Immittance findings in infants using different probe tone frequencies

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ALL INDIA INSTITUTE OF SPEECH & HEARING,

MANSAGANGOTHRI, MYSORE-570006

JUNE, 2011.

Dedicated to

AMMA

CERTIFICATE

This is to certify that this dissertation entitled **"Immitance findings in infants using different probe tone frequencies"** is a bonafide work in part of fulfillment for the degree of Master of Science (Audiology) of the student Registration number: 09AUD004. This has been carried under the guidance of a faculty of this institute and has not been submitted earlier to any other university for the award of any diploma or degree.

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CERTIFICATE

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DECLARATION

This is to certify that this master's dissertation entitled "Immittance findings in infants using different probe tone frequencies" is the result of my own study and has not been submitted earlier to any other university for the award of any degree or diploma.

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(James 1:5)

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1. Introduction

The hearing during the early years of life is critical for the development of speech, language, and cognition. It has long been recognized that failure to identify hearing loss early can adversely affect speech and language development as well as academic achievement and social-emotional development at later stages.

In the last few years, with the accelerating implementation of Universal new born hearing screening programs, there is rapid growth of pediatric population being tested and hence there is a crucial need for a test of middle ear function to distinguish sensorineural hearing loss from middle ear pathology. Nevertheless, the identification of hearing loss type is very important because the course of medical and audiological treatment differs for conductive and sensorineural hearing impairment. Although conductive hearing loss is often temporary in nature, if it is not treated during the early years of life, then it can have serious complications in speech and language development of the child.

Moreover, in young children, the incidence of middle ear problems is very high. Approximately 70 to 90 % of all children are affected by otitis media with effusion at some time before school age and more than 50 % in their first year of life (Harris, Hutchinson & Moravec, 2005). Recurrent otitis media with effusion can result in mild to moderate hearing loss because persistent middle ear fluid decreases the mobility of the tympanic membrane and ossicles.

Boone, Bower and Martin (2005) has pointed out that otitis media with effusion may contribute up to 67 % of the false positive newborn hearing screens. Doyle et al. (2004) found that 58 % of neonates identified with effusion within the first 48 hours of life had chronic otitis media during the first year of life with thresholds exceeding 25 dB HL at 1, 2 and 4 KHz by 9 months of age. As transient middle ear dysfunction may be more prevalent in neonatal and infant ears than sensorineural hearing loss there is critical need for better understanding of middle ear functioning in neonates and infants (Gorga & Norton, 2000)

Acoustic immittance testing represents a powerful tool in the clinician's armamentarium, by providing information regarding the presence of even a mild conductive pathology. Immittance measurement is comprised of two tests: tympanometry and acoustic reflexometry. Tympanometry is an objective and adequate test in detecting middle ear problems.

Conventional immittance evaluations of middle ear functioning with a 226 Hz probe tone have been demonstrated to be unreliable due to its poor sensitivity in identifying middle ear pathology in infants younger than 7 months of age, despite its successful application to adults and older children.

The sharp sensitivity contrast of 226 Hz tympanometry in young infants and adults has been attributed to the fact that the young infant's middle ear is mass dominated compared with a stiffness dominated system in adults. Low frequency probe tones are well suited to evaluate stiffness dominated systems and not the mass dominated middle ears of young infants (Holte et al, 1991; Keefe et al, 1996). Therefore for the non-stiffness dominated systems of neonates and young infants, a different probe tone should be used in assessing middle ear function (Marchant et al, 1986).

Both the Joint Committee on Infant Hearing (2007) and the American Speech-Language Hearing Association (2004) recommend high-frequency tympanometry for infants from birth to six months of age. Several high-frequency probe tones such as 660 Hz, 880 Hz & 1000 Hz have been studied. Recently, research has centered on a 1000 Hz probe tone which is currently the highest frequency available on commercial clinical immittance meters (Purdy & Williams, 2000). This 1000 Hz probe tone frequency has been shown to be more valid in identifying middle ear pathology in infants than other high-frequency probe tones such as 678 Hz according to Baldwin (2006), Rhodes et al (1999) and Williams et al (1995).

Apart from tympanometry, acoustic reflex thresholds are also important to assess middle ear status of infants. Acoustic reflexes have also proven a very good complement to tympanometry, since any conductive malfunction will diminish the reflex induced admittance change. Hence, a present reflex is a strong indicator that the middle ear is healthy (Gates et al., 1994). Both tympanometry and acoustic reflex measurements could be used to supplement the test battery (ASHA 2007).

Reliable acoustic reflex measures have potential value in the audiometric assessment of infants. Ipsilateral testing offers advantages over contralateral testing because the procedure is easier and possible in infants without the need for placement of the contralateral earphone, and results cannot be confounded by contralateral ear factors. Apart from these benefits, ipsilateral reflexes are very sensitive to middle ear pathology because ipsilateral reflexes are affected by both probe ear and stimulus ear effects of conductive disorders.

It has also been reported that acoustic reflexes cannot be reliably measured using a 226 Hz probe tone in neonates and infants as the presence of acoustic reflexes at this probe tone frequency was reported to be less when reflexes were measured at 226 Hz probe tone (Keith & Bench, 1978; McMillan et al, 1985; Weatherby & Bennett, 1980) This could be because of the lower resonant frequency of the infant ear. Hence, 1000 Hz probe tones have been recommended for tympanometric and acoustic reflex immittance measurements (Lantz, 2004).

Need for the study

The clinical utility of tympanometry has been clearly established for all populations except infants less than 1 year of age. Unfortunately, there has been little research and insufficient data comparing low (226Hz) and high frequency (668 Hz & 1000Hz) tympanometry and acoustic reflex threshold results obtained from infants at various developmental stages throughout the first year of life for routine use.

It is also clinically important to confirm that acoustic reflexes are present in all normal neonatal and infant ears when a probe tone of 1000 Hz is used. It is, therefore, not certain if the acoustic reflex thresholds obtained from healthy neonates at birth are different to those obtained at 1 year because during this period, rapid development of the ear takes place which may change the tympanometric values as well as acoustic reflex thresholds.

Hence there is a need for age specific data based on chronological age in months in infants to be used in assessing middle ear function and to improve the diagnostic utility of tympanometry in young infants.

Aim of the study

The present study attempts to:

a) Establish various tympanometric measures in infants using 1000 Hz probe tone
b) Know how the tympanometric measures differ in infants for 226 Hz, 668 Hz and 1000 Hz probe tone frequency
c) Document the changes in tympanometric findings across different age groups in various infants
d) Know how the acoustic reflex thresholds (ipsilateral) for 500 Hz, 2000 Hz and 4000 Hz pure tones varies with 226 Hz and 1000 Hz probe tone in infants.

2. Review of literature

Immittance measurements were first developed in the 1950s by Terkildsen and coworkers to measure middle ear pressure. Overtime, the contribution of immittance measurement to clinical diagnostics has become widely valued for its prominent role in the study of middle ear mechanics and it now forms a part of the routine basic hearing assessment battery.

In the last few years, audiologists have been faced with a very young patient population due to neonatal hearing screening programs. Audiologists are now faced with the goal of diagnosing the type and severity of an infant's hearing loss by three months of age (JCIH, 2007). The most common cause of referral on Universal new born hearing screening (UNHS) is conductive hearing loss, the majority of which is secondary to otitis media or middle ear effusion (Boone, Bower, & Martin, 2005; Doyle, Burggraff, Fujikawa, & Macarthur, 1997). Hence, it is of great importance on the part of audiologists that the infant's hearing can be accurately assessed by identifying or ruling out the middle ear disorders as soon as possible to provide adequate and appropriate rehabilitation.

Several studies have been done on the prevalence of different middle ear disorders in children. In a cohort study done by Alho, Oja, Koivu, & Sorri (1995) on infants followed from birth to 24 months of age, the prevalence of persistent otitis media with effusion was determined to be 4.4%. In the Netherlands, a longitudinal study carried out by Engel, Anteunis, Voloviks, Hendriks, & Marres (1999) assessed for the prevalence of otitis media with effusion on 150 healthy infants who were followed from birth through 24 months of age and their results revealed that the prevalence of otitis media with effusion increased from 19 % at birth to a peak of 4 9% at 9-12 months in healthy born babies.

In children, this high prevalence of otitis media with effusion underscores the need for pediatric acoustic immittance screening, since it can be associated with as much as a mild to moderate conductive hearing impairment (Silverman, 2010). Currently immittance measurement is a very promising physiologic test that could address the need to differentiate conductive hearing loss from sensorineural hearing loss.

Clinical immittance measures can be broadly separated into two general areas tympanometry and acoustic reflex measures. Tympanometic measures provide useful information regarding middle ear and eustachian tube function, including indicators of the presence or absence of middle ear effusion, patency of pressure equalization tubes and the presence of tympanic membrane perforations. Acoustic reflex measures are based on changes in acoustic admittance with stapedius muscle contractions. These acoustic reflex measures provide information related to the function of the middle ear and the sensory, neural and motor pathways associated with acoustic reflex arc.

2.1 Tympanometry

2.1.1 Tympanometry definition

Tympanometry is defined as the dynamic measure of acoustic immittance in the external ear canal as a function of changes in the air pressure in the external ear canal (ANSI, S3.39- 1987). The sound pressure level monitored at the probe tip provides an index of the ease with which acoustic energy flows into the middle-ear system, which is referred to as acoustic admittance (Y). The resulting display where acoustic admittance in mmhos or equivalent volume units in milliliters is plotted against air pressure in decapascals (daPa) is called a tympanogram.

2.1.2 Types of tympanometry

2.1.2.1 Tympanometry using 226 Hz probe tone frequency

The most commonly used probe-tone frequency is 226 Hz. This probe tone has some definitive advantages for testing the adult ear because the adult middle ear system is stiffness-controlled at this frequency. The 226 Hz probe tone is below the normal adult resonance, which lies between 650 Hz and 1400 Hz, so the effects of mass and friction are minor (Lantz et al., 2004).

The 226 Hz tympanograms are typically single-peaked and it is easy to interpret these tympanograms. A common classification system for interpreting tympanograms with a 226 Hz probe exists (Liden et al., 1969, modified by Jerger, 1970). Another advantage of this probe tone and the very reason for choosing 226 Hz originally, is that the true compliance value at this frequency is numerically equal to an enclosed volume of air. This is utilized for calibration purposes, since the admittance is 1 mmho when measured in a 1 cc cavity of air.

Static acoustic admittance, peak pressure and ear canal admittance are used routinely in interpreting standard low frequency tympanograms.

The peak compensated static acoustic admittance (Ytm) describes the height of the tympanogram measured at the plane of the tympanic membrane. This measure is useful because certain middle ear disorders can increase or decrease the normal height /

admittance of the tympanogram (Fowler & Shanks, 2002). Some of the disorders which increase the admittance of the tympanogram include ossicular chain discontinuity, ear drum pathology such as atrophic scarring / tympanosclerotic plaques and some disorders which decrease the admittance of the tympanogram include middle ear effusion, otosclerosis, thickened ear drum and malleus fixation.

Tympanometric peak pressure (TPP) is the pressure in deca Pascals at which peak of the tympanogram occurs (ANSI S3.39 - 1987). This measure provides an estimate of the pressure within the middle ear cavity (Fowler & Shanks, 2002). However TPP has not been found to be useful for the diagnosis of middle ear disorders and it is not recommended as a screening tool for assessing middle ear disorders (ASHA, 1997) as TPP was reported to lack diagnostic specificity. But, TPP can be used as one of the tympanometric parameters to assess middle ear function as children with high negative TPP are more likely to develop middle ear effusion than those with normal TPP values and hence should be monitored closely (Antonio et al., 2002).

Equivalent ear canal volume (V_{ea} or V_{ec}) is an estimate of the volume of air medial to the probe, which includes the volume between the probe tip and the tympanic membrane. The estimate of ear canal volume is useful for two reasons. First, an accurate determination of the compensated static admittance (Ytm) depends on an accurate estimation of the ear canal volume. If the ear canal volumes are overestimated, then the static admittance will be underestimated. Second, in case of flat tympanograms, the estimation of ear canal volume can provide a clue to the cause of the flat tympanogram, whether it is due to artifact / due to tympanic membrane perforation or middle ear effusion.

The use of conventional tympanometry using 226 Hz probe tone frequency to detect middle ear dysfunction for infants aged less than 7 months may produce erroneous test outcomes despite its successful application to adults and children. Paradise, Smith and Bluestone (1976) were the first to cast suspicion over the poor sensitivity (a high false negative rate) with the use of 226 Hz tympanometry with those children who are younger than 7 mnths when they published extensive data on 280 infants between the ages of 10 days to 5 years. For instance, Paradise, Smith & Bluestone (1976) have obtained type A tympanograms (normal static admittance and normal middle ear pressure) based on Liden / Jerger classification system (Liden, 1960; Jerger, 1970), coexisting with confirmed middle ear effusion in that age group and subsequent research has corroborated these findings (Shurin et al,1976, 1977; Weatherby & Bennett, 1980; Sprague et al, 1985; Hunter & Margolis, 1992; Keefe et al, 1993; Williams et al,1993; Meyer et al;1997; Rhodes et al,1999 Purdy & Williams, 2000; Sininger, 2003). In addition, low probe tone frequency has also produced flat tympanograms, indicating otitis media with effusion under the Jerger / Liden classification system, in neonates with normal middle-ear function (Keefe & Levi, 1996; Rhodes et al, 1999). Hence it is not recommended for infants aged less than 7 months due to poor sensitivity in identifying middle ear pathology (Meyer et al, 1997; Paradise et al, 1976; Petrak, 2002; Purdy & Williams, 2000; Rhodes et al, 1999; Sininger, 2003).

Furthermore, tympanograms obtained using 226 Hz probe tones produces different shaped tympanograms in infants compared with adults and older children. Low frequency probe tone tympanograms are more likely to show notching or complex patterns in infants (Holte et al., 1991; Keith 1975; McKinley, Grose & Roush, 1997;

Keith, 1973; Sprague et al, 1985; Holte et al, 1991). This makes it difficult to apply the traditional type A, B, C tympanogram classification scheme in infants. In the infant ear, tympanograms become simpler in shape as probe frequency increases. This is the reverse of what is found in adult ears, where tympanograms become more complex as probe tone frequency increases (Shahnaz, 2007, Margolis et al, 2003, Sprague et al., 1985).

Although the exact cause for the differences between tympanometric patterns remain unclear, these spurious middle-ear findings obtained with 226 Hz probe tones have been attributed to the fact that an infant's middle ear is a mass dominated system and has a lower resonant frequency, in sharp contrast to the adult ear, which is a stiffness dominated system (Holte et al, 1991).

Many investigations have documented the effects of maturation on the outer ear and middle ear in infants and children. Such effects include the development of the osseous external auditory meatus during the first 12 months of life (Anson, Bast, & Richany, 1955; Anson & Donaldson, 1981), rapid increases in all dimensions of the external auditory meatus during the first 2 years of life (Keefe et al, 1993; Saunders et al, 1983), increases in the size of the middle-ear cavity and mastoid with temporal bone growth and increases in the size of the antral and mastoid sinuses throughout childhood (Eby & Nadol, 1986; Anson & Donaldson, 1981; Saunders et al., 1983), fusion of the tympanic annulus with the temporal bone (Anson et al., 1955; Anson & Donaldson, 1981), increased orientation of the tympanic membrane within the vertical plane (Eby & Nadol, 1986), ossification of the bone marrow within parts of the malleus and incus that is followed by transformation into vascular channels by 25 months of age (Yokoyama, Ino, Kakizaki, & Murakami, 1999).

