanograms of 278 ears from 143 healthy neonates aged one to four weeks. They observed two types of tympanograms: single-peaked patterns (94% prevalence) and double-peaked patterns (6% prevalence). Sood et al. (2012) conducted initial DPOAE screening on 236 healthy full-term newborns aged 0 to 3 months. Only neonates who passed DPOAE were considered for further testing. The success rate for 1 kHz tympanometry was 84.53% compared to the original sample size. However, when considering the DPOAE pass criteria, the success rate for 1 kHz tympanometry increased to 95.4%.

Sanford et al. (2009) pioneered relating WBA to NHS and 1000 Hz tympanometry outcomes for neonates. They studied 375 healthy neonates passing DPOAE and 80 failing it within the first two days. Median absorbance in passing ears ranged from 0.39 to 0.67, while failing ears ranged from 0.20 to 0.40, with the best distinction around 1400-2500 Hz. Passing DPOAE neonates had higher absorbance, suggesting a more efficient conductive pathway.

Hunter et al. (2010) studied WBA in relation to NHS and 1000 Hz tympanometry outcomes in healthy neonates. Normative data for WBA were developed in 352 passing DPOAE neonates and compared to 141 failing DPOAE neonates. The WBA normative region was between the 0^{th} percentile (pass group) and 10^{th} percentile (refer group). Area indices over specific frequency ranges were also developed. The regions around 2000 Hz (1000 to 2000 Hz, 1000 to 4000 Hz, and 2000 Hz alone) showed the best discrimination between DPOAE pass and refer groups. No significant ear or gender effects were observed.

Merchant et al. (2010) examined 12 ears from seven neonates with passing DPOAE. Power absorbance was lowest (around 0.4) at 500 Hz, peaking at 0.82 around

2000 Hz, then declining beyond. No gender impact on absorbance was noted, although they found slight ear differences.

In a recent study, Aithal et al. (2023) aimed to present data on pressurized wideband absorbance at tympanometric peak pressure (WBATPP) and 0 daPa (WBA0) in healthy Caucasian neonates. A total of 249 neonates who passed hearing tests were included in the study. The study provided normative data for WBATPP and WBA0 in healthy neonates. No significant difference between the two measures was observed across frequencies. Both WBATPP and WBA0 showed distinct multipeaked patterns, with maxima at 1.25 to 1.5 kHz (0.80) and 6 kHz (0.72) and two minima at 0.4 to 0.5 kHz (0.45) and 4 kHz (0.49). Gender and ear had no significant impact on both WBA measures.

The studies above support the potential use of WBA for detecting middle ear dysfunction and interpreting neonatal screening results. However, a limitation is the absence of an easily accessible tool to confirm conductive hearing loss during the screening phase (Hunter et al., 2013). The issue is addressed in many studies, where they used DPOAE as a reference for normal middle ear status in neonates due to its usage in screening programs (Sangster, 2011). However, DPOAE alone cannot exclude effusion or abnormal pressure (Driscoll et al., 2000; Kemp, 2002). Sanford et al. (2009) and Hunter et al. (2010) acknowledged DPOAE's limitations in being a gold standard for middle ear function, as minor dysfunction may still present with DPOAEs. Consequently, using OAE as a gold standard hinders the clinical applicability of WBA in neonates (Sangster, 2011). Future WBA research should consider more robust gold standards, such as composite tests, to evaluate the clinical effectiveness of WBA.

Rhodes et al. (1999) investigated 87 NICU babies. They found that a significant percentage (30 to 67%) of infants who failed the 226 Hz and 678 Hz tympanometry

passed a series of electrophysiological tests, including OAE and ABR. However, the three ears that failed the 1000 Hz tympanometry also failed the OAE and ABR tests.

Alaerts et al. (2007) assessed probe tone frequencies (226 Hz and 1000 Hz) across different age groups, including NICU babies and various age ranges. Tympanometry at 1000 Hz was more accurate for infants under three months old, while 226 Hz was suitable from 9 months. Tympanometry's accuracy was consistent between 3 and 9 months. Using a two-stage evaluation with a 1000-to-226-Hz tympanometry sequence reduced the required tests.

Sanford et al. (2009) aimed to assess the effectiveness of wideband acoustic transfer functions and 1-kHz tympanometry in predicting the status of the sound conduction pathway in newborns undergoing universal hearing screening. They used a distortion-product otoacoustic emission test and evaluated 455 infant ears (375 passed, 80 referred). The study found that wideband tests performed better than 1-kHz tympanometry in predicting screening outcomes [WBA had an AROC (area under the operating curve) of 0.87, while HF had an AROC of 0.75]. WB measurements are helpful for quickly and objectively assessing newborn sound conduction. They can be used in UNHS programs, indicating that many referrals result from transient deficits. Additionally, WBT data show sound conduction pathway changes in the first two days of life.

Prieve et al. (2013) study aimed to assess the effectiveness of tympanometry and wideband reflectance (WBR) in detecting conductive hearing loss in young infants. The hearing loss type was determined in 84 ears from 70 infants using air and bone conduction auditory brainstem response. The results showed that both WBR and tympanometry using probe frequencies of 678 and 1000 Hz effectively detected CHL in young infants, with significant differences observed in these tests between ears with

CHL and normal hearing. However, tympanometry with a 226 Hz probe tone did not yield significant differences between CHL and normal hearing. The results of both wideband reflectance and 678 and 1000 Hz tympanometry were consistent with the type of hearing loss. It indicates that sensitivity is higher for these tests.

Aithal et al. (2015) aimed to evaluate wideband absorbance (WBA) for predicting neonatal outer and middle ear status. 298 ears of 192 neonates underwent various auditory tests, comparing WBA against nine reference standards, including single tests and test batteries. WBA demonstrated significant differences in ears that failed each test standard, indicating its effectiveness in identifying conductive conditions. However, the ears that passed the standards were the same. The frequency range between 1 and 4 kHz was beneficial for evaluating conductive status. Overall, WBA is considered a desirable measure for detecting conductive conditions in newborns, especially compared to the best-performing test combination.

A literature review on using HFT and WBA in neonates for newborn hearing screening protocols has identified some areas needing further study. Only a few studies have employed HFT and WBT for middle ear assessment in newborn hearing screening. These studies were conducted on well-nursery infants and reported that WBT is a more sensitive tool than HFT, although the effect size was insignificant (Sanford et al., 2009; Prieve et al., 2013; Hunter et al., 2010). The incidence of otitis media in neonates and young infants is very high (White et al., 1993; Gorga et al., 2001), and this condition is more prevalent in newborns who are referred from newborn hearing screening (Keefe et al., 2003; Boudewyns et al., 2011; Chang et al., 2015). Consequently, there is a high chance of false positive results in newborn hearing screening. Aithal et al. (2015) have noted that middle ear assessment in neonates at birth is still lacking. Considering the

limitations of OAE and AABR for middle ear conditions, it is vital to incorporate a middle ear assessment tool into the newborn hearing screening protocol.

While JCIH, in its 2019 position statement, has recommended using a middle ear assessment tool in screening newborn babies for hearing, there is currently a lack of reported data regarding the false positive rates and the prevalence of conductive hearing loss in the young infant population in India. The present study seeks to address all of these issues. To date, no studies have been conducted, as per our knowledge, on middle ear assessment using both 1000 Hz HFT and WBA in neonates, both with and without high risk, for those who are referred from newborn hearing screening.

CHAPTER 3

METHODS

3.1. Research Design: Standard Group Comparison

3.2. Subjects

A total of 76 neonates (152 ears), with a mean age of 2.05 days (SD = 0.69) were enrolled in the present study. Of these, 44 were male and 32 were female. These newborns were recruited from three distinct hospitals within the Mysuru district. Neonates were divided into two groups: Group 1 (Control group) comprised of 31 neonates (62 ears) without any high-risk, and passed both OAE and AABR hearing screening tests. Group 2 was target group of 45 neonates (90 ears) who were referred in both OAE and AABR hearing screening test. Group 2 was further subdivided into Group 2a with 21 neonates (42 ears) without high-risk and Group 2b with 24 (48 ears) neonates with high-risk. The details of neonates in Group 2b and their associated high-risk indicators are given in Appendix 1.

3.3. Test environment:

Testing was conducted in a silent noise-free room, specially designated for newborn hearing screening in three distinct hospitals in Mysuru.

3.4. Consent:

The written signed consent was taken from the parents/caretaker of the participants and the concerned hospital prior to the testing and data collection.

3.5. Instrumentation

The data was collected using the following equipment:

- Titan suite IMP440/WBT440 Advanced Research Module (Intracoustics, Middelfart, Denmark) ver. 3.5, connected to a laptop for measuring WBT and HFT.
- Ero-Scan DPOAEs (Maico Inc., Germany) for measuring DPOAE.
- Easy screen AABR (Maico Inc., Germany) for AABR screening.

All these equipment were calibrated according to the International Electroacoustic Commission standards for WBT, OAE & AABR, as recommended by the manufacturers.

3.6 Procedure

A structured interview was carried out with the parents and caretakers before screening the neonates. Detailed information regarding demographic data, any known medical history, and family history were obtained from the parents/caregivers. Following this, neonates from both groups underwent the following testing procedure.

3.6.1. High-risk register:

The modified high-risk register (HRR) developed by Anitha and Yathiraj (2001) was used to differentiate the neonates without high risk (Group 1 and Group 2a) and with high-risk (Group 2b). The HRR was administered by interviewing the parents/caregivers, and to the concerned hospital nurse. Additionally, relevant information was extracted from the neonates' medical records. The high-risk factors being included in the HRR is given in Appendix 2.

3.6.2. Otoscopic examination:

All neonates were subjected to a visual inspection of the ear canal using an otoscope (HEINE mini-3000) to examine the status of amniotic fluid. Neonates who had amniotic fluid or blocked ear canal were excluded from the study. Otoscopy is done before the test to find the status.

3.6.3. Otoacoustic emission:

DPOAEs was measured to assess the functionality of the outer hair cells in the cochlea. All neonates, regardless of the risk status underwent a screening DPOAE test. The stimulus used were pre-set consisting of pairs of pure tones ranging from 1.5 kHz to 6 kHz (4 bands), presented at sound pressure levels (SPL) of 65/55 dB (L1/L2) with an F2/F1 ratio of 1.22. The probe tip with a suitable size was firmly placed in the neonate's ear canal, and the stimulus was presented. The test duration ranged from 4 to 60 seconds. The neonates were considered as 'Pass', if the signal-to-noise ratio (SNR) was $\geq$6 dB, and a sound pressure level (SPL) of at least -5 dB detected in 3 out of 4 frequency bands (Maico, 2020). If SNR was $\leq$ 6dB, the neonates were considered as 'Refer'.

3.6.4. Automated Auditory Brainstem Response:

The test aims to analyse neural activity recorded from the auditory nerve to the brainstem. All the neonates with and without high-risk were screened using AABR screener. The procedure involved placing all neonates comfortably on a cot. Conduction gel was applied to both the testing area and the electrodes. The BERAphone was carefully positioned on the newborn's ear, ensuring precise electrode placement. Two electrodes were positioned—one at the vertex (located on the handle) and another at the mastoid (located in the lower area). While, a third electrode, situated between the vertex

and mastoid electrodes, served as the ground electrode and was positioned just above the pinna. The placement of the vertex electrode was adjusted to optimize its fit on heads of varying sizes.

Following this, an automated pre-test for impedance was measured which should be <5 kΩ to begin the test. Click stimuli was presented at a sound level of 35 dBnHL at a repetition rate of 90 per second. The test duration varied from 10 to 180 seconds, and artifact rejection was set at a threshold of 100 µV. For interpretation, Powerful Stimulus and Detection Algorithm (Maico, 2020) was used in the AABR system to detect a response from the auditory system. The algorithm complements with the click stimulus to determine the 'PASS/REFER' results within seconds. If a response is detected, the screening test produced a "PASS" result. However, if no response was detected within 180s, the screening test generated a "REFER" result.