There are also evidence regarding decrease in mass postnatally from resolution of residual mesenchyme in the middle ear with age (Yokoyama et al., 1999) or from disappearance of the amniotic fluid in the middle ear with age (Paparella et al., 1980), increase in middle-ear stiffness (according to Keefe et al., 1993, the middle-ear stiffness in infants less than 24 months of age represents 50% of that in adults) by tightening of the ossicular joints (Anson & Donaldson, 1981), loosening of the coupling between the stapes and annular ligament (Keefe et al., 1993); and decrease in resistance of external auditory meatus during the first 24 months of age, especially during the first 12 months (Keefe, 2010).

These developmental changes in the anatomical structures of external ear and middle ear may be accounted for differences in acoustic energy transmission through the middle ear between infants and adults. Some of the external and middle ear changes that take place after birth make the middle ear system more and more stiffness dominated as age increases. However at the younger age, since infant's ear is mass dominated / non-stiffness dominated, a different probe tone should be used in assessing middle-ear function (Marchant et al, 1986) other than conventional 226 Hz probe tone frequency.

The resonant frequency of the neonatal tympanic membrane is also reported to be much lower than in adults (Weatherby & Bennett, 1980; Holte, 1991). Meyer et al (1997) measured the resonant frequency of the ear of one infant from two weeks to six and a half months old and found that it remained below 550 Hz until she was 14 weeks old. Adult middle ear resonant frequency of 800 Hz - 1200 Hz (Silman & Silverman, 1991) was reached by three to four months. This supported the theory that the infant middle ear changes from a mass- to stiffness-dominated system, and that tympanometry using 226 Hz probe tone is not appropriate below this age. This is also supported by recent study done by Baldwin (2006) where it was reported that tympanometry using 226 Hz is invalid below 21 weeks of age in infants

2.1.2.2 Tympanometry using 678 Hz probe tone frequency

Previous investigations of tympanometry in infants have focused on the use of a higher frequency probe tone such as 660 or 678 Hz (Himelfarb et al., 1979; Marchant et al., 1986). This probe tone frequency was initially considered to be more relevant for neonates, as more accurate diagnosis of middle-ear effusion was achieved using the 660 / 678 Hz than using the traditional 226 Hz probe tone (Marchant et al., 1986).

Paradise et al. (1976), Marchant et al (1986) and Shurin et al. (1976, 1977) performed tympanometry using 678 Hz probe tone frequency on young infants less than 5 months of age and found it to be valid and also reported that susceptance tympanograms using a high probe tone frequency (660 Hz) correlated well with the middle ear diagnosis.

However, Keefe and Levi (1996) reported that probe tones higher than 668 Hz are likely to be more effective in diagnosing middle ear dysfunction in neonates. This was further supported by the research findings of Williams et al., (1995) and Rhodes et al., (1999), Margolis et al., (2003) who found that the use of a 1000-Hz probe tone yielded more accurate diagnosis of middle-ear pathology than 678 Hz.

2.1.2.3 Tympanometry using 1000 Hz probe tone frequency

Many studies on using high-frequency probe tones for tympanometry have been explored over the past 30 years .Tympanometry using 1000 Hz probe tone seems to be the most recommended (JCIH 2007; Karzon & Lieu, 2006; ASHA, 2004; Kei et al., 2003; Margolis et al., 2003; Baldwin, 2006; Kei et al., 2007). There are evidences to support the application of 1000 Hz probe tone frequency in children from birth to 4 months (Baldwin, 2006; Petrak, 2002). Currently, the recommended frequency of the probe tone for neonates is 1000 Hz (Baldwin et al, 2000).

Margolis et al (2003) established normative data from full-term infants aged 2 to 4 weeks who passed an otoacoustic emission screen for tympanometry using 1000 Hz probe tone. Nearly all infants tested were reported to have clearly defined, single peaked tympanogram, however a few infants had double peaked tympanograms. They suggested that 5^{th} percentile for static admittance compensated with negative tail (-/ 400 daPa) should be considered for a pass / fail criterion with cut off value of < 0.6 mmho. Then in the same study, they evaluated this cut off value on a group of babies with confirmed middle ear disorder and the sensitivity of their criterion was reported to be only 0.5 with specificity of 0.91.

More recent study done by Swanepoel et al., (2007) described high frequency immittance measurements using a 1000 Hz probe tone for a sample of 278 neonatal ears (birth - 4 weeks of age) in order to compile normative tympanometric criteria and reported that 1000 Hz probe tone tympanometry demonstrated a promising test for clarifying middle ear status in neonates.

Mazlan et al., (2007) compared the high frequency (1000 Hz) tympanometry (HFT) measures obtained from infants at birth and at 6-7 weeks of age. HFT results were obtained from 42 healthy full-term neonates (15 boys & 27 girls) at both test sessions, separated by six weeks. The results showed that the mean values of HFT test parameters obtained at 6-7 weeks were generally greater than those obtained at birth. In particular, the differences in mean values of uncompensated admittance at 200 daPa, uncompensated peak admittance, uncompensated peak susceptance and peak-compensated static admittance were found to be statistically significant. The findings from this study also suggest by supporting the need to have separate sets of normative for HFT data for infants at birth and 6 - 7 weeks.

2.1.2.4 Tympanometry using different probe tone frequencies

There are many studies done comparing the tympanometric measures across probe tone frequencies. More recently, Shahnaz, Miranda, and Polka (2008) reported that standard 226 Hz probe-tone tympanometry was often multipeaked, thus offering limited information with regard to middle ear effusion. Nonetheless, they reported that tympanograms acquired using a 1000 Hz probe tone were potentially more sensitive and more specific with regard to middle ear status. They also reported that, for newborns who passed ABR and OAE screenings, the 1000 Hz tympanograms were bell-shaped, had single peaks, and were well defined. Similarly, in newborns who failed TEOAEs, 1000 Hz tympanograms were most often flat, widened, or multi-peaked.

Kei et al., (2003) evaluated healthy and normal neonates of age 1-6 days with normal TEOAE results using 226 Hz and 1000 Hz probe-tones. For infants who passed the TEOAE screen, they found three different types of admittance tympanograms at 1000 Hz probe tone frequency. Type 1, the single peaked tympanogram was the most commonly occurring in about 92.2 % of neonates. Type 2, a flat tympanogram, was observed in 5.7 % of the infants and further, only 3 ears (1.2 %) produced a type, double-peaked tympanograms with the 1000 Hz probe tone. Interestingly, 116 of the 244 ears (47.5 %) demonstrated double-peaked tympanograms using the 226 Hz probe tone. They concluded that type 1 (single peak) tympanogram probably indicates normal middle ear function.

Alaerts, Lutz, and Woulters (2007) evaluated 226 Hz and 1000 Hz probe tone frequencies (226 and 1000 Hz) across six age groups (NICU babies, 0-3 months, 3-6 months, 6-9 months, 9-32 months, and adults). They determined that tympanometry using 1000 Hz probe tone frequency was the better when assessing neonates through 3 months of age. They found that for children less than 3 months of age, the 1000 Hz probe tone was easier to interpret and more reliable than 226 Hz. From 3 to 9 months, they concluded that it is wise to use both 226 and 1000 Hz. For babies older than 9 months, they recommended the 226 Hz tone.

Meyer et al., (1997) reported tympanometric changes in a single child using 226 Hz, 678 Hz and 1000 Hz probe tones from 2 weeks to until she was 6.5 months old. It was reported that both 226 Hz and 1000 Hz tympanogram show a maturational change in middle ear resonance from a mass to stiffness dominated system. They suggested performing both 226 Hz and 1000 Hz probe tone tympanometry in infants less than 6 months of age to increase diagnostic accuracy as the high frequency probe tone tympanometry increases the sensitivity of the test to the mechanics of middle ear when it is mass dominated.

Baldwin (2006) compared tympanometry using 226 Hz, 678 Hz, and 1000 Hz probe tones on two groups of babies, age 2 to 21 weeks. One group of babies had normal middle ear function as confirmed with normal ABR thresholds or TEOAEs and another group of infants had temporary conductive hearing loss based on the findings of a test battery. The majorities of tympanogram recorded in both groups using the 226 Hz probe tone were 'normal' Type A, with no significant difference in middle ear pressure or static admittance and hence concluded that tympanometry using 226 Hz is invalid below 21 weeks and 1000 Hz is the frequency of choice.

ASHA (2007) recommends tympanometry using 1000 Hz probe tones for infants and the Joint Committee on Infant Hearing (2007) also recommends tympanograms using a 1000 Hz probe tone for babies under 6 months of age.

Despite numerous studies indicating the relevance of high frequency tympanometry, limited normative data are available for 1000 Hz tympanometry. The clear clinical guidelines are not yet at hand, especially when considering infants between birth to 6 months of age (Calandruccio, Fitzgerald & Prieve, 2006; Kei et al., 2003; and Margolis et al., 2003). However, the use of these norms clinically is problematic for at least two reasons. First, test parameters such as compensated versus uncompensated tympanograms are not standardized. Second, it is not clear how development affects the need for age specific norms based on chronological age in weeks or months.

2.2 Multifrequency and multicomponent tympanometry

The multifrequency and multicomponent tympanometry assists in providing information on the middle ear status of neonates or infants which may be useful in identifying the early hearing loss. Several researches have suggested that the presence of any peak or notching is normal and that a flat unnotched tympanogram is suggestive of effusion.

Colletti (1975, 1976, & 1977) was one of the first to develop a system capable of recording multiple frequency tympanograms across a frequency range wide enough to observe immittance below and above the resonance of the middle ear. This system plotted the impedance values and this resulted in inverted tympanograms compared to the admittance tympanograms. Colletti (1975, 1976 & 1977) noticed that three distinct tympanometric patterns emerged as probe tone frequency was increased from 200 to 2000 Hz. The first pattern, recorded at low frequencies (< 1000 Hz), was a V-shaped tympanogram (the inverse of an admittance pattern) which is consistent with stiffness controlled middle ear. The second pattern, recorded at mid frequencies (650- 1400 Hz) and near the resonant frequency of the middle ear, was a W shaped or notched tympanogram.

Colletti (1975, 1976 & 1977) reported that an impedance tympanogram would notch near the middle ear resonant frequency. Thus, the onset of the W pattern coincides with middle ear resonance which is consistent with the Vanhuyse model (Vanhuyse, 1975). The third pattern, recorded at high frequencies (>1400 Hz) where the middle ear is mass controlled, was an inverted V shape tympanogram. Colletti (1977) recorded multifrequency impedance tympanograms in patients with different middle ear pathologies and noted that the transition from V shape to the notched pattern and then to the inverted V pattern occurs at different frequencies for various middle ear conditions. He found that the transition to the W pattern (coinciding with middle ear resonance) was the easiest to identify. In patients with otosclerosis, the W pattern emerged between 850 and 1650 Hz (mean of 1300 Hz), indicating an increase in resonant frequency due to an increase in stiffness of the middle ear transmission system. Conversely, in patients with ossicular discontinuity, the W pattern emerged between 500 and 900 Hz, indicating a decrease in resonant frequency due to an increase in the mass or a decrease in the stiffness of the middle ear transmission system.

Ferekidis et al., (1999) used multifrequency tympanometry to collect information about the mechanoacoustical changes occurring in the middle ear system after acute otitis media and to compare it with the results of conventional, low probe-tone tympanometry. Children with acute otitis media were followed up with both methods for 1 month after an episode of acute infection. Also, children with normal hearing were studied to establish normative data. Resonant frequency of the middle ear was found to be lower than normal even one month past the initial episode, for all types of 226 Hz tympanograms. Multifrequency tympanometry seemed to record changes in the middle ear after acute otitis media that 226 Hz tympanometry was unable to detect, implying persistence of pathology.

Margolis et al., (1993) evaluated the middle ear function using 226 Hz tympanometry (Static admittance, tympanometric width, tympanometric peak pressure and equivalent volume) and multifrequency tympanometry (MFT) from 226 Hz to 2000

Hz (Vanhuyse patterns across frequencies) in 98 ears. They found that tympanometric peak pressure is not related to presence or absence of middle ear effusion. Equivalent volume was found to be good predictor of perforation or patulous Eustachian tube patency in the absence of middle ear inflammation. MFT results showed 63% normal, 26% irregular, 11% low resonant frequency from children with otitis media histories. At the time of testing all the children had normal hearing and normal 226 Hz tympanograms and no visual evidence of active middle ear disease. Thus, MFT was found to be more sensitive to mechanical disturbances related to otitis media that are not detected by 226 Hz tympanometry and audiometry.

Vanhuyse, Creten, and Van Camp (1975) examined tympanometric patterns at various probe tone frequencies and developed a model which predicts the shape of susceptance (B) and conductance (G) tympanograms at 678 Hz in normal ears and in various pathologies. Later, this model was extended to higher probe tone frequencies (Margolis & Goycoolea, 1993). This model can be explained with reference to the relationship between susceptance and conductance tympanograms as probe tone frequency increases.

The Vanhuyse model classifies the tympanograms based on the number of peaks or extrema on the susceptance (B) tympanogram and the conductance (G) tympanogram and predicts four tympanometric patterns at 678 Hz. These patterns which are depicted in Figure 1.1 include the following:

a) 1B1G pattern (Figure A): Here both the susceptance tympanogram and conductance tympanogram has one peak each. The 1B1G pattern occurs when the middle ear is stiffness dominated and the absolute value of susceptance is greater than conductance at all ear canal air pressures, i.e., when the admittance phase angle is between 90° and 45°. In normal ears, the standard low frequency tympanometry yields a 1B1G pattern.

b) 3B1G pattern (Figure B): Here the susceptance (B) tympanogram has three extrema with two peaks on the side of a notch in the middle and has a single peak on the conductance (G) tympanogram. The admittance tympanogram will also have one peak. When this pattern is observed, the ear is either stiffness dominated or at resonance, i.e., the admittance phase angle is between 45° and 0°. In this pattern susceptance is still larger than conductance at extreme pressures; however, this relationship is reversed near the peak pressure. The central notch on the susceptance tympanogram occurs at the pressure corresponding to the peak value on the susceptance tympanogram. The middle ear is stiffness controlled when the central notch on the susceptance tympanogram is above either the positive or the negative tail, depending on which extreme is chosen to estimate ear canal volume.

c) 3B3G pattern (Figure C): Here the susceptance and the conductance tympanograms each have three peaks. The admittance tympanogram will also have three peaks, i.e., it will have a notch. When this pattern is observed, the ear is either at resonance or is mass dominated, i.e., the admittance phase angle is between 0° and -45°. This in turn results in a deep notch on the susceptance tympanogram. The middle ear is at resonance when the central notch on B tympanogram is equal to either the positive or the negative tail as this indicates that susceptance is zero, whereas it is mass controlled when the central notch falls below either the positive or the negative tail as this indicates that susceptance is negative.

d) 5B3G pattern (Figure D): Here the susceptance tympanogram has five peaks and the conductance tympanogram has three peaks. The admittance tympanogram will also have three peaks. In this pattern the ear is mass dominated and admittance phase angle is between -45° and -90°.

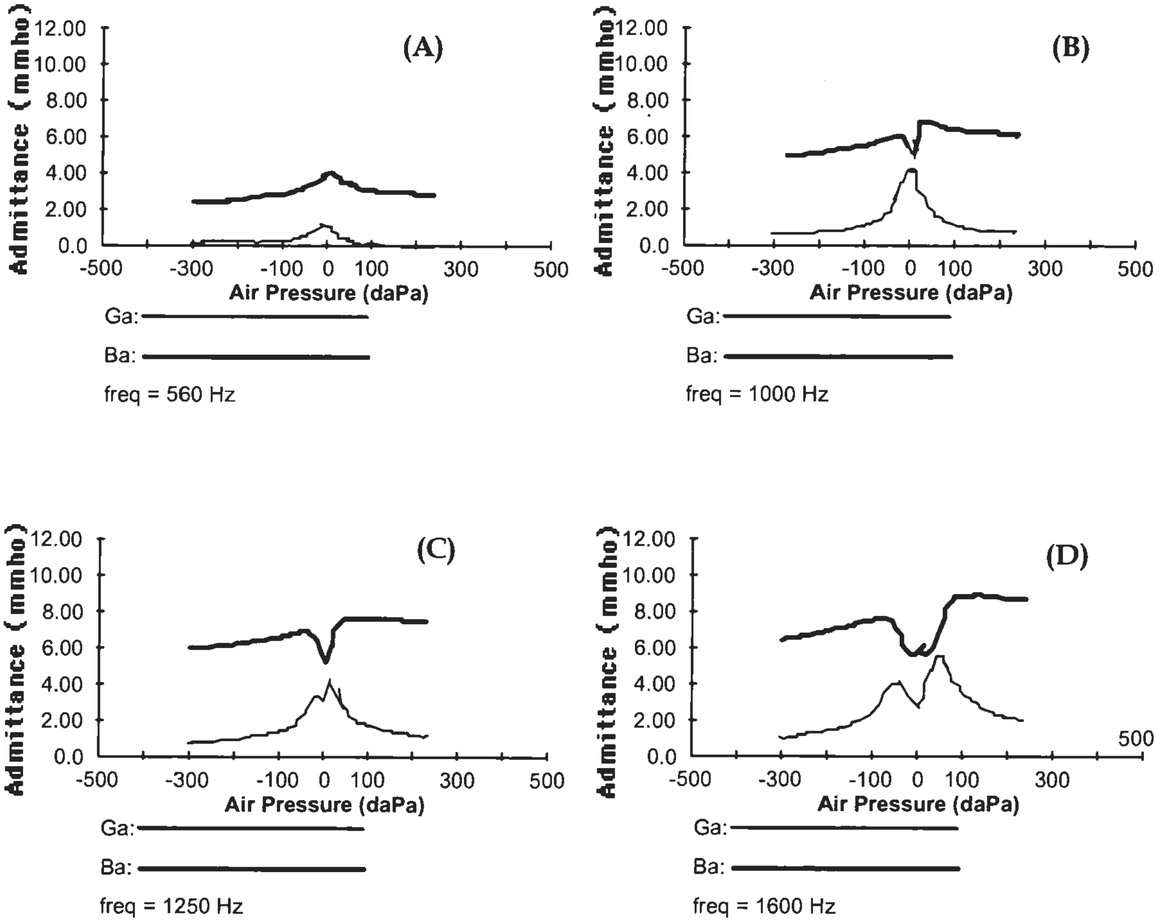


Figure 1.1: Vanhuyse et al. (1975) model showing four patterns for susceptance (B_a) and conductance (G_a) tympanograms, 1B1G (A); 3B1G (B); 3B3G (C); and 5B3G (D)

The sequence of patterns described in the model is seen as frequency is increased, in both normal and abnormal middle ears (Margolis et al., 1985). However, with pathologies the probe frequency at which each pattern occurs may be shifted higher or lower compared to normal ears. For example, in a stiffening pathology such as otosclerosis in which the resonant frequency is shifted upward, each of the various patterns can be expected to occur at higher probe tone frequencies compared to normal ears.

Apart from single and multifrequency tympanometry, the acoustic reflex measures have potential value in the audiological assessment of infants. Acoustic reflex measures reveal the integrity of middle ear and inner ear functions in infants.

2.3 Acoustic reflex measures

Acoustic reflex measures include measurement of acoustic reflex threshold. Acoustic reflex threshold refers to the minimum intensity at which the stapedius muscle contraction is exhibited which is measured as reduction in the admittance of the ear to a particular criterion value. The usually considered criterion value for the presence of acoustic reflex is change in the admittance value of 0.03 mmhos. Reflexes can be measured as ipsilateral and contralateral acoustic reflex thresholds. Ipsilateral acoustic reflex is the acoustic reflex elicited when the stimulus is presented to the same ear where the response is measured and contralateral acoustic reflex is elicited when the stimulus is presented to the opposite ear from where the response is measured.

The ability to elicit a reflex and to obtain acoustic reflex thresholds in neonates and infants depends on probe-tone frequency used. Early attempts to record acoustic reflexes from healthy neonates using a low frequency probe tone (i.e. either 220 or 226 Hz) were unsuccessful (Keith & Bench, 1978; McMillan et al., 1985; Weatherby & Bennett., 1980). For instance, Keith (1973) reported the presence of only 36% of acoustic reflexes in 40 neonates ranging in age from 36 to 151 hours after birth.

Many studies are being done on the measurement of contralateral acoustic reflexes (Keith, 1973; Keith & Bench, 1978; McCandless & Allred, 1978; Bennett & Weatherby, 1980, 1982). McCandless & Allred (1978) have reported elevated acoustic reflex thresholds in neonates compared to adults when using 660 Hz probe tone. Weatherby and Benett (1980) however found that acoustic reflex thresholds decreased with increased probe frequency.

A study done by Weatherby and Bennett (1981) where they measured contralateral reflex thresholds for pure tones, broadband noise, and filtered noise in 28 newborns aged 4–8 days. They obtained mean reflex threshold for the broadband noise to be 73 dB SPL, which is 4 dB lower than the reflex threshold for the 2600 Hz low and high pass filtered noise bands. They concluded that reflex activation at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz gave responses that closely followed the normal adult pattern although reflex thresholds were approximately 10 dB higher in infants.

McMillan, Bennett, Marchant & Shurin (1985) measured ipsilateral and contralateral acoustic reflex measurement in neonates using pure tones activators in octaves from 500 Hz to 4000 Hz and probe tones of 220 Hz and 660 Hz and concluded that ipsilateral thresholds are more sensitive than contralateral thresholds and ipsilateral testing with a 660 Hz probe tone detects a higher percentage of reflexes in neonates than testing with a 220 Hz probe tone.

Using a probe tone of 660 Hz, Sprague et al., (1985) was reported to have obtained a higher percentage of acoustic reflexes in neonates when their ears were stimulated by a 1000 Hz tone and a broadband stimulus ipsilaterally. Despite the higher percentage of presence of acoustic reflexes using the 660-Hz probe tone, the target of 100% presence of acoustic reflexes in healthy neonates has never been achieved in this study. However, when a high frequency probe tone (1000 Hz) and a broadband activator were used, Weatherby and Bennett (1980) were able to obtain acoustic reflexes in all 44 healthy neonates (100%), aged 10 to 169 hours.

Among some of the recent studies to support the measurement of acoustic reflexes using 1000 Hz probe tone in infants , a study done by Swanepoel et al., (2007) reported the high incidence of presence of acoustic reflexes (94%) in the neonates for 1000 Hz ipsilateral stimulus to the use of a 1000 Hz probe tone. Their results showed that the 1000 Hz probe tone reflex thresholds for a 1000 Hz stimulus in normal neonates are elevated by approximately 10 dB compared with conventional adult acoustic reflex thresholds using a 226 Hz probe tone. The mean acoustic reflex threshold reported was 93 dB ± 9 dB with a maximum of 110 dB and a minimum of 60 dB. The range (5^{th} to 95^{th} percentile) was 25 dB (80-105 dB).

Mazlan et al., (2009) investigated the test - retest reliability of acoustic reflex (AR) test measured from a group of 6 week old infants who passed a transient evoked otoacoustic emission test and an automated auditory brainstem response screening test. They recorded Ipsilateral acoustic reflex thresholds for a 2000 Hz pure tone and broadband noise from 70 infants using a Madsen Otoflex Diagnostic Immittance meter with a probe tone of 1000 Hz. The mean AR thresholds obtained in the first test were

67.3 and 80.9 dB HL for the broadband noise and 2000 Hz tone respectively. They also reported that the retest condition did not differ significantly from those of the first test. They concluded that The AR test also showed high test–retest reliability as demonstrated by intra-correlation coefficients across the test–retest conditions of 0.783 and 0.780 for the broadband noise and 2000 Hz pure tone stimuli respectively.

Mazlan et al., (2007) compared acoustic reflex (AR) measures for 1000 Hz probe tone frequency from infants at birth and at 6 -7 weeks of age. AR thresholds using a 2000 Hz tone and broadband noise activators were obtained from 42 healthy full-term neonates (15 boys and 27 girls) at both test sessions, separated by six weeks. The results of this study showed that the mean values of AR thresholds obtained at 6 -7 weeks were generally greater than those obtained at birth. In particular, AR thresholds with a 2 kHz tone and broadband noise were found to be statistically significant. The findings from this study suggest the need to have separate sets of normative data for infants at birth and 6 -7 weeks.

From the review of literature, it is evident that there has been little research done on comparing tympanometry and acoustic reflex threshold using different probe tone frequency in infants at various developmental stages throughout the first year of life. Hence the present study was taken with the purpose of obtaining age specific immittance finding and comparing tympanometric measures and ipsilateral reflex thresholds obtained from healthy infants from 2 to 12 months of age using probe tones of 226 Hz, 678 Hz and 1000 Hz.

3. Method

The present study was undertaken with the aim of establishing immittance findings by determining and comparing tympanometric measures using 226 Hz, 678 Hz and 1000 Hz probe tone and ipsilateral acoustic reflex thresholds for 500 Hz, 2000 Hz and 4000 Hz using both 226 Hz and 1000 Hz probe tones in infants. To accomplish the aim, the following method was employed:

Subjects:

The study was conducted on 70 infants (140 ears) in the age range of 2 to 12 months. 70 infants were further divided into 4 subgroups based on their age as shown in table 3.1.

Table 3.1: *Age range for the four groups and number of infants in each age group*

Age range	**Number of infants**
2 - 4 months	25
4 - 6months	15
6 - 8months	15
8 - 12 months	15

Selection criteria: The subjects were selected based on the following criteria

- Normal otoscopic examination indicating absence of external and middle ear pathology

- Healthy with no symptoms of cold or ear discharge at the time of assessment.
- No complaint and prior history of any high risk factors.
- No complaint and history of any neurological symptoms
- Age appropriate minimum response levels in behavioral observation audiometry
- Normal outer hair cell functioning ensured by recording TEOAEs
- Normal hearing sensitivity ensured by recording ABR

Instrumentation

The following instruments were used for the study:

- Otoscope to observe the status of external auditory canal and tympanic membrane
- A calibrated two channel diagnostic audiometer Madsen Orbiter-922 (version 2) with impedance matched loudspeakers to present stimuli for behavioral observation audiometry (BOA)
- A calibrated otoacoustic emission system ILO - V6 to record transient evoked otoacoustic emissions (TEOAEs) to examine the status of outer hair cells
- Intelligent Hearing Systems Smart EP version 3.94 evoked potentials system to record auditory brainstem responses (ABR).
- A Calibrated Grason Stadler Inc. - tympstar middle ear analyzer (version 2) to carry out tympanometry and acoustic reflexometry.

Test environment:

Testing was carried out in a sound treated room where ambient noise level was within the specified limits as per ANSI S3.1 (1991). The test room was made comfortable enough for the infants in terms of temperature and light

Test Procedure

***Case history*:**

Detailed information regarding the history of prenatal, natal and postnatal medical conditions was secured for all the infants. A detailed report regarding the auditory behavior of the infant at home for various environmental sounds like calling bell, voices from TV or radio, pressure cooker whistle etc was obtained from the parents or caregivers.

High Risk Register:

Medical reports were reviewed to make sure that all the infants were devoid of various risk factors and other medical conditions. This was done by administering the modified high risk register (HRR) developed by Anitha & Yathiraj, (2001) to rule the high risk factors in infants.

***Otoscopic examination*:**

The visual examination of the ear canal and the tympanic membrane of infant's ear were done using a hand held otoscope. This was done to rule out the presence of wax, foreign bodies in the ear canal and/or tympanic membrane pathologies.

Behavioral Observation Audiometry (BOA):

The behavioral responses (minimum response level) of the infants were observed in the free field condition using warble tones from 500 Hz to 4000 Hz separated in octaves and speech stimuli. It was carried out in a double room situation. The infants were seated comfortably on the caregivers lap at a distance of 1 meter from the loud speakers and at an azimuth of 45^0 in the observation room. One clinician was present in the observation room to draw the attention of the infant to the midline and to watch for the unconditioned responses. The other clinician in the test room, presented the test stimuli sequentially with the initiation level being decided below the level at which the infant is expected to exhibit some kind of auditory behavior, as reported by the parents. The lowest levels of presentation of each of the stimuli, at which the subject exhibited some sort of auditory behavior was noted down.

Transient evoked otoacoustic emissions (TEOAEs):

TEOAE were obtained using ILO - V6 instrument with a foam tip positioned in the external auditory canal so as to give a flat stimulus spectrum across the frequency range. Stimuli were clicks with a band pass filter encompassing 500 Hz - 6000 Hz. The duration of rectangular pulses (clicks) was 80μsec. The level was maintained at 80 dBpkSPL in the external auditory canal and the interstimulus interval was kept constant at 20 msec. A total of 260 averages above the automatic noise rejection level of instrument were stored for analysis. The presentation mode included a series of four stimuli; three at the same level and of same polarity and the fourth is of three times the level of either of the three and opposite in polarity. This is called nonlinear averaging which was used for artifact reduction during the response acquisition. The responses were considered as emissions based on the reproducibility and signal to noise ratio (SNR). The overall SNR of greater than or equal to +3dB and the reproducibility of greater than 50% were considered (Dijk & Wit, 1987) for the presence of otoacoustic emission to determine normal outer hair cell functioning.

Auditory brainstem response (ABR):

Single channel ABR was recorded in infants using IHS Smart EP system. Initially electrode sites were cleaned with the help of skin preparing gel. Electrodes were placed on the recording sites with the conduction paste and then were fixed with the help of surgical tape. It was ensured that the independent electrode impedance was less than 5 kΩ and inter electrode impedance was within 2 kΩ. The parameters used for recoding ABR are shown in tables 3.2 and 3.3.

Table 3.2: *Stimulus parameters used to record ABR*

Stimulus parameters	Stimuli:	Clicks
	Stimulus duration:	100 μs
	Number of stimuli:	1500
	Intensity:	30 dB nHL and 50 dB nHL
	Repetition rate:	11.1/sec
	Stimulus polarity:	Rarefaction
	Transducer:	Insert ear phone (ER- 3A)

Table 3.3: *Acquisition parameters used to record ABR*

Acquisition parameters	Analysis time:	15 msec
	Filter settings:	30- 3000 Hz
	Notch filter:	on
	Artifact rejection:	30 μV
	Number of channels:	Single
	Electrode placement: Inverting: Test ear mastoid, Noninverting: High forehead and Common: non test ear mastoid	

If the ABR wave V was clearly seen at 30 dB nHL then the subjects were considered to have normal hearing sensitivity

Tympanogram measurements:

Appropriate sized probe tip that comfortably fits into the ear canal of the infant was selected and gently placed into the ear canal to obtain hermetic seal. The tympanograms were recorded over a pressure range of +400 to -600 daPa with a positive to negative sweep with the pump speed of 200 daPa /sec for 3 different probe tone frequencies 226 Hz, 678 Hz & 1000 Hz. The probe intensity was 85 dB SPL for 226 Hz & 678 Hz probe tones and 75 dB SPL for 1000 Hz probe tone frequency. The following tympanometric measures as shown in the table 3.4 were found using 3 probe tone frequencies.