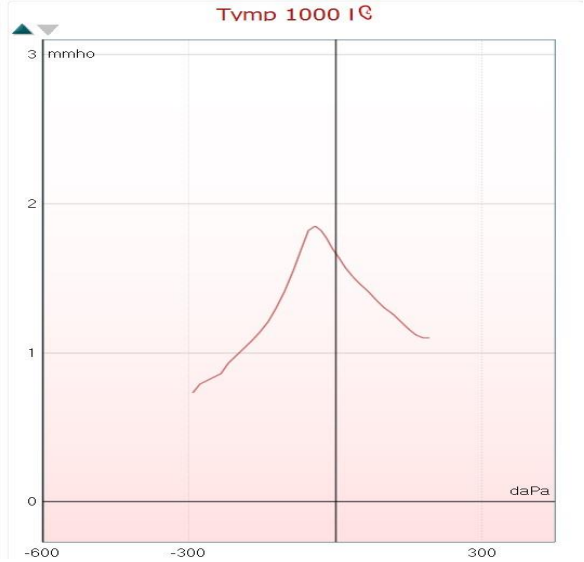
3.6.5. High-frequency tympanometry:

High frequency tympanometry is a test that assesses middle ear function using sound waves at higher frequencies typically at 1000 Hz probe tone. The HFT test provides a sensitive detection of middle ear disorders in neonates (Kilic et al., 2012). The procedure involved the inserting the probe tip of appropriate size into the neonate's ear canal. A high-frequency probe tone of 1000 Hz was delivered at an intensity level of 100 dB peSPL. The pressure was automatically varied between +200 daPa and -300 daPa at a medium-level pump speed of 200 daPa/sec. The static compliance (mmho), Ear canal volume (ml), Gradient (daPa) were measured. The accuracy of the immittance system was checked daily using the calibration cavity with volumes of 0.5, 2.0, and 5.0 cm3.

For interpretation, the “pass” criteria were defined as a single tympanogram displaying a positive peak (Baldwin 2006). This criterion applied to both the control group and the target group, with the control group data serving as the basis for establishing normative standards for HFT.

Figure 3.1

A sample 1000 Hz tympanogram recorded in right ear on a two-day-old neonate.



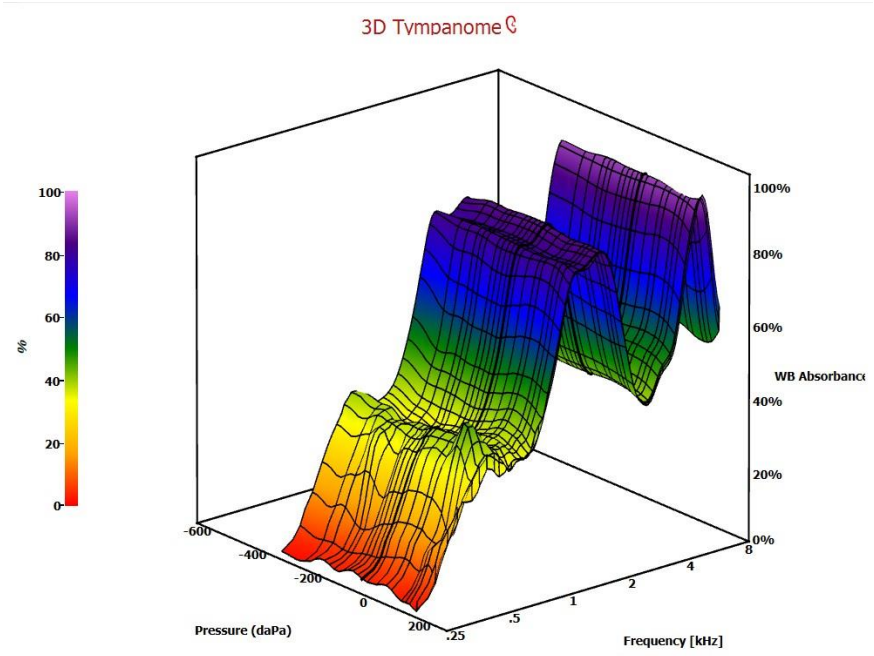
3.6.6. Wideband absorbance tympanometry:

Wideband absorbance tympanometry is an auditory assessment technique that measures how the middle ear absorbs sound across a range of frequencies. WBA was performed immediately after the HFT test with the same procedure for probe insertion methods. Before administrating the test, daily calibration of the WBA equipment was performed using four couplers as recommended by the manufacturer (Interacoustics Inc., 2016; Liu et al., 2008). The test involved delivering a broadband click stimulus ranging from 226 Hz to 8 kHz, at a stimulus level of 100 dBpeSPL (approximately 65

dBnHL), with a repetition rate of 21.5 Hz. Pressure was automatically swept between +200 daPa and -300 daPa at a medium-level pump speed of 200 daPa/sec, and the response was averaged over 32 sweeps (Interacoustics Inc., 2016).

Figure 3.2

A 3-Dimensional graph depicting wideband absorbance tympanometry of right ear in two-day-old healthy neonates who passed OAE and AABR screening tests.



The 3-dimensional graph was displayed in the Titan Suite that include frequencies on the x-axis (226 Hz to 8000 Hz), pressure on the y-axis (200 daPa to -300 daPa), and the absorbance values on the z-axis (0 to 100%) as shown in Figure 3.3. WBA values were measured across the frequencies that typically fall between 0.0 and 1.0, with '1' denoting that the middle ear absorbs all sound energy and '0' indicating that the middle ear reflects all sound energy (Stinson, 1990). Typically, the system generated WBA values for 1/24th octave frequencies (121 frequencies). For statistical analysis, the study

extracted WBA values for 1/3rd octave frequencies (16 frequencies) at the peak pressure using the excel spreadsheet.

In the present study, only peak pressure is considered due to the emphasis placed by numerous studies on the potential benefits of assessing sound absorption under pressurized conditions (Aithal, 2017b; Margolis, 1999; Pitaro, 2016). The application of pressurized WBA testing holds the promise of enhancing precision in detecting specific middle ear disorders (Keefe & Levi, 1996; Keefe & Simmons, 2003; Margolis, Saly, & Keefe, 1999). This approach is based on the premise that the middle ear is likely more effective at absorbing sound at the Tympanometric Peak Pressure (TPP), as the eardrum reaches its maximum mobility at that point (Feeney & Sanford, 2012; Katz, 2015, p. 157). Additionally, HFT is carried out only at peak pressure which would ease out for comparison with 1000Hz tympanometry.

3.7 Statistical analysis:

Statistical analysis was conducted using IBM SPSS (version 26) for Windows. Absorbance values across frequency for WBA and static compliance, peak pressure, gradient and ear canal volume for HFT was measured. Absorbance values were measured for each frequency, covering 1/3rd octave frequencies ranging from 226 to 8000 Hz, and a comparison was carried out.

To analyze the mean absorbance values across the frequencies ranging from 226 Hz to 8000 Hz (comprising 16 frequencies) for all the groups, descriptive statistics including the mean, median, standard deviation (SD), and range were calculated. Additionally, 10th and 90th percentile was calculated for Group 1 to establish the normative range and Kruskal-Walli's test was conducted to compare between the

groups for both HFT and WBA because some of the parameters exhibited a normal distribution while others did not.

ROC curve was plotted to show the trade-off between sensitivity and specificity across different interpretation conditions (C1 to C5). The effectiveness of each interpretation condition was measured using ‘d-prime’ and ‘area under the ROC curve (AUC).’ A custom MATLAB code was developed to plot ROC using ‘norminv’ function in the statistical and machine learning toolbox, and AUC was measured using ‘trapz-trapezoidal integration’ function.

CHAPTER 4

RESULTS

In the current study, Group 1 neonates were used to establish normative data for WBA. All the neonates in Group 1 had OAEs being present at all four frequencies, with SNR greater than 6 dB. Also, the AABR was present for all these neonates at an intensity level 35 dBnHL. The neonates were without any high-risk factors and were having normal external auditory canal, as tested using otoscopy. Middle ear function was evaluated using HFT that showed a single peaked tympanogram for all the neonates. Table 4.1 shows the mean, SD, and median values of 1000 Hz tympanometry measurements.

Table 4.1

Mean, standard deviation and median values for various parameters of 1000 Hz probe tone tympanometry.

Tympanometry parameters	Mean	SD	Median
Static compliance	1.43	0.34	1.37
Peak pressure	-44.87	114.35	-43.5
Gradient	170.18	38.16	168.5
Ear canal volume	0.31	0.08	0.32

Based on the findings from Otoscopy, OAE, AABR, and HFT, it was determined that the neonates in Group 1 indicated no signs of neural or cochlear hearing loss and exhibited normal middle ear function, without any signs of even subtle pathology. As a result, the neonates in Group 1 were categorized as the control group subjects, and it served as a reference for comparison with the target groups. WBA measurements were subsequently conducted on these neonates to establish normative values.

Establishing Norms for WBA in Healthy Neonates:

Descriptive statistics were performed on the WBA data of the Control group, which included mean, SD, median, range, and 10th and 90th percentile values for peak pressure, measured in 1/3rd octave bands across 16 frequencies. The 10th and 90th percentile criterion were adopted from Aital et al. (2015). The results for these statistics across the 16 frequencies are presented in Table 4.2.

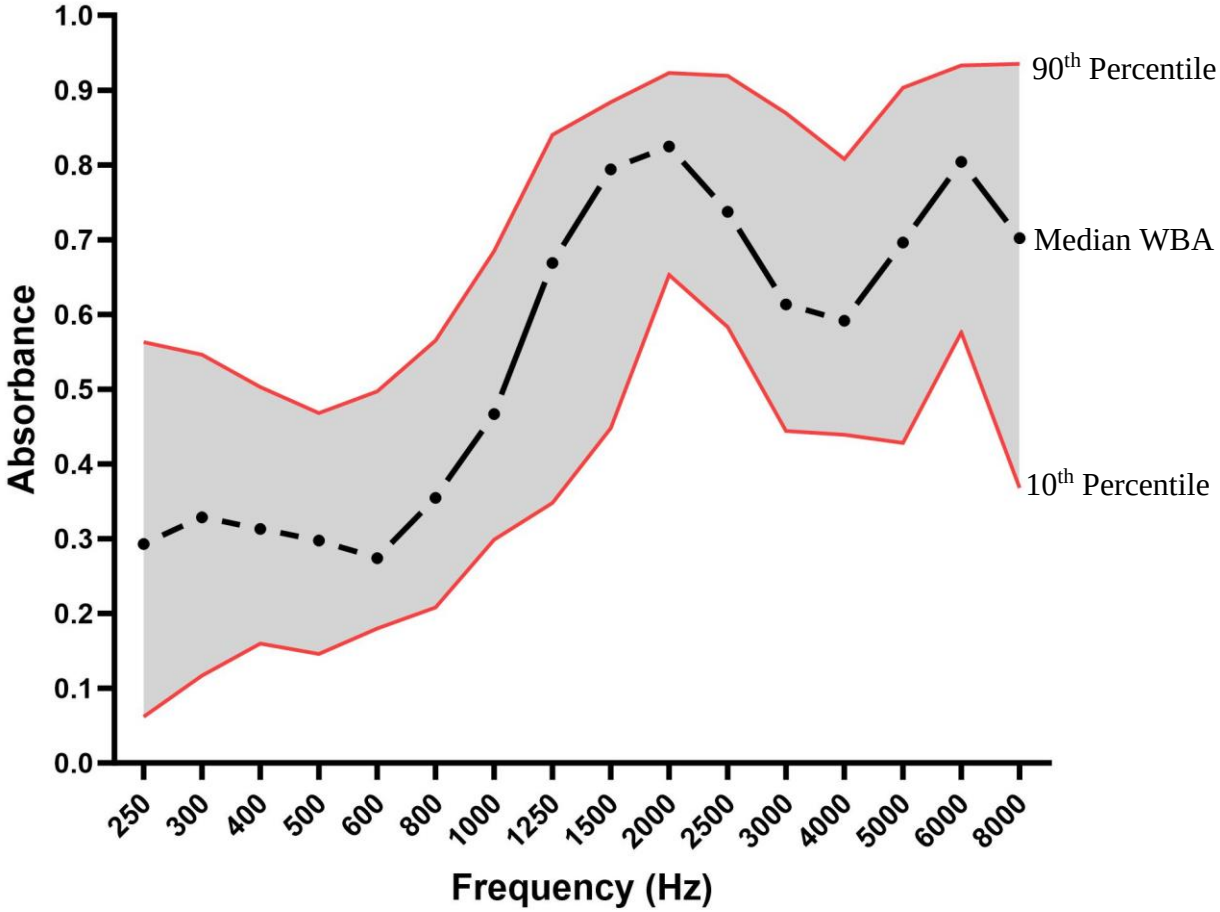
Table 4.2

Mean, SD, median, range and percentile values of WBA across the frequencies.