Table 3.4: *Tympanometric measures obtained using 226 Hz, 678 Hz and 1000 Hz probe tone frequency*

226 Hz	**678 Hz**	**1000 Hz**
Tympanometric peak pressure (TPP)	Tympanometric peak pressure (TPP)	Tympanometric peak pressure (TPP)
Peak compensated static admittance compensated at the +ve tail (Y_{+400})	Peak compensated static admittance compensated at the +ve tail (Y_{+400})	Peak compensated static admittance compensated at the +ve tail (Y_{+400})
	Peak compensated static admittance compensated at the –ve tail (Y_{-600})	Peak compensated static admittance compensated at the –ve tail (Y_{-600})
Ear canal volume (ECV)	-	-

These tympanometric parameters were obtained from tympanogram automatically when recorded using 226 Hz probe tone frequency where as when 678 Hz and 1000 Hz probe tone were used, TPP, Y_{+400} and Y_{-600} measures were obtained manually with the help of cursor since these measures could not be obtained automatically as compensated tympanograms. To obtain these parameters the following procedures were followed:

1) To obtain tympanometric peak pressure (TPP) the cursor was moved to the peak of the tympanogram at which the admittance value was maximum and the corresponding pressure in the x- axis was considered as TPP as shown in Figure 3.1

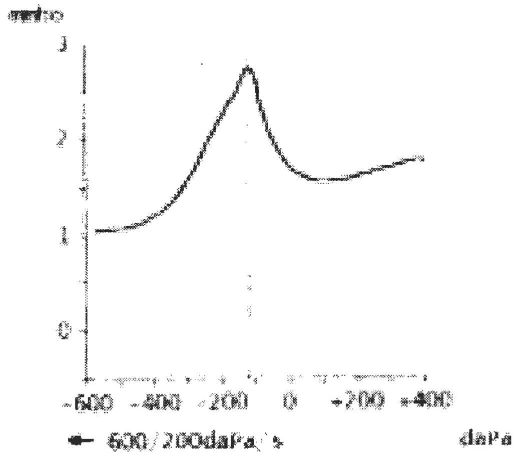


Figure 3.1: Tympanogram showing the tympanometric peak pressure (TPP)

2) Peak compensated static acoustic admittance compensated with +ve tail (Y_{+400}) was obtained by manual subtraction of static acoustic admittance at the +ve tail (Y_1) from the admittance at the peak pressure / peak admittance (Y).

3) Peak compensated static acoustic admittance at the -ve tail (Y_{-600}) was obtained by manual subtraction of static acoustic admittance at the -ve tail (Y_2) from the admittance at the peak pressure / peak admittance (Y).

To obtain Y_{+400} and Y_{-600}, the following values were recorded:

- Peak admittance (Y):

This was obtained by moving the cursor to peak of the tympanogram and the corresponding admittance at the y- axis was considered as peak admittance (Y) as shown in Figure 3.2.

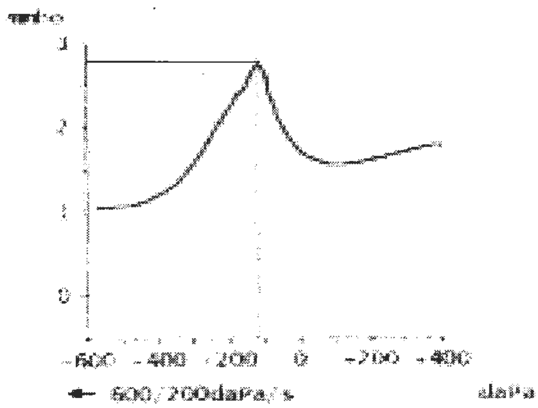


Figure 3. 2: Tympanogram showing peak admittance (Y)

- Static acoustic admittance at the +ve tail (Y_1):

This was obtained by moving the cursor to the +ve tail of the tympanogram at +400 daPa and the corresponding admittance at the y- axis was considered as Y_1

- Static acoustic admittance at the -ve tail (Y_2):

This was obtained by moving the cursor to the -ve tail of the tympanogram at -600 daPa and the corresponding admittance at the y- axis was considered as Y_2

Acoustic reflex measurements:

Ipsilateral acoustic reflex thresholds (ARTs) were measured in the present study. Reflexes were measured for pure tone stimuli of 500 Hz, 2000 Hz and 4000 Hz using 226 Hz and 1000 Hz probe tone. ART for 1000 Hz pure tone was not recorded as 1000 Hz probe tone might interact with the reflex activator signal frequency, causing an artifact (Wilson & Margolis, 2001).

Acoustic reflex thresholds were determined using an ascending technique by increasing the intensity of the activating stimuli in 5 dB steps from 60 dBHL as the staring intensity until reflex was obtained or equipment limit was reached. The minimum intensity at which the repeatable change in the admittance value is observed by taking the criterion as 0.03 mmhos was considered as an acoustic reflex threshold (ART).

Analysis:

To arrive at the goal, qualitative and quantitative analyses were done.

Qualitative analysis was done based on simple visual inspection system for tympanograms obtained for 226 Hz, 678 Hz and 1000 Hz probe tones and by obtaining percentage of presence of acoustic reflex thresholds

Quantitative analysis was done by obtaining the following values for different probe tone frequencies across age groups:

- Tympanometric peak pressure
- Peak compensated static acoustic admittance compensated to +ve tail (Y_{+400}) and –ve tail (Y_{-600}) of the tympanogram
- Ear canal volume
- Ipsilateral acoustic reflex thresholds at 500 Hz, 2000 Hz and 4000 Hz

The data obtained for various tympanometric measures and ART for each probe tone frequency across each age group was compared using appropriate statistical analysis.

4. Results and Discussion

The present study was conducted with the aim of establishing immittance findings by determining and comparing tympanometric measures using 226 Hz, 678 Hz and 1000 Hz probe tone and ipsilateral acoustic reflex thresholds for 226 Hz and 1000 Hz probe tones in infants. To accomplish the aim, qualitative analysis of various tympanometric patterns are done. Along with the qualitative analysis, the quantitative analysis was done by obtaining mean, standard deviation and range for various parameters such as tympanometric peak pressure (TPP), peak compensated static acoustic admittance compensated at + tail (Y_{+400}), peak compensated static acoustic admittance compensated at –ve tail (Y_{-600}), ear canal volume (ECV), ipsilateral acoustic reflex thresholds (ARTs) at 500 Hz, 2000 Hz and 4000 Hz.

The results of the present study are analysed and described both qualitatively as well as quantitatively.

4.1 Qualitative analysis

4.1.1 Qualitative analysis of tympanogram

Qualitative analysis was done based on simple visual inspection system for tympanograms obtained for 226 Hz, 678 Hz and 1000 Hz probe tones.

The qualitative analysis of tympanograms obtained from 140 healthy infant ears using 226 Hz probe tone revealed the following:

- Single peaked tympanogram in 36 ears (72 %), a double peaked tympanograms in 14 ears (28 %) were obtained in 2 – 4 mnths age group
- Single peaked tympanogram in 28 ears (93.3 %), a double peaked tympanograms in 2 ears (6.6 %) were obtained in 4 – 6 mnths age group
- Single peaked tympanogram in all 30 ears were obtained in 6 – 8 mnths and 8 – 12 mnths age group respectively

The different patterns of tympanogram obtained across different age groups when 226 Hz probe tone was used is depicted in Figure 4.1

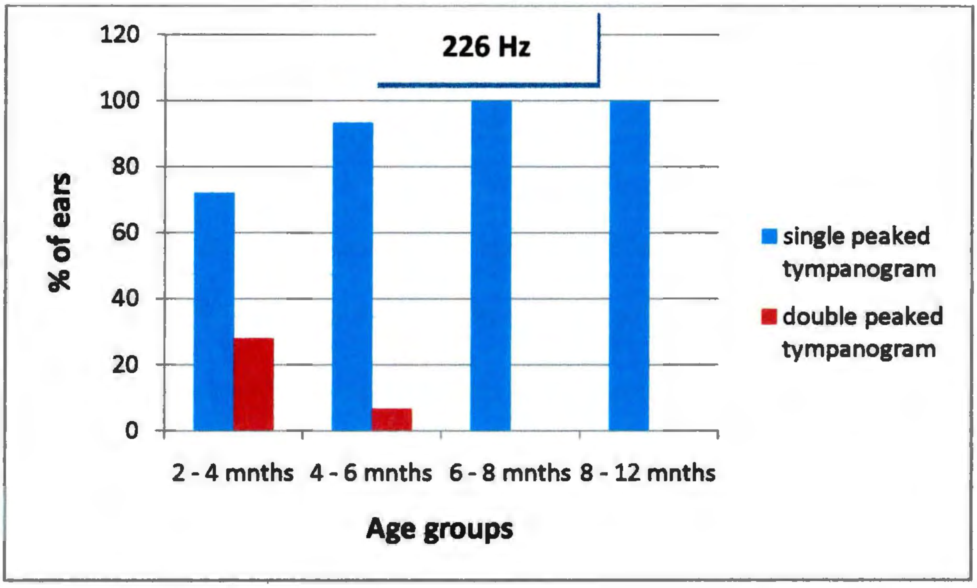


Figure 4.1: Graph depicting % of ears with different patterns of tympanogram obtained across different age groups when 226 Hz probe tone was used

It can be inferred from the graph 4.1, that when 226 Hz probe tone was used, the % of ears with single peaked tympanogram increases as the age increases. This type of single peaked tympanogram is similar to the type A tympanograms in the conventional

Liden / Jerger classification (Liden, 1969; Jerger, 1970) found in adults and older children with normal middle ear function. On the other hand, % of ears with double peaked tympanogram decreases as age increases. This could be attributed to the anatomical changes in the external and middle ears leading stiffness controlled middle ear system as age increases when 226 Hz probe tone was used. The % of ears with double peaked tympanogram at 226 Hz probe tone is more in younger age group (2 – 4 mnths) which could be reflective of increased mass and resistance with concomitant decreased resonant frequency in younger age groups (Himelfarb, 1979).

The qualitative analysis of tympanograms obtained from 140 healthy infant ears using 678 Hz probe tone revealed the following:

- Single peaked tympanogram in 17 ears (34 %), a double peaked tympanograms in 28 ears (56 %), inverted V shaped tympanogram in 3 ears (6%), more than two peaks in 2 ears (4 %) were obtained in 2 – 4 mnths age group
- Single peaked tympanogram in 13 ears (43.3 %), a double peaked tympanograms in 15 ears (50 %) , inverted / V shaped tympanogram in 2 ears (6.6 %) were obtained in 4 – 6 mnths age group
- Single peaked tympanogram in 22 ears (73 %), a double peaked tympanogram in 8 ears (27 %) were obtained in 6 – 8 mnths age group
- Single peaked tympanogram in 23 ears (76.6 %), a double peaked tympanogram in 7 ears (23.3 %) were obtained in 8 – 12 mnths age group

The different patterns of tympanogram obtained across different age groups when 678 Hz probe tone was used is depicted in Figure 4.2

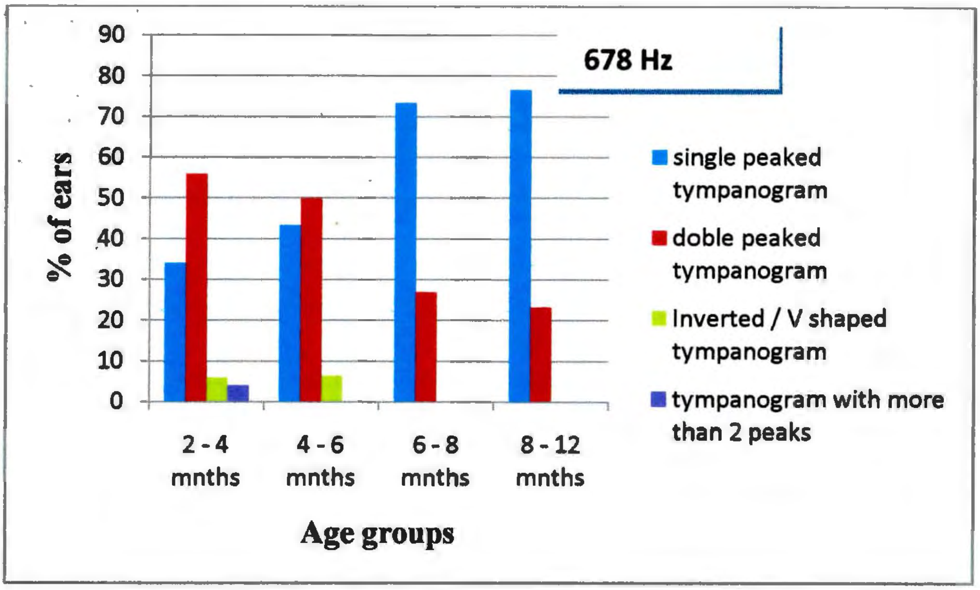


Figure 4.2: Graph depicting % of ears with different patterns of tympanogram obtained across different age groups when 678 Hz probe tone was used

It can be inferred from the graph 4.2, that when 678 Hz probe tone was used, the % of ears with single peaked tympanogram increases as the age increases. This type of single peaked tympanogram is similar to the type A tympanograms in the conventional Liden / Jerger classification (Liden, 1969; Jerger, 1970) found in adults and older children with normal middle ear function. On the other hand, % of ears with double peaked tympanogram decreases as age increases. This could be attributed to the anatomical changes in the external and middle ears leading stiffness controlled middle ear system as age increases when 678 Hz probe tone was used. The % of ears with double peaked tympanogram, tympanogram with two or more peaks and inverted V shaped tympanogram at 678 Hz probe tone is more in younger age groups (2 – 4 mnths and 4 – 6 mnths) which could be reflective of increased mass and resistance with concomitant decreased resonant frequency in younger age groups (Himelfarb, 1979).

The qualitative analysis of tympanogram obtained from 140 healthy infant ears using 1000 Hz probe tone revealed the following:

- Single peaked tympanogram in 44 ears (88 %), a double peaked tympanograms in 6 ears (12 %) were obtained in 2 – 4 mnths age group
- Single peaked tympanogram in 18 ears (60 %), a double peaked tympanograms in 12 ears (40 %) , were obtained in 4 – 6 mnths age group
- Single peaked tympanogram in 13 ears (43.3 %), a double peaked tympanogram in 17 ears (56.6 %) were obtained in 6 – 8 mnths age group
- Single peaked tympanogram in 13 ears (43.3 %), a double peaked tympanogram in 17 ears (56.6 %) were obtained in 8 – 12 mnths age group

The different patterns of tympanogram obtained across different age groups when 1000 Hz probe tone was used is depicted in Figure 4.3

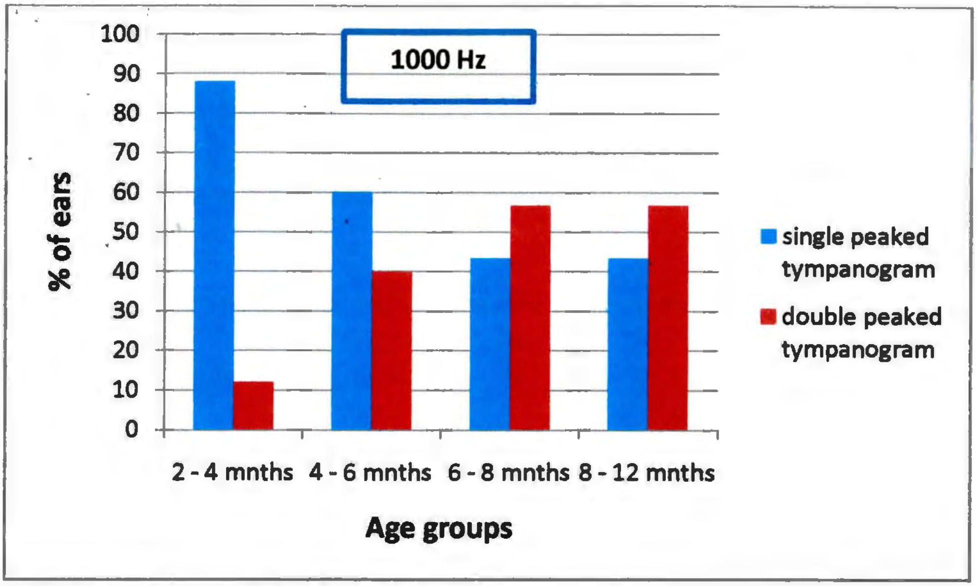
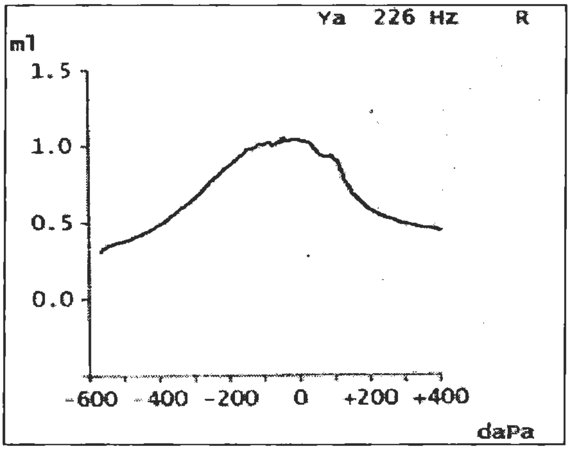


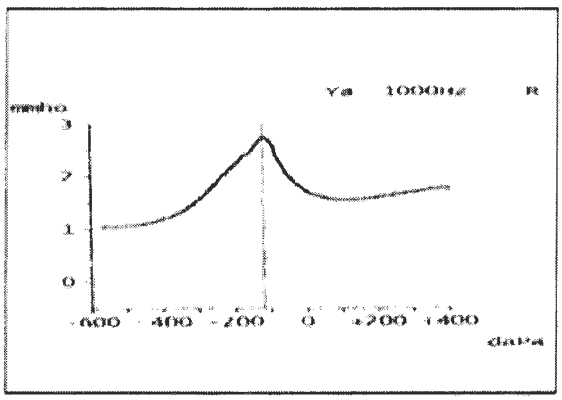
Figure 4.3: Graph depicting % of ears with different patterns of tympanogram obtained across different age groups when 1000 Hz probe tone was used

It can be inferred from the graph 4.3, that when 1000 Hz probe tone was used, the % of ears with single peaked tympanogram decreases as the age increases. On the other hand, % of ears with double peaked tympanogram increases as age increases. This could be attributed to the anatomical changes in the external and middle ears leading stiffness controlled middle ear system as age increases when 1000 Hz probe tone was used. The higher incidence of double peaked tympanograms (56.6 %) in the older (both 6 – 8 mnths and 8 – 12 mnths) age groups in the present study suggest that double peaked tympanograms are indicative of normal middle ear transmission and also correspond to previous reports suggesting that double peak tympanograms are not uncommon and are suggestive of normal middle ear transmission for 1000 Hz probe tone measurements. This occurrence of double peaked tympanograms using 1000 Hz probe tone in 1.2 % of normal neonates was also reported by Kei et al., (2003). However, in a more recent study done by Swanepoel et al (2007) also reports double peaked tympanograms in 6 % of ears in the normal neonates when 1000 Hz probe tone was used.

Some of the illustrative single peaked tympanometric patterns obtained for infants in the present study are shown below in Figure 4.4



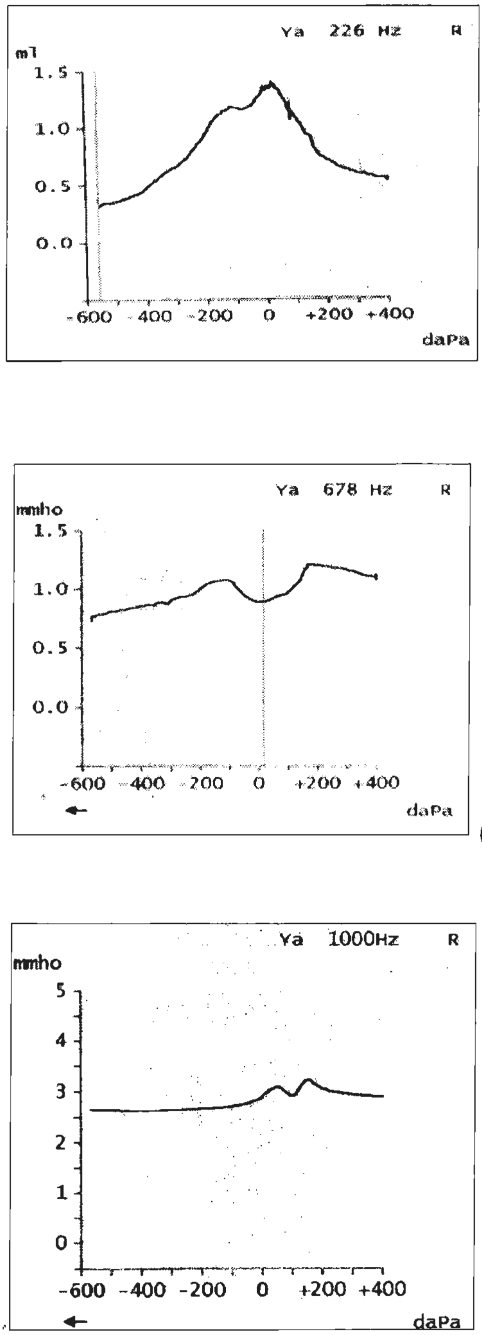
(A)



(B)

Figure 4.4: Single peaked tympanograms using 226 Hz (A) and 1000 Hz (B) probe tones

Some of the illustrative double peaked tympanometric patterns obtained for infants in the present study are shown below in Figure 4.5



(A)

(B)

(C)

Figure 4.5: Double peaked tympanograms using 226 Hz (A), 678 Hz (B) and 1000 Hz (C) probe tones

The illustrative tympanometric pattern with more than 2 peaks obtained for infants in the present study are shown below in Figure 4.6

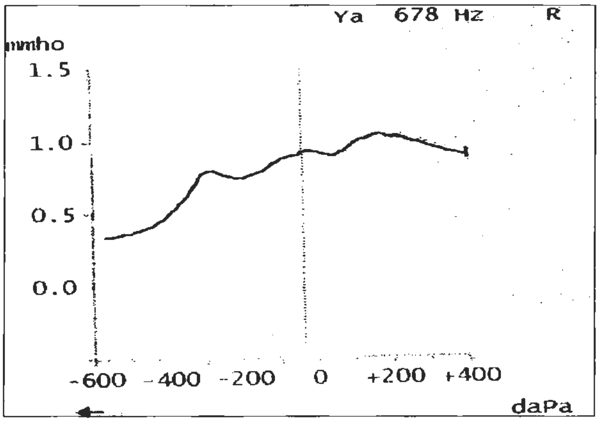


Figure 4.6: tympanogram with more than 2 peaks obtained using 678 Hz probe tones

4.1.2 Frequency of occurrence of acoustic reflex thresholds

The frequency of occurrence of ipsilateral acoustic reflex threshold from 140 healthy infant ears using 226 Hz and 1000 Hz probe tone revealed the following

- All infants had presence of acoustic reflex thresholds when 226 Hz and 1000 Hz probe tone was used with reflex activating signal of 500 Hz and 2000 Hz

- For reflex activating signal of 4000 Hz, reflexes could be elicited in 117 ears (83.6 %) and 121 ears (86.4 %) for 226 Hz and 1000 Hz probe tones respectively.

It can be seen that ART could be elicited using 226 Hz and 1000 Hz probe tones in all the infant ears for 500 Hz and 2000 Hz reflex activating signals in the present study. These results of the present study is not in agreement with the previous studies where the attempts to record acoustic reflexes from healthy neonates using a low frequency probe tone (i.e. either 220 or 226 Hz) were unsuccessful (Keith & Bench, 1978; McMillan et al., 1985; Weatherby & Bennett, 1980). For instance, Keith (1973) reported the presence of only 36% of acoustic reflexes in 40 neonates ranging in age from 36 to 151 hours after birth.

4.2 Quantitative measurements

Quantitative measurements are used together with qualitative data to characterize tympanograms with greater precision. For the quantitative measurements, according to the criteria employed by Shahnaz et al., (2008) for double-peaked tympanograms at 1000 Hz, the peak admittance were calculated from the notch between the maxima. In contrast, Margolis et al., (2003) calculated the peak admittance from the highest peak in double-peaked tympanograms. Sutton et al., (2002) recommended negative peak to calculate peak admittance. Since there were no standard consistent procedures to obtain admittance measures for tympanometric patterns other than single peaked tympanograms, the tympanograms which were nondiscernable such as double peaked, inverted V shaped and tympanogram with more than two peaks were not considered in the present study for statistical analysis.

From the tympanograms obtained from 70 infants (140 ears), 124 ears, 75 ears and 88 ears had single peaked tympanogram for 226 Hz, 678 Hz and 1000 Hz respectively. TPP, Y_{+400}, Y_{-600} and ECV values from single peaked tympanograms were obtained and were analysed using Statistical Package for the Social Sciences (SPSS) version 17 software.

Along with tympanometric measures, acoustic reflex thresholds from 140 ears for 500 Hz, 2000 Hz reflex activating signals were obtained using 226 Hz and 1000 Hz probe tone frequency and acoustic reflex thresholds from 117, 121 ears were obtained for 4000 Hz activating signal using 226 Hz and 1000 Hz respectively and they were statistically analysed (Since for 4000 Hz reflex activating signal the ipsilateral acoustic reflex thresholds were absent for 23 ears and 19 ears for 226 Hz and 1000 Hz probe tone respectively). The following statistical tools were used to analyse the obtained data:

- Descriptive statistics to calculate the mean, standard deviation and range for tympanometric peak pressure (TPP), the peak compensated static acoustic admittance compensated with +ve (Y_{+400}) & -ve (Y_{-600}) tail and ear canal volume (ECV) for 226 Hz, 678 Hz and 1000 Hz probe tones along with ipsilateral acoustic reflex thresholds at 500 Hz, 2000 Hz & 4000 Hz for 226 Hz and 1000 Hz probe tone frequency.
- One way analysis of variance (ANOVA) was done separately to study the overall age effects on TPP, Y_{+400}, Y_{-600}, ECV for 226 Hz, 678 Hz and 1000 Hz probe tones and also ipsilateral acoustic reflex thresholds at 500 Hz, 2000 Hz and 4000 Hz across the four age groups. (Since many unclear tympanograms were obtained for different probe tone frequencies, those data from the tympanograms where discernable peak was not obtained were not considered for analysis. Hence if repeated measure ANOVA was done then many subjects were eliminated from the analysis due to the absence of those data)
- Duncan Post Hoc analysis was done to study the age effects by performing pair wise comparison on TPP, (Y_{+200}), (Y_{-400}), ECV for 226 Hz, 678 Hz and 1000 Hz

probe tones, ipsilateral acoustic reflex thresholds at 500 Hz, 2000 Hz & 4000 Hz across the four age groups

- Paired sample t test was done to study the effects of different probe tone frequencies on TPP, Y_{+200}, Y_{-400}, ECV, reflex thresholds at 500 Hz, 2000 Hz and 4000 Hz across four age groups.

4.2. 1 Effect of age and probe tone frequency on TPP

To see the effect of age and probe tone frequency on TPP, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately and is shown in table 4.1

Table 4.1: *Mean, Standard Deviation (S.D) and Range for TPP in daPa across age groups for different probe tone frequencies*

Probe tone frequencies	**226 Hz**				**678 Hz**				**1000 Hz**			
	TPP (in daPa)											
Age groups	N	Mean	S.D	Range	N	Mean	S.D	Range	N	Mean	S.D	Range
2 - 4 mnths	36	20.69	28.38	-35 to 100	17	31.76	28.55	-10 to 90	44	7.27	48.84	-110 to160
4 - 6 mnths	28	10.35	32.74	-55 to 60	13	4.61	34.42	-85 to 45	18	2.36	60.58	-155 to 90
6 - 8 mths	30	-19.33	59.43	-140 to 85	22	-15.90	62.88	-130 to 55	13	-16.92	50.97	-120 to 50
8 - 12mths	30	-14.50	53.58	-145 to 80	23	-12.82	66.39	-160 to 95	13	8.07	77.25	-135 to110

N: number of ears

As it can been seen in table 4.1, there is no specific trend seen in mean TPP value obtained for different age groups and for different probe tone frequencies.

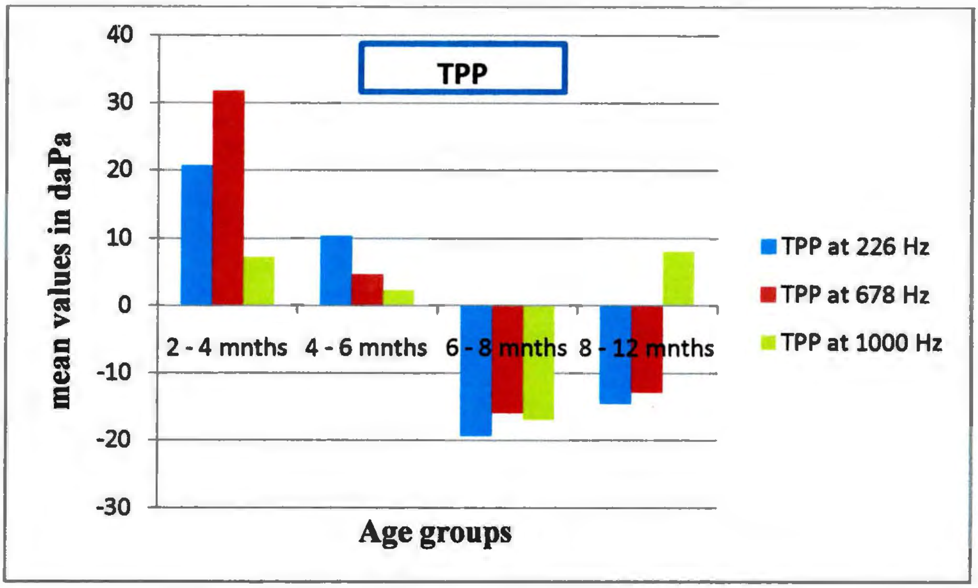


Figure 4.7: Graph depicting mean tympanometric peak pressure (TPP) values across different age groups for 226 Hz, 678 Hz and 1000 Hz probe tone frequencies

It can be inferred from the graph 4.7, that the mean values of TPP is more positive for infants of younger age groups (2 – 4 mnths & 4 – 6 mnths) and more negative for infants of older age groups (6 – 8 mnths & 8 – 12 mnths). However among the younger age groups, the mean TPP is more for 2 – 4 months than 4 – 6 months infants.

This slight positive mean TPP observed in the infants of 2 – 4 mnths and 4 – 6 mnths can be attributed to occurrence of positive middle ear pressure while sleeping. Since during the testing infants of 2 – 4 and 4 – 6 mnths were sleeping in the present study. It has been reported in adults by Hergils and Magnuson (1989) that while sleeping the partial pressure of carbon dioxide increases, thus contributing to positive pressure in the middle ear. Studies have not yet conducted in infants to prove this finding. Another possibility could be due to alterations in the anatomy of the infant ear which can also contribute to more positive TPP in the infants of 2 – 4 mnths and 4 – 6 mnths than in infants of other age group.