Frequency	Mean	SD	Median	Range	10th Percentile	90th Percentile
250	0.30	0.18	0.29	0.00 - 0.74	0.062	0.563
300	0.33	0.15	0.32	0.00 - 0.71	0.117	0.546
400	0.33	0.13	0.31	0.03 - 0.71	0.16	0.503
500	0.30	0.12	0.29	0.12 - 0.71	0.146	0.468
600	0.31	0.12	0.27	0.15 - 0.66	0.18	0.497
800	0.36	0.12	0.35	0.15 - 0.72	0.208	0.565
1000	0.47	0.15	0.46	0.17 - 0.87	0.299	0.685
1250	0.64	0.16	0.66	0.22 - 0.88	0.348	0.84
1500	0.74	0.15	0.79	0.28 - 0.93	0.448	0.884
2000	0.80	0.10	0.82	0.57 - 0.95	0.653	0.923
2500	0.73	0.12	0.73	0.48 - 0.97	0.583	0.919
3000	0.63	0.14	0.61	0.34 - 0.95	0.444	0.869
4000	0.61	0.13	0.59	0.38 - 0.88	0.439	0.808
5000	0.67	0.17	0.69	0.33 - 0.97	0.428	0.903
6000	0.77	0.13	0.80	0.44 - 0.97	0.576	0.933
8000	0.67	0.21	0.70	0.13 - 0.99	0.368	0.935

Figure 4.1

Graphical representation of median and the 10th - 90th percentile normative ranges of WBA for Control group.



The WBA values displayed a distinct pattern across the frequency range as displayed in Figure 4.1. WBA values were lowest at lower frequencies, ranging from 250 Hz to 600 Hz. As the frequency increased, there was a steep rise in WBA values, reaching a maximum at around 2000 Hz followed by slight decline up to 4000 Hz. The WBA values further increase to a second maximum at 6000 Hz followed by slight decline in absorbance at 8000 Hz.

In this study, the normative range for WBA was established using the 10th and 90th percentiles data from the control group as shown in Figure 4.1, indicated by the grey shaded across the frequencies. This normative range was used for further analysis

of WBA to compare with the target groups [in the group without high-risk factor [(Group 2a) and the group with high-risk factor (Group 2b)].

WBA in Referred Neonates without high risk factors:

The WBA was evaluated in neonates without high-risk factors of Group 2a who were referred in the OAE and AABR (n=42 ears). Table 4.3 shows the Mean, SD, median and range for neonates in Group 2a.

Table 4.3

Mean, SD, median and range obtained in referred neonates without high-risk factors (Group 2a).

Frequency	Mean	SD	Median	Range
250	0.09	0.13	0.03	0.00 - 0.45
300	0.12	0.14	0.06	0.00 - 0.51
400	0.18	0.13	0.19	0.00 - 0.59
500	0.20	0.12	0.21	0.00 - 0.50
600	0.22	0.11	0.23	0.01 - 0.49
800	0.25	0.10	0.24	0.03 - 0.49
1000	0.32	0.14	0.30	0.06 - 0.68
1250	0.40	0.20	0.42	0.07 - 0.77
1500	0.41	0.22	0.48	0.04 - 0.72
2000	0.31	0.21	0.31	0.00 - 0.97
2500	0.26	0.18	0.23	0.00 - 0.84
3000	0.23	0.17	0.20	0.00 - 0.63
4000	0.33	0.17	0.30	0.09 - 0.72
5000	0.34	0.23	0.27	0.03 - 0.95
6000	0.38	0.24	0.33	0.00 - 0.88
8000	0.40	0.26	0.30	0.00 - 0.91

In referred neonates without high-risk factors (Group 2a), WBA values were lowest at 250 Hz and increased steeply with increasing frequencies, reaching a maximum around 1500 Hz. Following the peak at 1500 Hz, there was a decrease in absorbance values until 3000 Hz, followed by a slight increase, and it remained relatively steady beyond 3000 Hz. In comparison to healthy neonates (Control group), the WBA for Group 2a (referred neonates without high-risk factors) has drastically reduced at all the frequencies as shown in Figure 4.2. However, the frequencies ranging from 400 Hz to 1500 Hz are within the lower borderline of the normative range.

Statistical analysis using the non-parametric Kruskal-Wallis H test indicated a significant difference between the control group and Group 2a (referred neonates without high-risk factors) for all the frequencies ($p<0.05$). The H-values and p-values for each frequency is given in Table 4.4. This suggests that the WBA values for Group 2a significantly deviated from those of the Control group across the frequencies, highlighting potential differences in middle ear function in the referred neonates without high-risk factors.

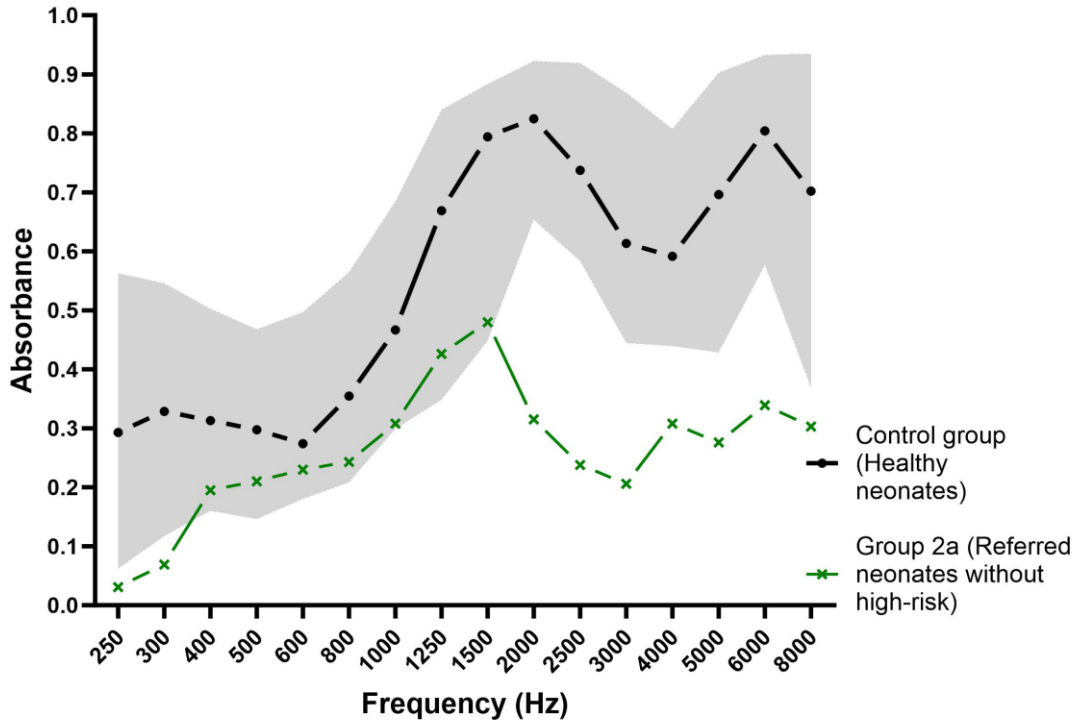
Table 4.4

The H-values and p-values of Kruskal wallis test across frequency for different groups.

WBA test	Control V/S Group 2a		Control V/S Group 2b		Group 2a V/S Group 2b	
Frequency	H - value	*p* - value	H - value	*p* - value	H - value	*p* - value
250	5.81	<0.001	3.30	<0.001	-2.50	0.01
300	5.89	<0.001	3.61	<0.001	-2.29	0.02
400	4.76	<0.001	2.76	0.01	-1.99	0.05
500	2.97	<0.001	2.02	0.04	-0.97	0.13
600	2.88	<0.001	1.73	0.09	-1.16	0.25
800	1.31	<0.001	3.95	0.19	-2.54	0.01
1000	4.31	<0.001	1.92	0.06	-2.36	0.02
1250	5.08	<0.001	4.20	<0.001	-0.99	0.32
1500	6.55	<0.001	6.44	<0.001	-0.33	0.74
2000	8.56	<0.001	7.31	<0.001	-1.45	0.15
2500	8.30	<0.001	7.33	<0.001	-1.18	0.24
3000	7.30	<0.001	7.84	<0.001	-0.77	0.44
4000	6.01	<0.001	6.18	<0.001	-0.82	0.41
5000	6.63	<0.001	5.62	<0.001	-1.11	0.27
6000	6.95	<0.001	5.92	<0.001	-1.19	0.23
8000	4.10	<0.001	3.45	<0.001	-0.73	0.46

Figure 4.2

Graphical representation of median WBA of control group and referred neonates without high-risk factors (Group 2a) across frequencies with 10th - 90th percentile normative ranges.



WBA in Referred Neonates with high-risk factors:

The WBA was evaluated in neonates with high-risk factors of Group 2b who were referred in the OAE and AABR (n=48 ears). Table 4.5 shows the Mean, SD, median and range for neonates in Group 2b.

Table 4.5

Mean, SD, median and range obtained in referred neonates with high-risk factors (Group 2b).

Frequency	Mean	SD	Median	Range
250	0.16	0.14	0.13	0.00 - 0.46
300	0.19	0.14	0.19	0.00 - 0.45
400	0.22	0.13	0.24	0.00 - 0.52
500	0.22	0.14	0.22	0.00 - 0.59
600	0.25	0.12	0.21	0.03 - 0.56
800	0.31	0.14	0.30	0.10 - 0.70
1000	0.39	0.15	0.40	0.07 - 0.75
1250	0.44	0.19	0.40	0.09 - 0.82
1500	0.44	0.20	0.40	0.04 - 0.81
2000	0.41	0.18	0.40	0.03 - 0.77
2500	0.33	0.16	0.33	0.06 - 0.94
3000	0.28	0.16	0.28	0.00 - 0.81
4000	0.37	0.14	0.34	0.14 - 0.68
5000	0.38	0.19	0.34	0.06 - 0.77
6000	0.45	0.26	0.42	0.03 - 0.93
8000	0.48	0.27	0.42	0.11 - 0.99

Figure 4.3 showed the WBA of referred neonates with high-risk factors (Group2b) in comparison to the Control group. The WBA values for Group 2b exhibited a pattern similar to that of Group 2a, with the lowest absorbance observed at 250 Hz. Furthermore, WBA values increased as the frequency increased, reaching their

maximum at 1000 Hz, followed by a stable absorbance value up to 2000 Hz. Beyond 2000 Hz, there was a slight decline in absorbance values until 3000 Hz, after which a gradual increase was observed. When comparing Group 2b (referred neonates with high-risk factors) to healthy neonates (Control group), it is evident that the WBA values for Group 2b have significantly decreased at all the frequencies, as depicted in Figure 4.3.

Statistical analysis using the non-parametric Kruskal-Wallis H test, revealed a notable difference between the control group and group 2b (referred neonates with high-risk factors) at most frequencies (p<0.05) except at 600 Hz, 800 Hz, and 1000 Hz (p>0.05). The H-values and p-values are given in Table 4.4, for all test frequencies.

Figure 4.3

Graphical representation of median WBA of control group and referred neonates without high-risk factors (Group 2b) across frequencies with 10th - 90th percentile normative ranges.

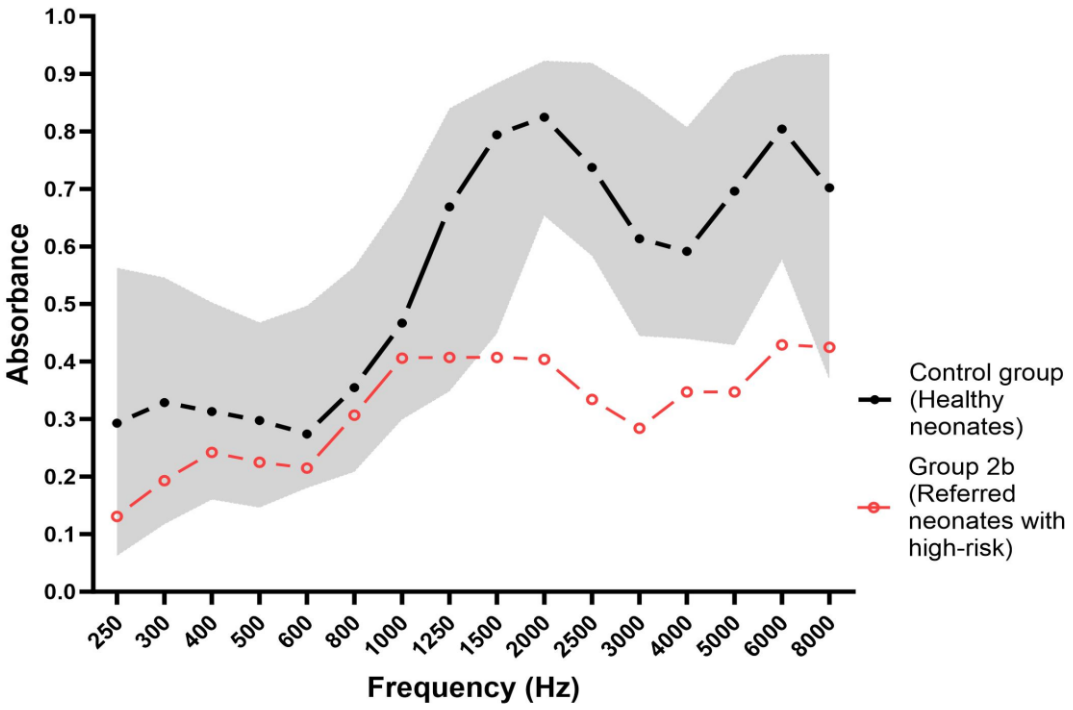


Figure 4.4

Graphical representation of median WBA of control group and referred neonates with high-risk factors (Group 2a) and without high-risk factors (Group 2a) across frequencies with 10th - 90th percentile normative ranges.

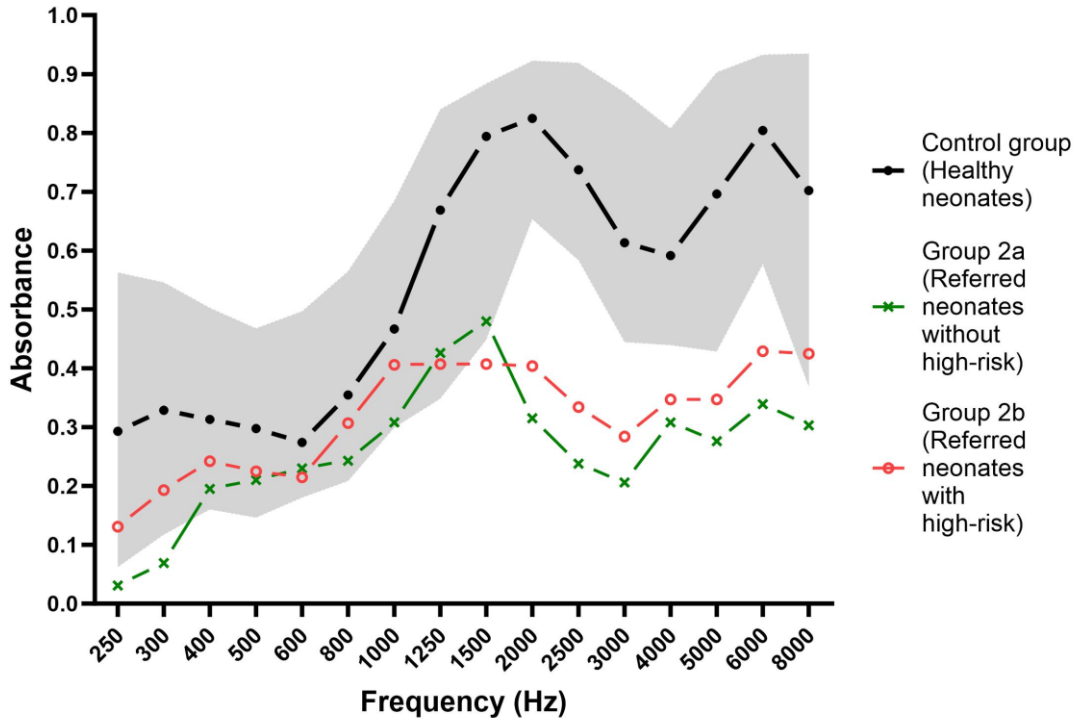


Figure 4.4 shows the median WBA of control group and referred neonates without high-risk factors (Group 2a) and with high-risk factors (Group 2b) across frequencies with 10th - 90th percentile normative ranges. Kruskal-Wallis H test statistics revealed no significant difference in the WBA between Group 2a and 2b. except 250, 300, 800 and 1000Hz.The test statistics are given in Table 4.4.

HFT in referred neonates without high-risk factors:

The HFT was measured in neonates without high-risk factors of Group 2a who were referred in the OAE and AABR (n=42 ears). Among these 42 ears, 09 ears exhibited a single peak tympanogram, similar to that of the control group, while the remaining 33 ears displayed either flat (27 ears) or abnormal tympanograms (07 ears).

Table 4.6 presents the static compliance, gradient, ear canal volume, and peak pressure data obtained from HFT for these 09 ears within Group 2a.

Table 4.6

Mean, standard deviation and median values for various parameters of 1000 Hz probe tone in neonates without high-risk factors (Group 2a).

Tympanometry parameters	Mean	SD	Median
Static compliance	0.98	0.4	0.81
Peak pressure	-111	136.40	-48
Gradient	125.33	41.40	139
Ear canal volume	0.27	0.08	0.27

Statistical analysis using the non-parametric Kruskal-Wallis H test was done for these 9 ears that revealed a significant difference between the Control group and Group 2a (Referred neonates without high-risk factors) only for static compliance, Peak pressure and ear canal volume ($p<0.05$) except for Gradient ($p>0.05$). These parameters exhibited slightly reduced values compared to the control group. However, it is important to note that these values remained within the normative range as reported in the literature, indicating normal middle ear functioning without any pathology for these 9 ears.

Statistical analysis was not carried out for the remaining 27 ears that displayed flat tympanograms due to the absence of a data for analysis. Since the tympanogram was flat, the instrument did not show any peak pressure or static compliance values. This suggests that these neonates likely have some form of middle ear pathology.

HFT in Referred Neonates with high-risk factors:

The HFT was measured in neonates with high-risk factors of Group 2b who were referred in the OAE and AABR (n=48 ears). Of the 48 ears, 38 ears had either flat (32 ears) or abnormal tympanogram (6 ears) and 10 ears had single peak tympanogram as similar to that of control group. The Table 4.7 showed the static compliance, gradient, ear canal volume and peak pressure obtained from HFT in Group 2b for those 10 ears.

Table 4.7

Mean, standard deviation and median values for various parameters of 1000 Hz probe tone in neonates with high-risk factors (Group 2b).

Tympanometry parameters	**Mean**	**SD**	**Median**
Static compliance	0.98	0.4	0.81
Peak pressure	-111	136.40	-48
Gradient	125.33	41.40	139
Ear canal volume	0.27	0.08	0.27

Statistical analysis using the non-parametric Kruskal-Wallis H test, revealed a significant difference between the Control group and Group 2a (Referred neonates with high-risk factors) only for static compliance, peak pressure and ear canal volume ($p<0.05$) except for gradient which showed no significant difference ($p>0.05$). These values were slightly reduced compared to the control group but remained within the normative range as reported in the literature. This suggests normal middle ear functioning without any pathology for these 10 ears.

However, statistical analysis was not feasible due to the lack of data for analysis for the remaining 38 ears that displayed flat tympanograms. This observation strongly suggests that these neonates likely have some form of middle ear pathology.

Comparison of middle ear problem using WBA:

Currently, there exists no consensus on the definitive reference standard for assessing the diagnostic accuracy of WBA) in identifying the middle ear pathology in neonates. Though, few studies have employed varying reference standards in their investigations. For instance, Sanford et al. (2009) and Hunter et al. (2010) employed Distortion Product Otoacoustic Emissions (DPOAE) as their reference standard, while Vander Werff et al. (2007), Shahnaz (2008), and Silva et al. (2013) utilized Transient Evoked Otoacoustic Emissions (TEOAE) to evaluate middle ear function. In these studies, the absence or presence of low-level OAEs was considered indicative of a potential middle ear disorder, particularly in cases where sensory hearing loss was not detected. The current study made a preliminary attempt to provide the 'Pass' and 'Refer' criteria of WBA. The criterion was calculated based on the normative range established from Group 1 (Control group). WBA was considered 'pass' if the absorbance values were falling within 10-90th percentile of the control group for at least 13/16 test frequencies. If WBA values fell below 10th percentiles, or above 90th percentile, for less than 13/16 test frequencies, it was considered 'Refer'.

The 13/16 criterion was based on the evaluation of sensitivity and specificity of different interpretation protocols: WBA pass at all frequencies (16/16; C1), 15/16 frequencies (C2), 14/16 frequencies (C3), 13/16 frequencies (C4), and 12/16 frequencies (C5). Sensitivity was calculated as true positive / (true positive + false negative), while specificity was calculated as true negatives / (true negative + false positive). True positive cases were those 'fail' in OAE+AABR and 'referred' in WBA;

False positive represented 'pass' in OAE+AABR but 'referred' in WBA; True negatives were those ‘pass’ in both OAE+AABR and WBA; and False negatives were those 'fail' in OAE+AABR but 'pass' on WBA. Table 4.8 presents sensitivity, specificity, d-prime, and AUC values across these conditions. It may be noted that higher d-prime and AUC values indicated better distinction between the positive and the negative instances.

Table 4.8

Sensitivity, specificity, d-prime and AUC values for each condition.

Conditions	Sensitivity	Specificity	d-Prime	AUC
C1	97.53	11.11	0.7461	0.4877
C2	96.29	22.22	1.0219	0.4815
C3	95.06	33.33	1.2209	0.4815
C4	91.35	55.55	1.5029	0.4568
C5	86.41	55.55	1.239	0.432

The sensitivity was highest for C1, but with very poor specificity. While considering the trade-off between sensitivity and specificity, C3 and C4 maintained good sensitivity and relatively better specificity. The d-prime values were highest for C4, whereas AUC is high and same for C1-C3. Considering the trade-off between d-prime and AUC, C4 seems to be reasonable condition. With all factors considered, C4 provided the best trade-off among the five. The Youden Index for C4 was 0.47, indicating 47% reduction in false positive rates by adding WBA in the NHS measurement. Other conditions had less than 0.47 Youden Index.

Therefore, it can be concluded that absorbance values should fall within the normative range for at least 13 out of the 16 frequencies to be categorized as 'Pass,'

signifying that the ears exhibit normal middle ear function without pathology. Conversely, if absorbance values deviate from the normative range for fewer than 13 frequencies, they should be categorized as 'Refer,' indicating the presence of middle ear pathology.

Based on the established criteria, each neonate from the target group was compared to the normative range established from the Control group and the details are shown in Table 4.9 In Group 2a (Referred neonates without high-risk factors), 37 out of the total 42 ears were referred, while the remaining 05 ears passed the WBA. These results indicate that 88.09% of non-high-risk neonates referred based on OAEs and AABRs were found to have middle ear pathology.

Similarly, in Group 2b, which consists of neonates with high-risk factors and referred in OAEs and AABRs, 42 out of the total 48 ears were referred, while six ears passed the WBA test. Based on these findings, it was determined that 87.5% of high-risk neonates referred based on OAEs and AABRs exhibited middle ear pathology.

Table 4.9

Number of ears with Pass and refer criteria in WBA for the target groups

Target groups	Pass	Refer
Group 2a (Referred neonates without High-risk factors)	05 (11.91%)	37 (88.09%)
Group 2b (Referred neonates with High-risk factors)	06 (12.5%)	42 (87.5%)

Comparison of Middle ear problem using HFT:

In using HFT, the neonates were classified as 'passed' if they exhibited a single positive peak and their measurements fell within the normative range for static compliance, peak pressure, gradient, and ear canal volume, similar to the Control group. Conversely, neonates whose measurements deviated from the normative range and displayed flat or abnormal tympanograms were categorized as 'referred' in HFT. The Table 4.10 provides details of the neonates who passed and referred using HFT in the target group.