4.2.1.1 Effect of age on TPP at each probe tone frequency

To see the effect of age on TPP, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed the following

- There was significant difference in TPP [$F (3, 120) = 5.91$, $p < 0.05$] across age groups for 226 Hz probe tone.
- There was significant difference in TPP [$F (3, 71) = 3.05$, $p < 0.05$] across age groups for 678 Hz probe tone
- However there was no significant difference in TPP [$F (3,85) = 0.658$, $p > 0.05$] across age groups for 1000 Hz probe tone

As one way ANOVA showed significant difference between age groups for 226 Hz and 678 Hz probe tones on TPP, further analysis using the Duncan post hoc analysis test was done to see between which two age groups, TPP differ significantly. The results of Duncan post hoc test for each probe tone frequency are shown in table 4.2

Table 4.2: *Results of Duncan post hoc analysis comparing different age groups for 226 Hz and 678 Hz probe tone frequencies*

Probe tone frequency	**Age groups (in months)**	**2 - 4 mnths**	**4 - 6 mnths**	**6 - 8 mnths**	**8 -12 mnths**
	2 - 4 mnths		>0.05	**<0.05***	**<0.05***
	4 - 6 mnths	>0.05		**<0.05***	**<0.05***
226Hz	6 - 8 mnths	**<0.05***	**<0.05***		>0.05
	8 - 12 mnths	**<0.05***	**<0.05***	>0.05	
	2 - 4mnths		**<0.05***	**<0.05***	**<0.05***
	4 - 6 mnths	**<0.05***		>0.05	>0.05
678Hz	6 - 8mnths	**<0.05***	>0.05		>0.05
	8 - 12 mnths	**<0.05***	>0.05	>0.05	

Note. * Indicates significant difference

As it is depicted in table 4.2, the results of the present study show that when 1000 Hz probe tone was used there was no age effect seen on TPP. When 226 Hz probe tone was, there was age effect seen with significant difference between younger (2 – 4 mnths & 4 – 6 mnths) and older (6 – 8 mnths & 8 – 12 mnths) infants. When 678 Hz probe tone was used, there was age effect seen with significant difference between infants of 2 – 4 months age group and other age groups.

These results of the present study are in accordance with the previous studies done by Swanepoel et al., (2007) where they reported statistically no significant age effects for TPP values when compared between neonates at birth and at 4 weeks of age when 1000 Hz probe tone frequency was used. However, when they compared the 90 % TPP range (5^{th} to 95^{th} percentile) for newborn neonates and older neonates (2-4 weeks of age), they demonstrated an increasing range of normative tympanic peak pressure values with increasing age.

Similar study done by Mazlan et al., (2007) compared TPP measures between neonates at birth and at 6 weeks of age using 1000 Hz probe tone and reported that the TPP range for neonates was -88 daPa to +98 daPa and for infants of 6 weeks it ranged from -254 daPa to +80 daPa and that the TPP did not show any significant change with age.

The results of the present study can also be supported by results of study done by Alaerts et al., (2007) where they reported no significant differences between the 3 to 9 month and the 9 to 32 mnths age groups with regard to TPP with 1000 Hz probe tone.

In the present study there was age effect seen between age groups when 226 Hz probe tone was used which is contradicting to the previous research done by Alaerts et al., (2007) where there was no effect seen between 3 to 9 month and the 9 to 32 months age groups.

4.2.1.2 Effect of probe tone frequency on TPP for each age group

To see the effect of probe tone frequency on TPP, Paired sample t test was done at each age group. The results are shown in table 4.3

Table 4.3: *Results of paired t test comparing different probe tone frequencies on TPP for each age group*

Age groups (months)	**Parameters**	**df**	**t-value**	**Sig (2-tailed)**
2 - 4 mnths	TPP at 226 Hz- TPP at 678 Hz	15	-0.63	>0.05
	TPP at 226 Hz- TPP at 1000 Hz	29	0.56	>0.05
	TPP at 678 Hz- TPP at 1000 Hz	16	-0.11	>0.05
4 – 6 mnths	TPP at 226 Hz- TPP at 678 Hz	12	1.73	>0.05
	TPP at 226 Hz- TPP at 1000 Hz	16	-0.56	>0.05
	TPP at 678 Hz- TPP at 1000 Hz	9	-1.59	>0.05
6 - 8 mnths	TPP at 226 Hz- TPP at 678 Hz	21	-0.25	>0.05
	TPP at 226 Hz- TPP at 1000 Hz	12	-0.86	>0.05
	TPP at 678 Hz- TPP at 1000 Hz	12	-0.07	>0.05
8 – 12 mnths	TPP at 226 Hz- TPP at 678 Hz	22	-1.60	>0.05
	TPP at 226 Hz- TPP at 1000 Hz	12	-3.13	**< 0.05***
	TPP at 678 Hz- TPP at 1000 Hz	10	-4.24	**< 0.05***

Note. * Indicates significant difference

Results of paired t test comparing different probe tone frequencies on TPP for each age group showed that,

- There was significant difference for TPP between 226 Hz & 1000 Hz [t (12)= -3.13, p < 0.05)] and 678 Hz & 1000 Hz [t (10)= -4.25, p < 0.05] at 8 - 12 mnths

- However no significant difference for TPP between probe tone frequencies were seen at 2 - 4 mnths, 4 - 6 mnths and 6 - 8 mnths

This difference in TPP between 226 Hz & 1000 Hz and 678 Hz & 1000 Hz probe tone frequencies at 8 - 12 mnths could be attributed to alterations in the middle ear pressure when probe tone frequency of 1000 Hz of probe intensity 75 dB SPL is presented. The possible explanation for difference in TPP between 226 Hz & 1000 Hz and 678 Hz & 1000 Hz at 8 – 12 mnths may be related to sound pressure level delivered to the infant's ear. As the 1000 Hz probe tone was calibrated using 2 ml cavity in the present study, which is larger than ear canal of infants, the sound pressure actually presented to the ear canal would have been greater than 75 dB SPL, which in turn could have led to increase in TPP.

4.2.2 Effect of age and probe tone frequency on Y_{+400}

To see the effect of age and probe tone frequency on Y_{+400}, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately as given in table 4.4.

Table 4.4: *Mean, Standard Deviation (S.D) and Range for Y_{+400} in mmho across age groups for different probe tone frequencies*

Probe tone frequencies	**226 Hz**				**678 Hz**				**1000 Hz**			
	Y_{+400} (in mmho)											
Age groups	N	Mean	S.D	Range	N	Mean	S.D	Range	N	Mean	S.D	Range
2 – 4 mnths	36	0.55	0.64	0.2- 1.3	17	0.47	0.24	0.24-1.29	44	1.23	0.46	0.38- 2.23
4 - 6 mnths	28	0.50	0.29	0.2- 1.3	13	0.85	0.55	0.20- 2.21	18	0.82	0.37	0.34- 1.67
6 – 8 mnths	30	0.69	0.34	0.4- 1.4	22	0.85	0.44	0.28- 1.89	13	1.01	0.37	0.34- 1.65
8 - 12 mnths	30	0.44	0.19	0.2- 0.8	23	0.82	0.44	0.11-1.75	13	0.88	0.46	0.11- 1.97

N: number of ears

As it can been seen in table 4.4, there was no specific trend seen in mean Y_{+400} values obtained for different age groups and for different probe tone frequencies.

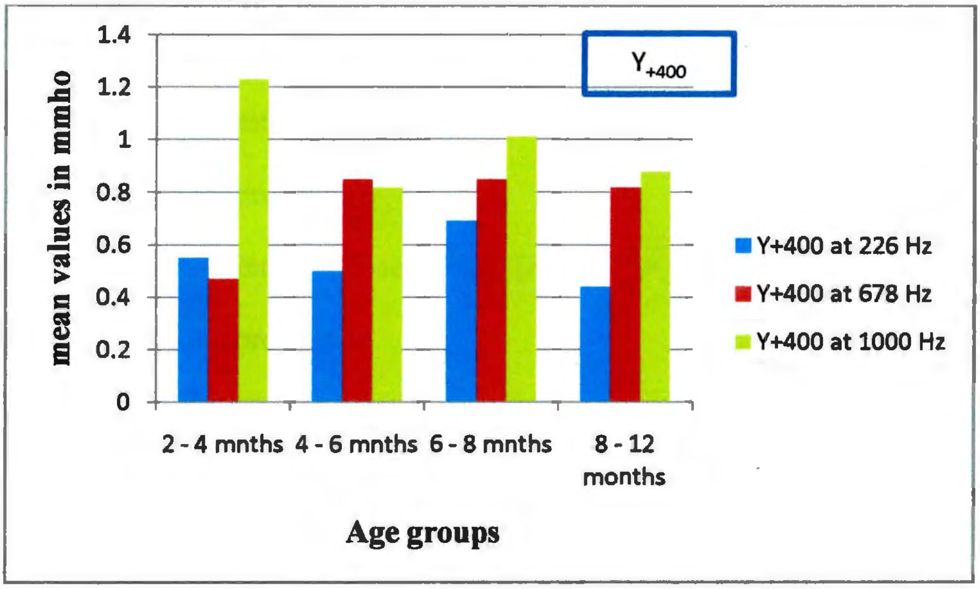


Figure 4.8: Graph depicting mean peak compensated static acoustic admittance compensated with + ve tail (Y_{+400}) values across different age groups for 226 Hz, 678 Hz and 1000 Hz probe tone frequencies

It can be inferred from the graph 4.8, that the mean values of Y_{+400} increases as probe tone frequency increases for infants of older age groups (6 – 8 mnths & 8 – 12 mnths). However among the younger age groups (2 – 4 mnths & 4 – 6 mnths) there was no specific trend of increase or decrease in admittance seen with increase in probe tone frequency.

4.2.2.1 Effect of age on Y_{+400} at each probe tone frequency

To see the effect of age on Y_{+400}, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed the following

- There was significant difference in Y_{+400} [$F (3, 120) = 4.24$, $p < 0.05$] across age groups for 226 Hz probe tone.
- There was significant difference in Y_{+400} [$F (3, 71) = 3.30$, $p < 0.05$] across age groups for 678 Hz probe tone
- There was significant difference in Y_{+400} [$F (3,84) = 4.89$, $p > 0.05$] across age groups for 1000 Hz probe tone

As one way ANOVA showed significant difference between age groups 226 Hz, 678 Hz and 1000 Hz probe tones on Y_{+400}, further analysis using the Duncan post hoc analysis test was done to see between which of the two age groups, Y_{+400} differ significantly. The results of Duncan post hoc test for each probe tone frequency are given in table 4.5

Table 4.5: *Results of Duncan post hoc analysis comparing different age groups for 226 Hz, 678 Hz and 1000 Hz probe tone frequencies*

Probe tone frequency	Age groups (months)	2 - 4 mnths	4 - 6 mnths	6 - 8 mnths	8 - 12 mnths
226 Hz	2 – 4 mnths		>0.05	**<0.05***	>0.05
	4 - 6 mnths	>0.05		**<0.05***	>0.05
	6 - 8 mnths	**<0.05***	**<0.05***		**<0.05***
	8 - 12 mnths	>0.05	>0.05	**<0.05***	
678Hz	2 – 4 mnths		**<0.05***	**<0.05***	**<0.05***
	4 - 6 mnths	**<0.05***		>0.05	>0.05
	6 - 8 mnths	**<0.05***	>0.05		>0.05
	8 - 12 mnths	**<0.05***	>0.05	>0.05	
1000Hz	2 - 4mnths		**<0.05***	**<0.05***	**<0.05***
	4 - 6 mnths	**<0.05***		>0.05	>0.05
	6 – 8 mnths	**<0.05***	>0.05		>0.05
	8 - 12 mnths	**<0.05***	>0.05	>0.05	

Note. *Indicates significant difference

The results of the present study show that, there was significant difference seen between infants in the age range of 2 - 4 months and older age groups (4 – 6 mnths , 6 – 8 mnths & 8 - 12 mnths) on Y_{+400} values ,when 678 Hz and 1000 Hz probe tones were used. When 226 Hz probe tone was used, Y_{+400} values in infants in the age range of 6 – 8 months, differed significantly from other age groups (2 - 4 mnths, 4 - 6 mnths & 8 – 12 mnths). However, in the present study the Y_{+400} values did not show any specific trend of increase / decrease with increase in age.

The results of the present study are contradicting to the study done by Mazlan et al., (2007) where they reported that there was significant age effect seen on Y_{+200} i.e. with increase in age Y_{+200} values increases. They reported that the mean Y_{+200} values of neonates at birth were 0.78 mmho and over the 6-week period, it increased to 1.01 mmho when 1000Hz probe tone was used. Similar results were reported by Swanepoel et al., (2007) where there were statistically significant differences in peak admittance values reported for neonates at birth and older neonates of 4 weeks of age indicating a general increase in admittance with increasing age for 1000 Hz probe tone tympanometry. A study done by Alaerts et al., (2007) also reported significant differences between the < 3 months and 3- to 9 months age groups with regard to Y_{+200} with a 226-Hz probe tone and 1000Hz probe tone and there was increase in Y_{+200} values with age for both 226 Hz and 1000 Hz tympanometry.

However, this trend of increase in Y_{+400} with age which is reported in the previous studies is not observed in the present study. The possible reasons for the discrepancy between the present study and previous research could be that in the present study the static acoustic admittance is compensated with +ve tail of the tympanogram at +400 daPa. However in the previous studies, the static acoustic admittance is compensated with +ve tail of the tympanogram at +200 daPa. This difference could have led to contradicting results in the present study. However there are no supporting studies comparing Y_{+400} values between age groups.

4.2.2.2 Effect of probe tone frequency on Y_{+400} for each age group

To see the effect of probe tone frequency on Y_{+400}, Paired sample t test was done at each age group. The results are shown in table 4.6

Table 4.6: *Results of paired t test comparing different probe tone frequencies on Y_{+400} for each age group*

Age groups (months)	Parameters	df	t-value	Sig (2-tailed)
2 - 4 mnths	Y_{+400} at 226 Hz- Y_{+400} at 678 Hz	15	0.97	>0.05
	Y_{+400} at 226 Hz- Y_{+400} at 1000 Hz	29	-5.77	**< 0.05***
	Y_{+400} at 678 Hz- Y_{+400} at 1000 Hz	16	-7.81	**< 0.05***
4 - 6 mnths	Y_{+400} at 226 Hz- Y_{+400} at 678 Hz	12	-1.66	>0.05
	Y_{+400} at 226 Hz- Y_{+400} at 1000 Hz	15	-1.70	>0.05
	Y_{+400} at 678 Hz- Y_{+400} at 1000 Hz	9	-0.40	**< 0.05***
6 - 8 mnths	Y_{+400} at 226 Hz- Y_{+400} at 678 Hz	21	-0.92	>0.05
	Y_{+400} at 226 Hz- Y_{+400} at 1000 Hz	12	-1.98	**< 0.05***
	Y_{+400} at 678 Hz- Y_{+400} at 1000 Hz	12	-2.37	**< 0.05***
8 – 12 mnths	Y_{+400} at 226 Hz- Y_{+400} at 678 Hz	22	-3.90	**< 0.05***
	Y_{+400} at 226 Hz- Y_{+400} at 1000 Hz	12	-3.58	**< 0.05***
	Y_{+400} at 678 Hz- Y_{+400} at 1000 Hz	10	0.26	>0.05

Note. *Indicates significant difference

- There was significant difference between 226 Hz & 1000 Hz [t (29)= -5.77, $p < 0.05$)] and 678 Hz & 1000 Hz [t (16)= -7.81, $p < 0.05$] at 2 - 4 mnths

- There was significant difference between 678 Hz & 1000 Hz [t (9)= -4.06, $p < 0.05$] at 4 - 6 mnths
- There was significant difference between 226 Hz & 1000 Hz [t (12)= -1.98, $p < 0.05$) and 678 Hz & 1000 Hz [t (12)= -2.37, $p < 0.05$] at 6 - 8 mnths
- There was significant difference between 226 Hz & 678 Hz [t (22)= -3.90, $p < 0.05$] and 226 Hz & 1000 Hz [t (12)=-3.58, $p < 0.05$] at 8 - 12 mnths

This difference in Y_{+400} values between 226 Hz, 678 Hz and 1000 Hz probe tone frequencies as seen in the present study are in agreement with results obtained by Alaerts et al., (2007) where they reported that there is increase in Y_{+200} values with increase in probe tone frequencies in infants of 3 – 9 mnths age group. However there are no supporting studies comparing Y_{+400} values between probe tone frequencies i.e. when the static acoustic admittance is compensated with the + ve tail of the tympanogram at +400 daPa.