Table 4.10

Number of ears with Pass and refer using HFT in the target groups

Target groups	Pass	Refer
Group 2a (Referred neonates without High-risk factors)	09 (21.43%)	33 (78.57%)
Group 2b (Referred neonates with High-risk factors)	10 (20.84%)	38 (79.16%)

Out of the 42 ears tested in Group 2a, 33 ears were referred, indicating that they had middle ear pathologies, while 9 ears successfully passed in the HFT. Among the 33 referred ears, 26 exhibited flat tympanograms, and 7 displayed abnormal peaked tympanograms. This implies that HFT identified middle ear pathologies in 78.57% of the neonates within Group 2a.

In the case of neonates in Group 2b, HFT results indicated 'referred' for 38 ears, with 32 of them having flat tympanograms and 6 showing abnormal peaked tympanograms. Consequently, HFT identified middle ear pathologies in 79.16% of the ears of neonates within Group 2b. While, 10 ears showed ‘Pass’ in HFT.

Comparisons of WBA and HFT across groups:

When comparing the results of WBA and HFT across the Group 2a and Group 2b, a higher percentage of neonates were referred rather than passing in both WBA and HFT test. However, Group 2a displayed marginally better pass rates compared to Group 2b in both WBA and HFT tests, suggesting that neonates without high-risk factors had a slightly lesser likelihood of middle ear pathologies.

In more specific terms, within Group 2a, approximately 11.91% passed the WBA test, while 21.43% passed the HFT test. In contrast, in Group 2b, the pass rates were slightly lower, with 12.5% passing the WBA test and 20.84% passing the HFT test. These results indicate that while a larger proportion of neonates in both groups were referred for further evaluation, the presence of high-risk factors in Group 2b appeared to have a slightly more pronounced impact on the pass rates of both WBA and HFT.

These results also indicate that compared to HFT, WBA showed a higher referral rate for both the target groups. It suggested that WBT is more sensitive in identifying middle ear pathologies than HFT and hence, may be preferred over HFT to reduce the false positive rate in new-born hearing screening. However, these results should be further confirmed using detailed diagnostics middle ear assessments before concluding. It is essential to understand the implications of these findings and their relevance in the neonatal hearing screening.

CHAPTER 5

DISCUSSION

Newborn hearing screening results are affected significantly by middle ear problems. Fluid in the middle ear causes false positive hearing screening results. Such results can lead to unnecessary concerns for parents and additional follow-up evaluations. Middle ear problems also reduce the sensitivity of the hearing screening. Fluid or other obstructions in the middle ear can interfere with sound transmission to the inner ear. This interference can make it more challenging for the screening equipment to accurately detect auditory responses, leading to reduced sensitivity in identifying potential hearing issues. OAE testing is a standard method used in newborn hearing screening. Middle ear problems affect the transmission, making it challenging to obtain reliable OAE responses. In such cases, it is essential to assess the middle ear function in neonates.

Newborns are at a significantly higher risk of experiencing conductive hearing loss, which occurs thirty times more frequently than sensorineural hearing loss. This is often due to congenital middle ear effusion or more severe outer and middle ear issues (Gorga et al., 2001). Therefore, it is essential to incorporate a middle ear assessment tool into newborn hearing screening protocols. Two non-invasive techniques, Wideband Tympanometry (WBT) and High-Frequency Tympanometry (HFT), are commonly employed to evaluate middle ear function in this context.

Wideband Tympanometry (WBT) is a sophisticated method for assessing middle ear functionality. Unlike traditional tympanometry, which relies on a single-frequency probe tone, WBT employs a multifrequency approach, typically spanning from 226 Hz to 8000 Hz. It measures both reflectance (sound energy bouncing back from the middle ear) and absorbance (sound energy absorbed by the middle ear) across

this frequency range, offering a comprehensive evaluation of middle ear function. During the procedure, the patient sits comfortably while an audiologist or skilled healthcare provider inserts a small probe into the ear canal. This probe emits a series of acoustic signals, spanning frequencies from 226 Hz to 2000 Hz or higher, simultaneously recording the ear's response by capturing sound energy reflected from the middle ear (Katz, 2015).

High-Frequency Tympanometry (HFT), also known as 1000 Hz tympanometry, assesses the flexibility and pressure dynamics of the eardrum and middle ear system at the specific frequency of 1000 Hz. This examination involves inserting a probe into the ear canal. The probe emits a high-frequency tone and measures the sound pressure within the ear canal while adjusting the pressure, often through a small pump. During these pressure changes, the instrument records how the middle ear system's impedance or compliance responds at this specific high frequency. The resulting data is typically represented graphically as a tympanogram (Katz, 2015).

WBT and HFT offer objective assessments of middle ear condition, which proves especially valuable in neonates who are unable to offer subjective feedback. These tests are efficient and non-intrusive, rendering them ideal for neonates who might be sensitive or easily disrupted by invasive procedures. Research has demonstrated that WBT and HFT exhibit superior sensitivity compared to traditional tympanometry for identifying conditions like otitis media with effusion and other middle ear issues, particularly in the neonatal population.

A review of literature regarding the use of WBT and HFT in newborns as part of hearing screening protocols has revealed areas requiring further investigation. Limited studies have applied WBT and HFT for assessing the middle ear in newborn hearing screening, primarily involving healthy infants. These studies suggest that WBT

may be slightly more sensitive than HFT, though the difference is not statistically significant (Sanford et al., 2009; Prieve et al., 2013; Hunter et al., 2010). Given the high incidence of otitis media in neonates and its association with referrals from newborn hearing screening, there's a risk of false positive results (Keefe et al., 2003; Boudewyns et al., 2011; Chang et al., 2015). Thus, there's a need to incorporate middle ear assessment tools into screening protocols, as recommended by the Joint Committee on Infant Hearing (JCIH) in 2019. However, data on false positive rates and conductive hearing loss prevalence in young infants in India is lacking. This study aims to address these gaps by conducting middle ear assessments using both WBT and 1000 Hz HFT in neonates, both with and without high-risk factors, who are referred from newborn hearing screening. To the best of our knowledge, such a comprehensive study has not been conducted previously.

The present cross-sectional study aimed to assess the middle ear status of neonates. Two primary groups were examined: Group 1, consisting of neonates without hearing loss risk factors who passed the standard hearing test, and Group 2, further divided into two subgroups. Group 2a comprised neonates without risk factors, and Group 2b included neonates with risk factors referred from the standard test. All subjects underwent WBA and HFT for both ears.

Comparing Group 2a and Group 2b to Group 1, both WBA and HFT identified a significant incidence of middle ear pathology in the former groups, with no significant difference between them. WBA appeared more effective in detecting middle ear issues, possibly because it assesses function across a broad frequency range, while HFT relies on a single frequency.

WBT in neonates.

Group 1 neonates exhibited multipeaked tympanograms with two peaked absorbance responses: one in the range of 1 to 2.5 kHz and another at 6 kHz. Additionally, two notches were observed, one around 0.4 to 0.8 kHz and the other at 3 - 4 kHz. The absorbance pattern in the present study was similar to that found in Aithal et al. (2014) in their cross-sectional study on healthy neonates.

The study involved a comparison of WBA measurements obtained at peak pressure in neonates from Group 2a (without high-risk factors) and Group 2b (with high-risk factors) who were referred from the reference standard tests (OAE and AABR) with neonates from Group 1 (control group). Notably, both Group 2a and Group 2b neonates, referred from the reference standard tests, displayed nearly flat absorbance curves. In contrast, the control group neonates exhibited a distinct WBA pattern characterized by a smooth, broad, double-peaked configuration typically associated with healthy middle ear function. Furthermore, it was observed that both Group 2a and Group 2b neonates had lower absorbance levels across all frequencies when compared to the control group neonates.

The present study findings are consistent with the outcomes of Abbott (2018), which demonstrated that neonates who failed a newborn hearing screening test battery had significantly lower WBA than those who passed. The results of the present study were found to be similar to that of the study conducted by Aithal (2014a), indicating that Australian Aboriginal and Caucasian neonates who failed HFT, DPOAE, and AABR test battery had notably lower WBA compared to those who passed. Results found in our study are further supported by Keefe et al's. (2000) research, demonstrating that WBA measured in healthy babies was significantly higher compared to neonates with risk factors and NICU graduates.

Scientific literature demonstrates the presence of fluid in neonates during the early postnatal period. Roberts et al. (1995) found that 20% of neonates had this fluid after three days. Negative middle ear pressure was observed in some neonates (Bennett, 1975), leading to reduced tympanic membrane mobility (Jaffe et al., 1970). Stuart et al. (1994) reported hearing loss in neonates due to residual amniotic fluid, resulting in ABR Air-Bone Gap (ABG) >12 dB within 48 hours of birth. Kok et al. (1992) observed that TEOAEs could not be recorded in 50% of neonatal ears within 3-51 hours but improved after 24 hours due to fluid clearance. Indeed, these findings strongly support the notion that amniotic fluid in the middle ear at birth leads to temporary conductive hearing loss. Which typically resolves as the fluid naturally clears, followed by negative middle pressure (Priner et al., 2003b). These findings collectively highlight the importance of considering fluid's impact on neonatal hearing screening.

A noteworthy distinction in WBA was observed between neonates who successfully passed the test battery and those who did not. This disparity suggests that the neonates who failed the tests exhibited lower absorbance levels, particularly in speech-relevant frequencies. This phenomenon could be attributed to increased middle ear mass and resistance, which may indicate otitis media with effusion (Beers et al., 2010; Hunter et al., 2010; Shahnaz, 2010). Ears that passed the screening test battery exhibited higher absorbance levels, signifying that neonates who passed had a more efficient acoustic conduction pathway (Aithal, 2014b).

In conclusion, Group 2a and Group 2b neonates who did not pass the reference standard test exhibited lower absorbance levels across all frequencies when compared to the control group. As previously discussed and supported by existing literature, this phenomenon may be associated with the persistence of amniotic fluid or vernix in the neonate's ear canal or middle ear, resulting in increased mass and resistance and reduced

sound absorption by the middle ear. Overall, WBA has proven to be a sensitive tool for detecting middle ear issues. It can serve as an adjunctive tool in newborn hearing screening, aiding the differentiation between conductive and sensorineural hearing loss and thereby reducing false positive results. Ultimately, this contributes to more timely interventions and improved outcomes in neonatal hearing healthcare.

HFT in Neonates

The study included a comparison of HFT measurements taken from neonates in Group 2a and Group 2b, both referred for the reference standard tests, with neonates in Group 1 (the control group). It is crucial to note that neonates in Groups 2a and 2b exhibited nearly flat tympanograms. Conversely, the neonates in the control group displayed a distinct HFT pattern characterized by a smooth, single-peaked tympanogram typically associated with healthy middle ear function. Furthermore, it was observed that the Group 2a and 2b neonates had flat and abnormal tympanograms, along with lower statistical compliance and variable peak pressure values, compared to the control group neonates.

Results found in our studies were supported by Kei et al. (2003) and Margolis et al. (2003), whose studies proved that single peaks obtained out of HFT at 1 kHz were considered to be normally functioning middle ears. In newborns with fluid in the middle ear, Priner et al. (2003b) observed a flat tympanogram, followed by a negative peak with fluid clearance, transitioning to a positive peak or reaching ambient pressure. It was accomplished by increasing OAE magnitude and improving ABR air conduction (AC) thresholds.

These results were also seconded by Swanepoel et al. (2006), who found out that 86% of the ears with OAE pass also had HFT single peaks present, which was labeled

positive correspondence, 6% of those ears that were referred through OAE screening tests had flat curves on HFT which was labeled negative correspondence. Furthermore, 8% of those ears that passed OAE tests showed no peaks in the HFT tests. Hence, the HFT test is a good tool for detecting middle ear problems.

Contrary to these findings, Pestalozza et al. (1980) reported that a smooth-notched curve was recorded in 12 out of 14 ears for cases involving purulent or serous-hematic secretion. The W-shaped curve was observed in one case, and the flat curve occurred in another. In a study conducted by Hoffmann et al. (2013), they examined the test results of 1000 Hz tympanometry in both normal neonates and neonates with craniofacial anomalies. The aim was to determine the most effective stimulus frequency for assessing the middle ear function in newborns. The findings of the study revealed that 1000 Hz was a superior choice compared to lower-frequency stimuli when it came to confirming the normal functioning of the middle ears in these newborns. However, they also mentioned that the sensitivity of the test varies with the types of classification used.

Ravicz et al. (2004) found that umbo velocity exhibits frequency-dependent behavior in the normal middle ear. It is compliance-controlled at low frequencies (up to 1 kHz) and resistance-controlled between 1 and 3 kHz. At higher frequencies, umbo velocity indicates potential middle ear mass effects but lacks consistency. The reduced umbo velocity at low frequencies is associated with middle-ear fluid, which decreases middle-ear volume. At high frequencies, umbo velocity reduction depends on the location of the fluid relative to the tympanic membrane, with fluid on the membrane causing reduction. The extent of reduction increases as more of the membrane is covered by fluid.

In conclusion, fluid in the middle ear can lead to flat or abnormal tympanograms, affecting the movement of the tympanic membrane and resulting in decreased umbo velocity. The extent of this impact varies depending on the quantity of fluid present in the middle ear. It is worth noting that a few ears passed the 1000Hz tympanometry test despite being referred to in the reference standard test because the fluid in the middle ear did not significantly impair the mobility of the tympanic membrane in those particular cases.

Middle ear problems in high-risk and non-high-risk neonates.

In the current study, the incidence of middle ear problems between Group 2a and Group 2b was compared using WBT and HFT test findings. The results indicate no marked difference in the prevalence of middle ear pathologies between high-risk and non-high-risk neonates.

In a contradictory study by White et al. (1993), they discovered that among healthy infants, 17 out of 1000 had conductive hearing loss. In contrast, among infants in the newborn intensive care unit (NICU), the prevalence was higher, with 36 out of 1000 experiencing conductive hearing loss**.**

In conclusion, the current study did not identify a significant difference between high-risk and non-high-risk neonates. This lack of distinction might be attributed to the study focusing exclusively on neonates referred to in newborn hearing screening rather than including the general population of neonates.

The prevalence of middle ear pathologies in both high-risk and non-high-risk neonates can be attributed to various factors and considerations. The observed similarity in prevalence may be primarily linked to the methodology used in selecting the study population. This population encompasses a diverse mix of high-risk and non-

high-risk neonates, with the sampling process lacking strict controls, resulting in a more representative representation of the broader neonatal population. Consequently, the prevalence of middle ear pathologies may appear comparable since it mirrors the wider neonatal demographic.

Middle ear pathologies can stem from a multitude of factors, including infections, structural irregularities, and genetic predispositions. While high-risk neonates may indeed exhibit a higher propensity for specific medical conditions or risk factors, other elements such as genetic factors and exposure to environmental influences can significantly contribute to the development of middle ear issues. These additional factors may also affect non-high-risk neonates, thereby contributing to a more balanced prevalence.

The advancement of neonatal care has yielded improved outcomes for high-risk neonates. This progress encompasses enhanced management of conditions that can contribute to middle ear problems. Consequently, the gap in prevalence between high-risk and non-high-risk neonates may have diminished over time.

High-risk neonates frequently undergo more rigorous medical monitoring and intervention, including regular hearing screenings. This proactive approach facilitates the early identification and management of middle ear issues, potentially mitigating long-term differences in prevalence.

It is imperative to underscore that while the prevalence of middle ear pathologies may exhibit similarities between high-risk and non-high-risk neonates in the current study, this should not diminish the significance of early detection and appropriate intervention, particularly for high-risk neonates. Middle ear problems can exert a profound impact on a child's development, including speech and language proficiency. Hence, early identification and management remain imperative for all newborns.

Comparison of WBT and HFT in identifying the middle ear function.

The current study evaluated the effectiveness of WBT and HFT in assessing middle ear function. The results indicate that WBT is a more effective tool for identifying middle ear problems when compared to HFT. These findings are consistent with the outcomes of Sanford et al. (2009). They reveal that the WBA test outperformed 1-kHz tympanometry in predicting screening outcomes. Specifically, WBA demonstrated an AROC of 0.87, while HFT had an AROC of 0.75. It suggests that WBA predicts screening outcomes more accurately than HFT. Similar findings were also reported by Prieve et al. (2013), indicating that the results of both WBT and HFT are consistent with the type of hearing loss, but WBT is a more effective tool.

The literature demonstrates that the frequency range between 1 and 4 kHz was beneficial for evaluating conductive status (Aithal et al., 2015). Hunter et al. (2010) reported that the AROC was determined to be 0.90 for WBA at 2 kHz and 0.82 for WBA at 1 kHz.

In conclusion, WBT is more effective for detecting middle ear problems than HFT. This superiority is attributed to WBT employing a wide range of frequencies for middle ear assessment. In contrast, HFT relies on a single frequency, providing a more comprehensive evaluation of middle ear function.

Chapter 6

SUMMARY AND CONCLUSION

The external ear and middle ear are crucial components in the process of sound conduction to the inner ear. When fluid accumulates in the middle ear, it can significantly impact the transmission of sound to the inner ear. It occurs because the presence of fluid hampers the mobility of the tympanic membrane and subsequently reduces the velocity of the ossicles. The interference in the mechanical chain of sound conduction can lead to hearing difficulties or impairment. The primary aim of newborn hearing screening is to identify true cases of hearing loss in neonates. However, studies in the literature have indicated that the prevalence of middle ear pathology tends to be higher in neonates who are referred from newborn hearing screening. This higher incidence of middle ear issues can contribute to an increased rate of false positive results in newborn hearing screening programs. This emphasizes the importance of incorporating middle ear assessment tool to accurately distinguish between conductive hearing loss and sensorineural hearing loss in neonates. Early identification of hearing loss in infants is crucial because it allows for prompt and effective intervention during a critical period of language development. It, thus, justify the need to incorporate middle ear assessment tool investigates the efficacy of WBT and/or HFT in reducing false positive rates in newborn hearing screening.

The present study had six primary objectives:

1. To examine middle ear function using WBT in non-high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.
2. To examine the middle ear function using WBT in high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.

3. To examine the middle ear function using HFT in non-high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.
4. To examine the middle ear function using HFT in high-risk babies referred for detailed audiological evaluation based on OAE/AABR evaluation.
5. To compare the middle ear problem in high-risk non-high-risk neonates.
6. To compare the output of WBT and HFT in identifying the middle ear function.

The present study employed a cross-sectional research design with standard group comparison. Two distinct groups of neonates were included in the study. Group 1 comprised neonates without any high-risk factors for hearing loss who successfully passed the reference standard test (OAE and AABR). In contrast, Group 2 was the target group, which was further divided into two subgroups: Group 2a consisted of neonates without any high-risk factors, and Group 2b included neonates with high-risk factors, both of whom were referred from the reference standard test. All the neonates had undergone HFT and WBA for both ears.

The study conducted a comparison of WBA at peak pressure and HFT of the Group 2a and Group 2b neonates with Group 1 neonates. Pass criteria were established by referencing the control group. For WBA, the pass criteria were set within the 10th to 90th percentile range at peak pressure. For HFT, the criteria included a single positive peaked tympanogram falling within the range defined by the mean and standard deviation of the control group.

In comparison with Group 1 WBA data, it was observed that in Group 2a, out of 42 ears, 37 ears were referred, and 5 ears passed. This indicates that 88.09% of neonates in Group 2a had middle ear pathology. Similarly, in Group 2b, out of 48 ears, 42 ears were referred, and 6 ears passed, this indicates that 87.5% of neonates in Group 2b having middle ear pathology. The neonates' ears in both Group 2a and Group 2b who

were referred in WBA displayed lower absorbance across all frequencies. It could be attributed to a reduced acoustic transfer function along the auditory pathway, possibly due to transient middle ear conditions. These transient conditions in the middle ear might have temporarily affected the ear's ability to efficiently transmit sound, leading to the observed lower absorbance levels across the frequency spectrum in the WBA measurements.

In comparison with Group 1 HFT data, in Group 2a, it was observed that out of 42 ears, 33 ears were referred, and 9 ears passed. This implies that 78.57% of neonates in Group 2a had middle ear pathology. Likewise, in Group 2b, out of 48 ears, 38 ears were referred, and 10 ears passed, indicating that 79.16% of neonates in Group 2b had middle ear pathology. The neonates' ears in both Group 2a and Group 2b who were referred in HFT displayed flat or abnormal tympanogram. It could be attributed to extent and quantity of fluid in the middle ear affecting mobility of tympanic membrane.

No significant difference in the incidence of middle ear problems was observed between Group 2a and Group 2b neonates. The lack of a notable difference in middle ear problem between Group 2a and Group 2B neonates may be explained by the fact that the study focused solely on referred neonates from newborn hearing screening, rather than the entire population of neonates.

The comparison between WBA and HFT revealed that WBA is more effective at identifying middle ear pathology. This superiority of WBA may be attributed to its ability to assess middle ear function across a wide range of frequencies, whereas HFT relies on just one frequency for evaluation.

6.1 CONCLUSION:

HFT and WBA both are middle ear function assessing tool. Incorporating WBA into newborn hearing screening, alongside OAE and AABR, could enhance the screening process by reducing false positive results. WBA's superior ability to assess middle ear pathology makes it a valuable addition to ensure more accurate and comprehensive newborn hearing assessments.

6.2 IMPLICATIONS OF THE STUDY

1. One of the critical applications would be that this study's findings might help us understand the need to incorporate a middle ear assessment tool in newborn hearing screening.
2. The result of the present study would also help clinician verify and correlate OAE and AABR results with WBA findings in newborn hearing screening.
3. Results would help clinician to council the parents or caretakers of newborn about test findings of the newborn hearing screening.
4. The findings of the present study suggest the selection of WBA as a middle ear assessment tool over HFT in newborn hearing screening due to its high predictability of OAE and AABR test findings.

6.3 STRENGTH AND LIMITATIONS OF THE STUDY

1. To the best of our knowledge, this is the first study conducted in India on healthy neonates who have passed the newborn hearing screening test, as well as on neonates with and without high-risk factors who have been referred from newborn hearing screening.

2. The current study validated the HFT and WBA outcomes in high-risk and non-high-risk neonates referred from newborn hearing screening and compared them with the normative range of a control group. It effectively distinguished between newborns with and without transient middle ear conditions.
3. The study compared the HFT findings with the WBA findings, which allowed for a more comprehensive comparison of results.
4. Small Sample Size: a small sample size for the control group (31 neonates with 62 ears) used to establish the normative range can be a limitation in a study.
5. In the present study, neonates were grouped based on OAE and AABR test results, even though these tests assess the inner ear. This was done under the assumption that if there is a middle ear pathology, the results of these tests will be affected.

6.3 FUTURE DIRECTIONS

1. Incorporating a larger sample size when establishing a normative range would aid in generalizing the results to the population.
2. Neonates grouped based on Otomicroscopy or Pneumatic otoscopy has with and without middle ear effusion used as a reference for HFT and WBA measurement.

REFERENCE

Abbott, L. (2018). *Wideband acoustic immittance measures as part of a newborn hearing screening program in Canadian First Nations and Metis, Caucasian and other ethnicity neonates* (Doctoral dissertation, University of British Columbia).

Aidan, D., Avan, P., & Bonfils, P. (1999). Auditory screening in neonates by means of transient evoked otoacoustic emissions: a report of 2,842 recordings. *Annals of Otology, Rhinology, and Laryngology*, *108*(6), 525–531. https://doi.org/10.1177/000348949910800601

Aithal, S. (2014b). Wideband Absorbance Measures in Neonates and Young Infants. (Doctor of Philosophy), The University of Queensland, Queensland, AU.

Aithal, S. (2017b). Normative Study of Wideband Acoustic Immittance Measures in Newborn Infants. Journal of speech, language, and hearing research, 60(5), 1417-1426. doi:10.1044/2016_JSLHR-H-16-0237

Aithal, S., Aithal, V., Kei, J., & Wilson, M. (2023). Wideband tympanometry findings in healthy neonates. *Journal of the American Academy of Audiology*.