4.2.3 Effect of age and probe tone frequency on Y_{-600}

To see the effect of age and probe tone frequency Y_{-600}, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately as given in table 4.7

Table 4.7: *Mean, Standard Deviation (S.D) and Range for Y_{-600} in mmho across age groups for different probe tone frequencies*

Probe tone frequencies	**678 Hz**				**1000 Hz**			
	Y_{-600} (in mmho)							
Age groups	N	Mean	S.D	Range	N	Mean	S.D	Range
2 - 4 mnths	17	0.98	0.40	0.37- 1.87	43	1.77	0.61	0.59- 3.06
4 – 6 mnths	13	1.22	0.70	0.41- 2.90	18	1.40	0.47	0.53- 2.06
6 – 8 mnths	22	1.21	0.43	0.55- 2.23	13	1.49	0.43	0.86- 2.29
8 –12 mnths	23	1.07	0.46	0.22- 2.10	13	1.25	0.53	0.17- 3.06

N: number of ears

As it can been seen in table 4.7, there was no specific trend seen in mean Y_{-600} values obtained for different age groups for different probe tone frequencies. There was general trend of decreasing Y_{-600} values with age when 1000 Hz probe tone was used. But however there was no similar trend observed when 678 Hz probe tone was used.

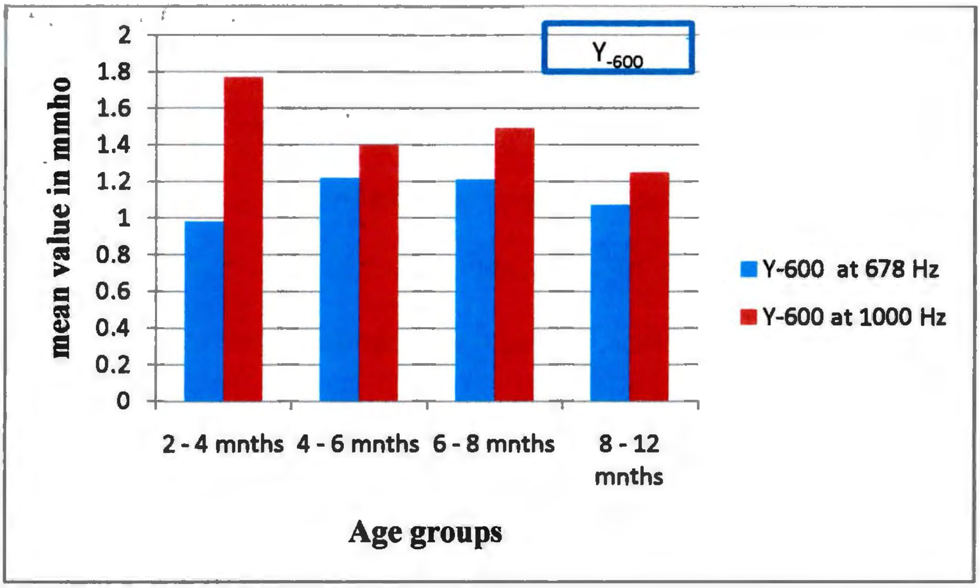


Figure 4.9: Graph depicting mean peak compensated static acoustic admittance compensated with - ve tail (Y_{-600}) values across different age groups for 678 Hz and 1000 Hz probe tone frequencies

It can be inferred from the graph 4.9 that Y_{-600} values are more for when 1000 Hz probe tone was used than when 678 Hz probe tone was used.

4.2.3.1 Effect of age on Y_{-600} at each probe tone frequency

To see the effect of age on Y_{-600}, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed the following

- There was significant difference in Y_{-600} [$F(3, 83) = 3.84$, $p < 0.05$] across age groups for 1000Hz probe tone.
- However there was no significant difference in Y_{-600} [$F(3, 91) = 0.91$, $p > 0.05$] across age groups for 678Hz probe tone

As one way ANOVA showed significant difference between age groups for 1000Hz probe tone on Y_{-600}, further analysis using the Duncan post hoc analysis test was done to see between which of the two age groups, Y_{-600} differ significantly. The results of Duncan post hoc test for each probe tone frequency are shown in table 4.8

Table 4.8: *Results of Duncan post hoc analysis comparing Y_{-600} across different age groups for 1000 Hz probe tone frequency*

Probe tone frequency	**Age groups (months)**	**2 - 4 mnths**	**4 - 6 mnths**	**6 - 8 mnths**	**8 - 12 mnths**
	2 - 4mnths		>0.05	>0.05	**<0.05***
	4 - 6 mnths	>0.05		>0.05	>0.05
1000Hz	6 - 8 mnths	>0.05	>0.05		>0.05
	8 - 12 mnths	>0.05	>0.05	>0.05	

Note. * Indicates significant difference

The present study shows that there was significant difference between 2 - 4 mnths and 8 - 12 mnths for Y_{-600} values when 1000 Hz probe tone was used. This could be attributed to the middle ear stiffness and mass contributions on Y_{-600}. At 8 - 12 mnths middle ear becomes more stiffness dominated and at 2 – 4 mnths middle ear is mass dominated. Hence when 1000 Hz probe tone was used the change in admittance could have been different between two groups of infants. When 678 Hz probe tone was used, there were no age effects seen.

However, none of the other studies have reported age effects on Y_{-600} using 678 Hz and 1000 Hz probe tones.

4.2.3.2 Effect of probe tone frequency on Y_{-600} for each age group

To see the effect of probe tone frequency on Y_{-600}, Paired sample t test was done at each age group. The results are shown in table 4.9:

Table 4.9: *Results of paired t test comparing 678 Hz and 1000 Hz probe tone frequencies on Y_{-600} for each age group*

Age groups (months)	**Parameters**	**df**	**t-value**	**Sig (2-tailed)**
2 - 4 mnths	Y_{-600} at 678 Hz- Y_{-600} at 1000 Hz	16	-6.57	**< 0.05***
4 - 6 mnths	Y_{-600} at 678 Hz- Y_{-600} at 1000 Hz	9	-7.35	**< 0.05***
6 - 8 mnths	Y_{-600} at 678 Hz- Y_{-600} at 1000 Hz	12	-3.59	**< 0.05***
8 – 12 mnths	Y_{-600} at 678 Hz- Y_{-600} at 1000 Hz	10	-0.39	>0.05

Note. * Indicates significant difference

- There was significant difference between 678 Hz & 1000 Hz [t (16)= -6.57, p < 0.05] at 2 - 4 mnths
- There was significant difference between 678 Hz & 1000 Hz [t (9)= -7.35, p < 0.05] at 4 – 6 mnths
- There was significant difference between 678 Hz & 1000 Hz [t (12)= -3.59, p < 0.05] at 6 - 8 mnths
- There was no significant difference between 678 Hz & 1000 Hz [t (10)= -0.39, p > 0.05] at 8 - 12 mnths

The present study shows that there was significant difference between 678 Hz and 1000 Hz probe tone for Y_{-600} values at 2 - 4 mnths, 4 – 6 mnths and 6 – 8 mnths. However on the other hand there was no significant difference between 678 Hz and 1000 Hz probe tones for Y_{-600} values at 8 – 12 mnths.

This could be attributed to the middle ear stiffness and mass contributions along with the resonant frequency of the middle ear on Y_{-600}. At 8 - 12 months middle ear become more stiffness dominated and the resonant frequency becomes high and in the younger age group infants (2 - 4 mnths, 4 - 6 mnths & 6 - 8 mnths) middle ear is mass dominated and resonant frequency of the middle ear is low. Hence the change in admittance for 678 Hz probe tone and for 1000 Hz probe tone could have been different between infants of 8 – 12 months and younger age groups. However, none of the studies have evaluated effects of different probe tone frequency on Y_{-600}.

4.2.4 Effect of age on ECV

To see the effect of age on ECV using 226 Hz probe tone frequency, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately as given in table 4.10

Table 4.10: *Mean, Standard Deviation (S.D) and Range for ECV across age groups*

Age groups	ECV using 226 Hz probe tone			
(mnths)	N	Mean (ml)	S.D	Range (ml)
2 - 4 mnths	50	0.43	0.10	0.3 - 0.6
4 - 6 mnths	30	0.52	0.15	0.3 - 0.8
6 – 8 mnths	30	0.59	0.12	0.4 - 0.8
8 – 12 mnths	30	0.66	0.13	0.5 - 0.9

N: number of ears

As it can been seen in table 4.10, there was specific trend seen in mean ECV values obtained for different age groups. There was general trend of increasing ECV values with age when 226 Hz probe tone was used.

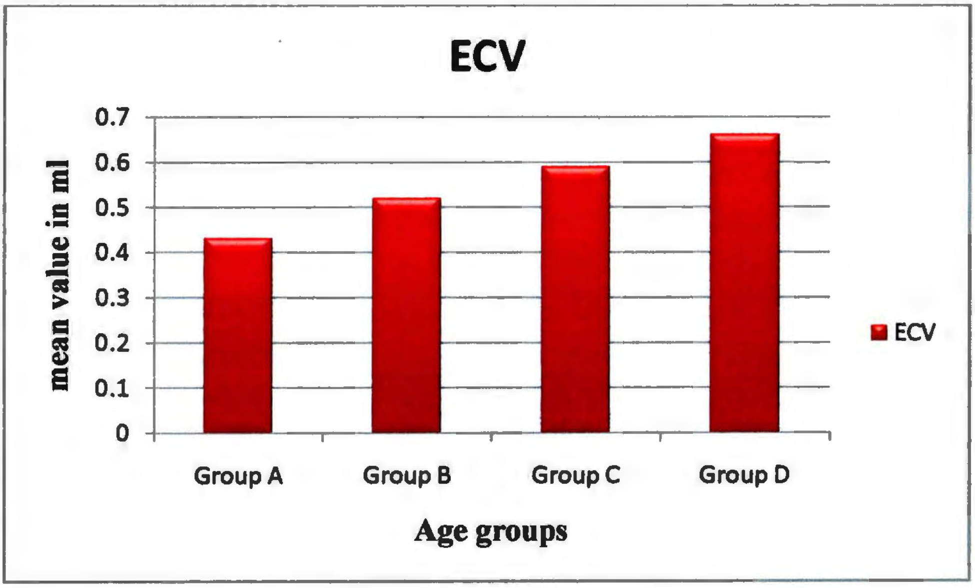


Figure 4.10: Graph depicting mean ear canal volume (ECV) values comparing different age groups for 226 Hz probe tone frequency

It can be inferred from the graph 4.10, as the age clearly increases with age with increase in mean ECV value as age increases from 2 – 12 mnths.

To see the effect of age on ECV, one way analysis of variance (ANOVA) was done. The results showed that there was significant difference in ECV [$F(3, 136) = 22.41$, $p < 0.05$] across age groups.

As one way ANOVA, showed significant difference between age groups, further analysis using the Duncan post hoc analysis test was done to see between which of the two age groups, ECV differ significantly. The results of Duncan post hoc test are shown in table 4.11.

Table 4.11: *Results of Duncans post hoc analysis comparing ECV across age groups for226 Hz probe tone*

Age groups (months)	**2 - 4 mnths**	**4 - 6 mnths**	**6 - 8 mnths**	**8 -12 mnths**
2 - 4 mnths		**<0.05***	**<0.05***	**<0.05***
4 - 6 mnths	**<0.05***		**<0.05***	**<0.05***
6 - 8 mnths	**<0.05***	**<0.05***		**<0.05***
8 - 12 mnths	**<0.05***	**<0.05***	**<0.05***	

Note. * Indicates significant difference

As it is shown in table 4.11, the mean ear canal volume values clearly increases from 0.43 ml to 0.66 ml as age increases from 2 – 12 mnths in the present study. The mean ECV values in the present study are in agreement with previous studies done by Margolis

& Heller (1987) and Shanks et al, (1992) where ECV values were reported to be 0.3 ml to 1 ml between children of 6 weeks to 7 years. Holte et al (1991) also reported that mean ECV values to be 0.3 ml at 4 mnths of age which is also in accordance to the present study.

The increase in EVC values with increase in age could be attributed to anatomical developmental changes in the external ear in terms of its increase in length and diameter in the first few years of life that influence its acoustical properties (Shanks and Lilly, 1981). Such developmental changes include the development of the osseous external auditory meatus during the first 12 months of life (Anson, Bast, & Richany, 1955; Anson & Donaldson, 1981), rapid increase in all dimensions of the external auditory meatus during the first 2 years of life (Keefe et al, 1993; Saunders et al, 1983).

4.2.5 Effect of age and probe tone frequency on ART at 500 Hz

To see the effect of age and probe tone frequency on ART at 500 Hz, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately as given in table 4.12.

Table 4.12: *Mean, Standard Deviation (S.D) and Range of ART at 500 Hz across age groups for different probe tone frequencies*

Probe tone frequencies	**226 Hz**				**1000 Hz**			
	ART_{500} (in dBHL)							
Age groups	N	Mean	S.D	Range	N	Mean	S.D	Range
2 - 4 mths	50	92.10	6.31	75 - 105	50	81.30	6.68	70 - 95
4 - 6 mths	30	92.66	6.91	80 - 105	30	82.16	7.50	70 - 95
6 - 8 mths	30	92.66	6.39	80 - 105	30	82.66	5.68	70 - 95
8 -12 mths	30	89.33	5.37	80 - 100	30	82.50	6.12	75 - 95

N: number of ears

As it is shown in table 4.12, the mean ART at 500 Hz inferred no specific trend with respect to increase / decrease in ARTs across age groups.