Aithal, S., Kei, J., & Driscoll, C. (2014a). Wideband Absorbance in Australian Aboriginal and Caucasian Neonates. *Journal of the American Academy of Audiology*, *25*(05), 482–494. https://doi.org/10.3766/jaaa.25.5.7

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*(05), 471–481. https://doi.org/10.3766/jaaa.25.5.6.

Aithal, S., Kei, J., Driscoll, C., Khan, A., & Swanston, A. (2015). Wideband absorbance outcomes in newborns. *Ear And Hearing*, *36*(5), e237–e250. https://doi.org/10.1097/aud.0000000000000175.

Aithal, S., Kei, J., Driscoll, C., Khan, A., & Swanston, A. (2015). Wideband absorbance outcomes in newborns: a comparison with high-frequency tympanometry, automated brainstem response, and transient evoked and distortion product otoacoustic emissions. *Ear and Hearing*, *36*(5), e237-e250.

Alaerts, J., Luts, H., & Wouters, J. (2007). Evaluation of middle ear function in young children: clinical guidelines for the use of 226-and 1,000-Hz tympanometry. *Otology & Neurotology*, *28*(6), 727-732.

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*(4s), 63. https://doi.org/10.1682/jrrd.2005.04.0064

Anitha, T., & Yathiraj, A. (2001). Modified High Risk Registers (HRR) for Professional and Non-professional Formulation and its Efficacy. *Unpublished Independent Project submitted to Univ. of Mysore, as a part fulfillment of M. Sc.(Sp. & Hg.)*.

Baldwin, M. (2006). Choice of probe tone and classification of trace patterns in tympanometry undertaken in early infancy: Selección de la sonda de prueba y clasificación de la curva de timpanometría en la infancia temprana. *International Journal of Audiology*, *45*(7), 417-427.

Beers, A. N., Shahnaz, N., Westerberg, B. D., & Kozak, F. K. (2010). Wideband reflectance in normal Caucasian and Chinese school-age children and in children with otitis media with effusion. Ear and Hearing, 31, 221–223.

Bennett, M. J. (1975). Acoustic Impedance Bridge Measurements with the Neonate. *British Journal of Audiology*. https://doi.org/10.3109/03005367509079122.

Boone, R. T., Bower, C. M., & Martin, P. F. (2005). Failed newborn hearing screens as presentation for otitis media with effusion in the newborn population. *International Journal of Pediatric Otorhinolaryngology*, *69*(3), 393-397.

Boskabadi, H., Zakerihamidi, M., Moradi, A., & Bakhshaee, M. (2018). Risk Factors for Sensorineural Hearing Loss in Neonatal Hyperbilirubinemia. *Iranian journal of otorhinolaryngology*, *30*(99), 195–202.

Boudewyns, A., Declau, F., Van den Ende, J., Van Kerschaver, E., Dirckx, S., Hofkens-Van den Brandt, A., & Van de Heyning, P. (2011). Otitis media with effusion: an underestimated cause of hearing loss in infants. *Otology & neurotology: official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology*, *32*(5), 799–804. https://doi.org/10.1097/MAO.0b013e31821b0d07

Campo, P., Morata, T. C., & Hong, O. (2013). Chemical exposure and hearing loss. *Disease-a-month*, *59*(4), 119-138.

Chang, S. J., Chen, C. J., Lien, C. H., & Sung, F. C. (2006). Hearing loss in workers exposed to toluene and noise. *Environmental health perspectives*, *114*(8), 1283-1286.

Chang, Y., Ryu, G., Kim, K., & Cho, Y. (2019). Normative wideband absorbance measures in healthy neonates in Korea: A preliminary study. *International Journal of Pediatric Otorhinolaryngology*, *117*, 6–11. https://doi.org/10.1016/j.ijporl.2018.11.012.

Choi, Y. H., & Kim, K. (2014). Noise-induced hearing loss in Korean workers: co-exposure to organic solvents and heavy metals in nationwide industries. *PloS one*, *9*(5), e97538.

De Necker, A., Biagio-de Jager, L., & Stoltz, A. C. (2020). Auditory brainstem response test at different stimulus rates in normal-hearing adults living with HIV. *American Journal of Audiology*, *29*(4), 873-886.

De Schrijver, L., Topsakal, V., Wojciechowski, M., Van de Heyning, P., & Boudewyns, A. (2019). Prevalence and etiology of sensorineural hearing loss in children with down syndrome: a cross-sectional study. *International journal of pediatric otorhinolaryngology*, *116*, 168-172.

De, D. (1973). Infection and amniotic aspiration of middle ear in stillbirths and neonatal deaths. *Archives of Disease in Childhood*, *48*(11), 872–880. https://doi.org/10.1136/adc.48.11.872

Doyle, K. J., Burggraaff, B., Fujikawa, S., Kim, J., & MacArthur, C. J. (1997). Neonatal Hearing Screening with Otoscopy, Auditory Brain Stem Response, and Otoacoustic Emissions. *Otolaryngology-Head and Neck Surgery*, *116*(6), 597–603. https://doi.org/10.1016/s0194-5998(97)70234-1

El-Refaie, A., Parker, D., & Bamford, J. (1996). Otoacoustic emission versus ABR screening: The effect of external and middle ear abnormalities in a group of SCBU neonates. *British Journal of Audiology*, *30*(1), 3–8. https://doi.org/10.3109/03005369609077924

Emmett, S. D., & West, K. P., Jr (2014). Gestational vitamin A deficiency: a novel cause of sensorineural hearing loss in the developing world?. *Medical hypotheses*, *82*(1), 6–10. https://doi.org/10.1016/j.mehy.2013.09.028

Engel, J., Anteunis, L., Volovics, A., Hendriks, J., & Marres, E. (1999). Prevalence rates of otitis media with effusion from 0 to 2 years of age: healthy-born versus high-risk-born infants. *International journal of pediatric otorhinolaryngology*, *47*(3), 243-251.

Feeney, M., & Sanford, C. (2012). Application of wideband acoustic transfer functions to the assessment of the infant ear. In J. K. F. Zhao (Ed.), Assessing Middle Ear Function in Infants (pp. 131-161). San Diego CA: Plural Publishing.

Gorga, M. P., Preissler, K., Simmons, J., Walker, L., & Hoover, B. (2001). Some issues relevant to establishing a universal newborn hearing screening program. *Journal of the American Academy of Audiology*, *12*(02), 101–112. https://doi.org/10.1055/s-0042-1745585

Gravel, J. S. (1994). Auditory assessment of infants. *Seminars in Hearing*, *15*(02), 100–113. https://doi.org/10.1055/s-0028-1083759.

Güven, S. G., Taş, M., Bulut, E., Tokuç, B., Uzun, C., & Karasalihoğlu, A. R. (2019). Does noise exposure during pregnancy affect neonatal hearing screening results. *Noise & Health*, *21*(99), 69.

Heidari, S., Manesh, A. O., & Rajabi, F. (2015). The sensitivity and specificity of automated auditory brainstem response and otoacoustic emission in neonatal hearing screening: a systematic review. *Auditory and vestibular research*, *24*(3), 141-151.al, S., Aithal, V., Kei, J., & Wilson, M. (2023). Wideband tympanometry findings in healthy neonates. *Journal of the American Academy of Audiology*.

Hirsch, A. (1991). Behavioural Tests: Applications and Limitations in Comparison with Brainstem Response Audiometry. *Acta Oto-laryngologica*, *111*(sup482), 118–125. https://doi.org/10.3109/00016489109128034

Hoffmann, A., Deuster, D., Rosslau, K., Knief, A., Zehnhoff-Dinnesen, A. A., & Schmidt, C. (2013). Feasibility of 1000Hz tympanometry in infants: Tympanometric trace classification and choice of probe tone in relation to age. *International Journal of Pediatric Otorhinolaryngology*, *77*(7), 1198–1203. https://doi.org/10.1016/j.ijporl.2013.05.001

Hood, L. J. (1998). Clinical applications of the auditory brainstem response. Singular Pub. *Group*.

Hunter, L. L., & Margolis, R. H. (1992). Multifrequency tympanometry. *American Journal of Audiology*, *1*(3), 33–43. https://doi.org/10.1044/1059-0889.0103.33

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., Prieve, B. A., Kei, J., & Sanford, C. A. (2013). Pediatric applications of wideband acoustic immittance measures. *Ear and hearing*, *34*, 36s-42s.

Hunter, L., Feeney, P., Lapsley-Miller, J., Jeng, P., & Bohning, S. (2010). Wideband Reflectance in Newborns: Normative Regions and Relationship to Hearing-Screening Results. Ear & Hearing, 31, 599-610.

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

Jaffe, B. F., F, H., & Hurtado, E. (1970). TYMPANIC MEMBRANE MOBILITY IN THE NEWBORN (WITH SEVEN MONTHS?? FOLLOW-UP). *Laryngoscope*, *80*(1), 36–48. https://doi.org/10.1288/00005537-197001000-00004

Jaffe, B. F., F, H., & Hurtado, E. (1970b). TYMPANIC MEMBRANE MOBILITY IN THE NEWBORN (WITH SEVEN MONTHS?? FOLLOW-UP). *Laryngoscope*, *80*(1), 36–48. https://doi.org/10.1288/00005537-197001000-00004

Joint Committee on Infant Hearing. (2019). Year 2019 position statement: Principles and guidelines for Early Hearing Detection and Intervention programs. Journal of Early Hearing Detection and Intervention, 4(2),1–44. https://doi.org/10.15142/fptk-b748

Juliano, A. F., Ginat, D. T., & Moonis, G. (2015). Imaging Review of the Temporal Bone: Part II. Traumatic, Postoperative, and Noninflammatory Nonneoplastic Conditions. *Radiology*, *276*(3),655–672. https://doi.org/10.1148/radiol.2015140800

Katz, J., Chasin, M., English, K. M., Hood, L. J., & Tillery, K. L. (Eds.). (2015). *Handbook of clinical audiology* (Vol. 7). Philadelphia, PA: Wolters Kluwer Health.

Keefe, D. H., & Levi, E. C. (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., & 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.

Keefe, D. H., Bulen, J. C., Arehart, K. H., & Burns, E. B. (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., 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.

Kei, J., Allison-Levick, J., Dockray, J., Harrys, R., Kirkegard, C., Wong, J. Y. H., Maurer, M., Hegarty, J., Young, J., & Tudehope, D. (2003b). High-Frequency (1000 Hz) tympanometry in normal neonates. *Journal of the American Academy of Audiology*, *14*(01), 020–028. https://doi.org/10.3766/jaaa.14.1.4

Kei, J., Allison-Levick, J., Dockray, J., Harrys, R., Kirkegard, C., Wong, J. Y. H., Maurer, M., Hegarty, J., Young, J., & Tudehope, D. (2003). High-Frequency (1000 Hz) tympanometry in normal neonates. *Journal of the American Academy of Audiology*, *14*(01), 020–028. https://doi.org/10.3766/jaaa.14.1.4

Kemp D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *The Journal of the Acoustical Society of America*, *64*(5), 1386–1391. https://doi.org/10.1121/1.382104

Kemp, D. T. (2002). Otoacoustic emissions, their origin in cochlear function, and use. *British medical bulletin*, *63*(1), 223-241.

Kilic, A., Baysal, E., Karatas, E., Baglam, T., Durucu, C., Deniz, M., ... & Mumbuc, S. (2012). The role of high frequency tympanometry in newborn hearing screening programme. *European review for medical and pharmacological sciences*, *16*(2), 220-3.

Kimberling, W. J., Hildebrand, M. S., Shearer, A. E., Jensen, M. L., Halder, J. A., Trzupek, K., ... & Smith, R. J. (2010). Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. *Genetics in Medicine*, *12*(8), 512-516.

Kok, M. R., Van Zanten, G. A., & Brocuar, M. P. (1992). Growth of evoked otoacoustic emissions during the first days postpartum: A preliminary report. *Audiology*, *31*(3), 140-149.

Kutz, J. W., Simon, L. M., Chennupati, S. K., Giannoni, C. M., & Manolidis, S. (2006). Clinical predictors for hearing loss in children with bacterial meningitis. *Archives of otolaryngology--head & neck surgery*, *132*(9), 941–945. https://doi.org/10.1001/archotol.132.9.941

Lenneberg, E.H. (1967). Biological Foundations of Language. New York: Wiley. ISBN 978-0-89874-700-3.

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

Mahdieh, N., Rabbani, B., Wiley, S., Akbari, M. T., & Zeinali, S. (2010). Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. *Journal of human genetics*, *55*(10), 639-648.

Maico. (2020). easyScreen Operation Manual.

Maico. (2020). Operaion Manual EROSCAN Screening and Diagnostic Version.

Marchant, C. D., McMillan, P. M., Shurin, P. A., Johnson, C. E., Turczyk, V. A., Feinstein, J. C., & Panek, D. M. (1986). Objective diagnosis of otitis media in early infancy by tympanometry and ipsilateral acoustic reflex thresholds. *The Journal of pediatrics*, *109*(4), 590-595.

Margolis, R. H., Bass-Ringdahl, S., Hanks, W. D., Holte, L., & Zapala, D. A. (2003). Tympanometry in newborn infants—1 KHz norms. *Journal of the American Academy of Audiology*, *14*(07), 383–392. https://doi.org/10.1055/s-0040-1715757

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.

Mason, J. A., & Herrmann, K. R. (1998). Universal infant hearing screening by automated auditory brainstem response measurement. *Pediatrics*, *101*(2), 221-228.

Mauk, G. W., White, K. R., Mortensen, L. B., & Behrens, T. R. (1991). The effectiveness of screening programs based on high-risk characteristics in early identification of hearing impairment. *Ear and hearing*, *12*(5), 312-319.

Meadow-Orlans, K. P., & Steinberg, A. G. (1993). Effects of infant hearing loss and maternal support on mother-infant interactions at 18 months. *Journal of Applied Developmental Psychology*, *14*(3), 407-426.

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.

Meyer, S., Jardine, C. A., & Deverson, W. (1997). Developmental changes in tympanometry: a case study. *British Journal of Audiology*, *31*(3), 189–195. https://doi.org/10.3109/03005364000000021

Murhekar, M., Verma, S., Singh, K., Bavdekar, A., Benakappa, N., Santhanam, S., Sapkal, G., Viswanathan, R., Singh, M. P., Nag, V. L., Naik, S., Munivenkatappa, A., Abraham, A. M., Devika, Sabarinathan, R., Verghese, V. P., George, S., Sachdeva, R. K., Kolekar, J., . . . Gupta, N. (2020b). Epidemiology of Congenital Rubella Syndrome (CRS) in India, 2016-18, based on data from sentinel surveillance. *PLOS Neglected Tropical Diseases*, *14*(2), e0007982. https://doi.org/10.1371/journal.pntd.0007982

NORTHERN, J. L., & DOWNS, M. P. (1978). Hearing in children (2nd ed.). Baltimore: Williams & Wilkins.

Olusanya B. O. (2010). Is undernutrition a risk factor for sensorineural hearing loss in early infancy?. *The British journal of nutrition*, *103*(9), 1296–1301. https://doi.org/10.1017/S0007114509993059

Palva, T., Northrop, C., & Ramsay, H. (1999). Spread of amniotic fluid cellular content within the neonate middle ear. *International journal of pediatric otorhinolaryngology*, *48*(2), 143-153.

Park, M. K. (2017). Clinical applications of wideband tympanometry. *Korean Journal of Otorhinolaryngology-Head and Neck Surgery*, *60*(8), 375-380.

Pati, S. K., Pinninti, S., Novak, Z., Chowdhury, N., Patro, R. K., Fowler, K., Ross, S., Boppana, S., & NIDCD CHIMES Study Investigators (2013). Genotypic diversity and mixed infection in newborn disease and hearing loss in congenital

cytomegalovirus infection. *The Pediatric infectious disease journal*, *32*(10), 1050–1054. https://doi.org/10.1097/INF.0b013e31829bb0b9

Pestalozza, G., & Cusmano, G. (1980). Evaluation of tympanometry in diagnosis and treatment of otitis media of the newborn and of the infant. *International journal of pediatric otorhinolaryngology*, *2*(1), 73-82.

Pinna, M. H., Bento, R. F., & Neto, R. V. (2012). Vestibular schwannoma: 825 cases from a 25-year experience. *International archives of otorhinolaryngology*, *16*(4), 466–475. https://doi.org/10.7162/S1809-97772012000400007

Pitaro, J. (2016). Wideband reflectance measurements in newborns: Relationship to otoscopic findings. International Journal of Pediatric Otorhinolaryngology, 86, 156-160. doi:10.1016/j.ijporl.2016.04.036

Piza, J., Gonzalez, M., Northrop, C. C., & Eavey, R. D. (1989). Meconium contamination of the neonatal middle ear. *The Journal of pediatrics*, *115*(6), 910-914.

Prieve, B. A., Vander Werff, K. R., 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.

Priner, R., Freeman, S., Perez, R., & Sohmer, H. (2003b). The Neonate Has a Temporary Conductive Hearing Loss due to Fluid in the Middle Ear. *Audiology and Neuro-otology*, *8*(2), 100–110. https://doi.org/10.1159/000068997

Raghuwanshi, S. K., Gargava, A., Kulkarani, V., & Kumar, A. (2019). Role of otoacoustic emission test in neonatal screening at tertiary center. *Indian Journal of Otolaryngology and Head & Neck Surgery*, *71*(Suppl 2), 1535-1537.

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.

Rhodes, M. C., Margolis, R. H., Hirsch, J. E., & Napp, A. P. (1999). Hearing screening in the newborn intensive care nursery: comparison of methods. *Otolaryngology–Head and Neck Surgery*, *120*(6), 799-808.

Roberts, D. G., Johnson, C., Carlin, S. A., Turczyk, V. A., Karnuta, M. A., & Yaffee, K. (1995). Resolution of middle ear effusion in newborns. *Archives of Pediatrics & Adolescent Medicine*, *149*(8), 873. https://doi.org/10.1001/archpedi.1995.02170210047008

Sanford, C. A., Hunter, L. L., Feeney, M. P., & Nakajima, H. H. (2013). Wideband acoustic immittance. *Ear And Hearing*, *34*(Supplement 1), 65s–71s. 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. (2009b). 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

Sangster, L. (2011). Critical review: Can wideband energy reflectance be used in newborn hearing screening to detect transient middle ear dysfunction and to interpret screening results. *Consulted*, *25*, 2011-12.

Sanord, 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

Selander, J., Albin, M., Rosenhall, U., Rylander, L., Lewné, M., & Gustavsson, P. (2016). Maternal occupational exposure to noise during pregnancy and hearing dysfunction in children: a nationwide prospective cohort study in Sweden. *Environmental Health Perspectives*, *124*(6), 855-860.

Shahnaz, N. (2010). Clinical application of wideband reflectance (WBR) in infants, children and adults. Canadian Hear Rep, 5, 23-29.

Sheffield, A. M., & Smith, R. J. (2019). The epidemiology of deafness. *Cold Spring Harbor perspectives in medicine*, *9*(9).

Sheng, H., Zhou, Q., Wang, Q., Yu, Y., Liu, L., Liang, M., ... & Huang, Z. (2021). Comparison of two-step transient evoked otoacoustic emissions and one-step automated auditory brainstem response for universal newborn hearing screening programs in remote areas of China. *Frontiers in Pediatrics*, *9*, 655625.

Smith, R. J., Shearer, A. E., Hildebrand, M. S., & Van Camp, G. (1993). Deafness and hereditary hearing loss overview. *GeneReviews*.

Sood, A. S., Bons, C. S., & Narang, G. S. (2013). High frequency tympanometry in neonates with normal otoacoustic emissions: measurements and

interpretations. *Indian Journal of Otolaryngology and Head & Neck Surgery*, *65*, 237-243.

Stinson, M. R. (1990). Revision of estimates of acoustic energy reflectance at the human eardrum. *The Journal of the Acoustical Society of America*, *88*(4), 1773-1778.

Stuart, A., Yang, E. Y., & Green, W. B. (1994). Neonatal auditory brainstem response thresholds to air-and bone-conducted clicks: 0 to 96 hours postpartum. *Journal of the American Academy of Audiology*, *5*(3), 163-172.

SURG1995, O. H. (1995). Joint Committee on Infant Hearing 1994 Position Statement. *Otolaryngology-Head and Neck Surgery*, *113*(3), 191-196.

Sutton, G. J., Gleadle, P., & Rowe, S. J. (1996). Tympanometry and otoacoustic emissions in a cohort of special care neonates. *British Journal of Audiology*, *30*(1), 9–17. https://doi.org/10.3109/03005369609077925

Swanepoel, D. W., Von Hugo, R., & Louw, B. (2006). Infant hearing screening at immunization clinics in South Africa. *International Journal of Pediatric Otorhinolaryngology*, *70*(7), 1241–1249. https://doi.org/10.1016/j.ijporl.2006.01.002

Teixeira, M. H., Borges, V. M. S., Riesgo, R. D. S., & Sleifer, P. (2020). Hyperbilirubinemia impact on newborn hearing: a literature review. *Revista da Associação Médica Brasileira*, *66*, 1002-1008.

Thangavelu, K., Martakis, K., Feldmann, S., Roth, B., & Lang-Roth, R. (2023). Referral rate and false-positive rates in a hearing screening program among high-risk newborns. *European Archives of Oto-Rhino-Laryngology*, 1-11.

Tong, Y. C., Clark, G. M., Seligman, P. M., & Patrick, J. (1980). Speech processing for a multiple-electrode cochlear implant hearing prosthesis. *Journal of the Acoustical Society of America*, *68*(6), 1897–1899. https://doi.org/10.1121/1.385184

Van Straaten, H. L. M. (1999). Automated auditory brainstem response in neonatal hearing screening. *Acta Paediatrica*, *88*, 76-79.

Venkatesh, M. D., Moorchung, N., & Puri, B. (2015). Genetics of non syndromic hearing loss. *medical journal armed forces india*, *71*(4), 363-368.

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

Wali, H. A., Mazlan, R., & Kei, J. (2017). Pressurized wideband absorbance findings in healthy neonates: a preliminary study. *Journal of Speech Language and Hearing Research*, *60*(10), 2965–2973. https://doi.org/10.1044/2017_jslhr-h-17-0120.

Wet Swanepoel, D., Werner, S., Hugo, R., Louw, B., Owen, R., & Swanepoel, A. (2007). High frequency immittance for neonates: a normative study. *Acta Oto-Laryngologica*, *127*(1), 49-56.

White, K. R. (1996, May). Universal newborn hearing screening using transient evoked otoacoustic emissions: Past, present, and future. In *Seminars in Hearing* (Vol. 17, No. 02, pp. 171-182). Copyright© 1996 by Thieme Medical Publishers, Inc..

Appendix

Appendix 1.

Subject No.	Risk factors
1	3rd degree consanguinity.
2	3rd degree consanguinity.
3	Delayed birth cry.
4	Neonatal jaundice.
5	Premature, low birth weight, NICU.
6	Elderly pregnancy, low birth weight, high BP.
7	Delayed birth cry.
8	Delayed birth cry.
9	2nd degree consanguinity.
10	2nd degree consanguinity.
11	High BP, low birth weight, premature, NICU.
12	Delayed birth cry, history of abortion.
13	3rd degree consanguinity.
14	Family history of childhood hearing loss, delayed birth cry.
15	Family history of childhood hearing loss and speech disorder.
16	Low birth weight, premature, NICU.
17	Low birth weight, 3rd degree consanguinity.
18	Family history of childhood hearing loss.
19	Family history of ID, 3rd degree consanguinity.
20	Elderly pregnancy, high BP.

21	3rd degree consanguinity.
22	Delayed birth cry.
23	2nd degree consanguinity.
24	3rd degree consanguinity.

NOTE: 10 neonates had more than one risk factor.

Appendix 2.

High-risk factors are as follows:

- Consanguinity.
- Family history of childhood SN hearing loss.
- Delayed birth cry.
- Incubator/ICN admission.
- Pre-maturity.
- Maternal illness during pregnancy.
- Illness to the child.
- Hyper-bilirubinemia in the child.
- Drug intaken by mother during pregnancy.
- Birth asphyxia.
- Drug given for illness to child.
- Congenital craniofacial anomalies.