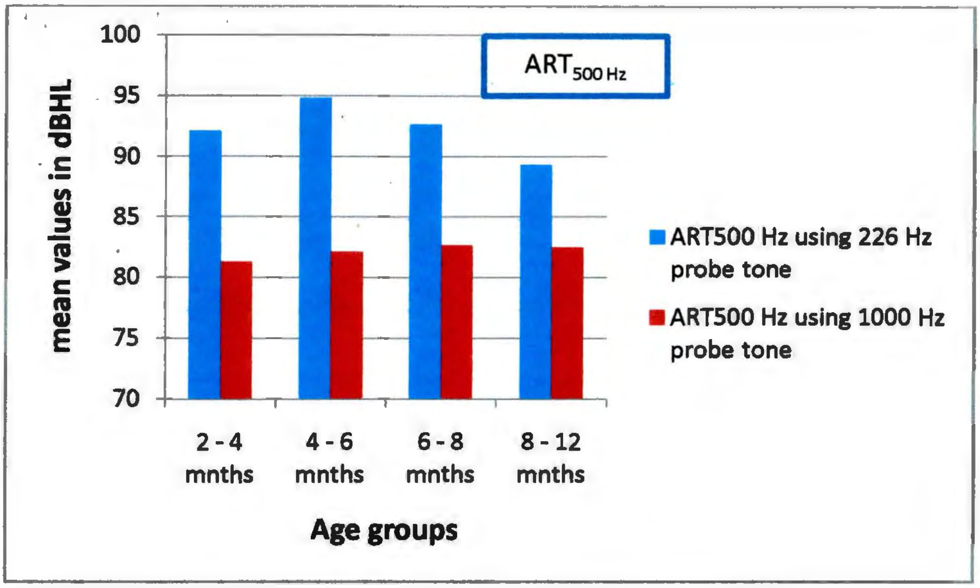


Figure 4.11: Graph depicting mean ARTs at 500 Hz across different age groups for 226 Hz and 1000 Hz probe tone frequencies

It can also be inferred from the graph 4.8 that ART at 500 Hz for 226 Hz probe tone is much higher than for 1000 Hz probe tone frequency across all the age groups.

4.2.5.1 Effect of age on ART at 500 Hz at each probe tone frequency

To see the effect of age on ART at 500 Hz, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed that there was no significant difference in 500 Hz ART across age groups for 226 Hz [$F(3, 136) = 1.95$, $p > 0.05$] and 1000 Hz [$F(3, 136) = 0.35$, $p > 0.05$] probe tone frequencies.

4.2.5.2 Effect of probe tone frequency on ART at 500 Hz for each age group

To see the effect of probe tone frequency on ART at 500 Hz, Paired sample t test was done at each age group. The results are shown in table 4.13:

Table 4.13: *Results of Paired t test comparing ART at 500 Hz between 226 Hz and 1000 Hz probe tone frequencies*

Age groups (months)	**Parameters**	**df**	**t-value**	**Sig (2-tailed)**
2 - 4 months	ART_{500} for 226 Hz - ART_{500} for 1000 Hz	49	11.22	**< 0.05***
4 - 6 months	ART_{500} for 226 Hz - ART_{500} for 1000 Hz	29	11.17	**< 0.05***
6 - 8 months	ART_{500} for 226 Hz - ART_{500} for 1000 Hz	29	11.14	**< 0.05***
8 – 12 months	ART_{500} for 226 Hz - ART_{500} for 1000 Hz	29	6.01	**< 0.05***

Note. * Indicates significant difference

- There was significant difference between 226 Hz & 1000 Hz [t (49)= 11.22, $p < 0.05$] at 2 - 4 mnths
- There was significant difference between 226 Hz & 1000 Hz [t (29)= 11.17, $p < 0.05$] at 4 – 6 mnths
- There was significant difference between 226 Hz & 1000 Hz [t (29)= 11.14, $p < 0.05$] at 6 - 8 mnths
- There was significant difference between 226 Hz & 1000 Hz [t (29)= 6.01, $p < 0.05$] at 8 - 12 mnths

4.2.6 Effect of age and probe tone frequency on ART at 2000 Hz

To see the effect of age and probe tone frequency on ART at 2000 Hz, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately as given in table 4.14

Table 4.14: *Mean, Standard Deviation (S.D) and Range of ART at 2000 Hz across age groups for different probe tone frequencies*

Probe tone frequencies	**226 Hz**				**1000 Hz**			
	ART at 2000Hz (in dBHL)							
Age groups	N	Mean	S.D	Range	N	Mean	S.D	Range
2 - 4mnths	50	93.60	6.85	80 -105	49	81.02	6.45	65 - 95
4 - 6mnths	30	94.83	7.36	80 -105	30	84.00	7.70	70 - 95
6 - 8mnths	30	95.00	6.82	80 -105	30	83.50	7.44	70 -100
8 -12mnths	30	91.83	6.62	80 -105	30	82.66	7.03	70 - 95

N: number of ears

As it shown in table 4.14, the mean ART at 2000 Hz inferred no specific trend with respect to increase / decrease in acoustic reflex thresholds across age groups. However, it was more evident that when 1000 Hz probe tone was used the acoustic reflex thresholds were obtained at lesser intensity than when 226 Hz probe tone was used.

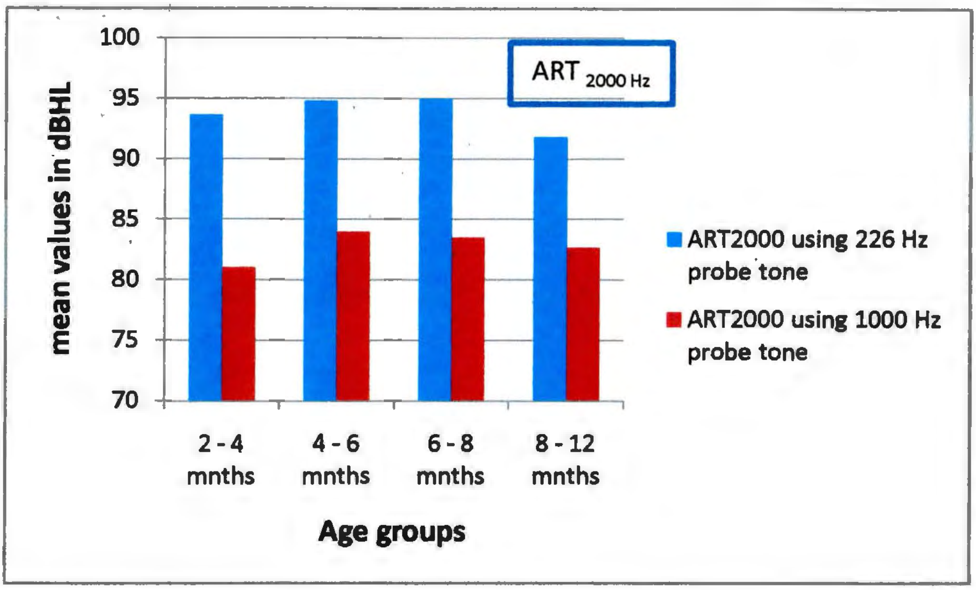


Figure 4.12: Graph depicting mean ARTs at 2000 Hz across different age groups for 226 Hz and 1000 Hz probe tone frequencies

It can be inferred from the graph 4.9 also that ARTs at 2000 Hz for 226 Hz probe tone is much higher than for 1000 Hz probe tone frequency across all the age groups.

4.2.6.1 Effect of age on reflex at 2000 Hz at each probe tone frequency

To see the effect of age on reflexes at 2000 Hz, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed that there was no significant difference in 2000 Hz reflex across age groups for 226 Hz [$F (3, 136)= 1.34$, $p > 0.05$] and 1000 Hz [$F (3, 135)= 1.36$, $p > 0.05$] probe tone frequencies.

4.2.6.2 Effect of probe tone frequency on ART at 2000 Hz for each age group

To see the effect of probe tone frequency on ART at 2000 Hz, Paired sample t test was done at each age group. The results are shown in table 4.15

Table 4.15: *Results of Paired t test comparing ART at 2000 Hz between 226 Hz and 1000 Hz probe tone frequencies*

Age groups (months)	**Parameters**	**df**	**t-value**	**Sig (2-tailed)**
2 - 4 mnths	ART_{2000} for 226 Hz – ART_{2000} for 1000 Hz	48	13.71	**< 0.05***
4 - 6 mnths	ART_{2000} for 226 Hz – ART_{2000} for 1000 Hz	29	9.02	**< 0.05***
6 - 8 mnths	ART_{2000} for 226 Hz – ART_{2000} for 1000 Hz	29	9.20	**< 0.05***
8 – 12 mnths	ART_{2000} for 226 Hz – ART_{2000} for 1000 Hz	29	7.09	**< 0.05***

Note. * Indicates significant difference

- There was significant difference between 226 Hz & 1000 Hz [t (48)= 13.7, p < 0.05)] at 2 - 4 mnths
- There was significant difference between 226 Hz & 1000 Hz [t (29)= 9.02, p < 0.05] at 4 - 6mnths
- There was significant difference between 226 Hz & 1000 Hz [t (29)= 9.20, p < 0.05] at 6 - 8 mnths
- There was significant difference between 226 Hz & 1000 Hz [t (29)= 7.09, p < 0.05] at 8 - 12 mnths

4.2.7 Effect of age and probe tone frequency on ART at 4000 Hz

To see the effect of age and probe tone frequency on ART at 4000 Hz, descriptive statistics was done to obtain mean, standard deviation and range for each age range and each probe tone frequency separately as given in table 4.16.

Table 4.16: *Mean, Standard Deviation (S.D) and Range of ART at 4000 Hz across age groups for 226 Hz and 1000 Hz probe tone frequencies*

Probe tone frequencies	**226 Hz**				**1000 Hz**			
	ART_{4000} (in dBHL)							
Age groups	N	Mean	S.D	Range	N	Mean	S.D	Range
2 – 4 mnths	45	96.22	5.65	80-105	42	83.09	7.95	70-95
4 – 6 mnths	23	94.56	6.72	80-105	25	84.60	8.40	70-95
6 -8 mnths	23	96.08	4.99	85-105	24	84.58	8.32	70-100
8 -12 mnths	26	95.00	6.32	85-105	30	83.50	7.32	70-95

N: number of ears

As it shown in table 4.16, the mean ART at 4000 Hz inferred no specific trend with respect to increase / decrease in ARTs across age groups. However, it was more evident that when 1000 Hz probe tone was used the ARTs were obtained at lesser intensity than when 226 Hz probe tone was used.

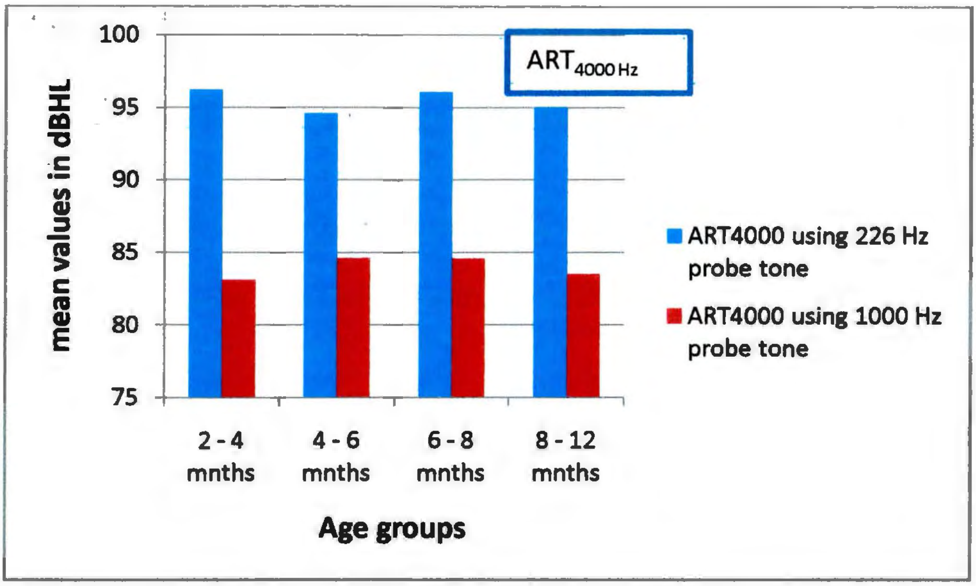


Figure 4.13: Graph depicting mean ART at 4000 Hz across different age groups for 226 Hz and 1000 Hz probe tone frequencies

It can be inferred from the graph 4.13 also that ART at 4000 Hz for 226 Hz probe tone is much higher than for 1000 Hz probe tone frequency across all the age groups.

4.2.7.1 Effect of age on ART at 4000 Hz at each probe tone frequency

To see the effect of age on ART at 4000 Hz, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed that there was no significant difference in 4000 Hz reflex across age groups for 226 Hz [$F(3, 113) = 0.54$, $p > 0.05$) and 1000 Hz [$F(3, 117) = 0.28$, $p > 0.05$] probe tone frequencies.

4.2.7.2 Effect of probe tone frequency on ART at 4000 Hz for each age group

To see the effect of probe tone frequency on ART at 4000 Hz, Paired sample t test was done at each age group. The results are shown in table 4.17

Table 4.17: *Results of Paired t test comparing ART at 4000 Hz between 226 Hz and 1000 Hz probe tone frequencies*

Age groups (months)	**Parameters**	**df**	**t-value**	**Sig (2-tailed)**
2 - 4 mnths	ART_{4000} for 226 Hz – ART_{4000} for 1000 Hz	40	13.48	**< 0.05***
4 - 6 mnths	ART_{4000} for 226 Hz – ART_{4000} for 1000 Hz	22	6.48	**< 0.05***
6 - 8 mnths	ART_{4000} for 226 Hz – ART_{4000} for 1000 Hz	22	7.24	**< 0.05***
8 – 12 mnths	ART_{4000} for 226 Hz – ART_{4000} for 1000 Hz	25	8.63	**< 0.05***

Note. * Indicates significant difference

- There was significant difference between 226 Hz & 1000 Hz [$t(40)= 13.48$, $p < 0.05$] at 2 - 4 mnths
- There was significant difference between 226 Hz & 1000 Hz [$t(22)= 6.48$, $p < 0.05$] at 4 - 6 mnths
- There was significant difference between 226 Hz & 1000 Hz [$t(22)= 7.24$, $p < 0.05$] at 6 - 8 mnths
- There was significant difference between 226 Hz & 1000 Hz [$t(25)= 8.63$, $p < 0.05$] at 8 - 12 mnths

In general, the results of the present study showed that the reflexes obtained with 1000 Hz probe tones were less compared to those obtained with 226 Hz probe tone. This could be because with 1000 Hz probe tone frequency, the probe intensity used was 75 dB SPL. As the infant's ear canal is smaller than the 2cc cavity which was used for calibration of 1000 Hz probe tone in the present study, the sound pressure actually presented to the ear canal is greater than probe tone intensity of 75 dB SPL used for 1000 Hz probe tone frequency. At this level, possibly the decrease in admittance is seen at a much lower intensity with 1000 Hz probe tone than is seen with 226 Hz probe tone.

5. Summary and conclusions

With the advent of universal new born hearing screening programs, there is accelerating growth of pediatric population being tested. Hence there is a crucial need for identification of type of hearing loss. The most common cause of referral on Universal new born hearing screening (UNHS) is conductive hearing loss, the majority of which is secondary to otitis media or middle ear effusion (Boone, Bower, & Martin, 2005; Doyle, Burggraff, Fujikawa, & Macarthur, 1997). Hence, it is of great importance on the part of audiologists that the infant's hearing can be accurately assessed by identifying or ruling out the middle ear disorders as soon as possible to provide adequate and appropriate rehabilitation.

Acoustic immittance testing represents a powerful tool in the clinician's armamentarium, by providing information regarding the presence of even a mild conductive pathology. Immittance measurement is comprised of two tests: tympanometry and acoustic reflexometry.

The clinical utility of immittance measurements has been clearly established for all populations except infants less than 1 year of age. Unfortunately, there has been little research and insufficient data comparing low (226 Hz) and high frequency (668 Hz & 1000Hz) tympanometry and acoustic reflex thresholds obtained from infants at various developmental stages throughout the first year of life for routine use. Hence there is a need for age specific data based on chronological age in months in infants to be used in assessing middle ear function and to improve the diagnostic utility of tympanometry in young infants.

The present study aimed at providing tympanometry findings and acoustic reflex thresholds in infants in the age of 2 – 12 mnths of age. The study was conducted on 70 (140 ears) normal hearing infants confirmed by battery of tests in the age range of 2 to 12 mnths. 70 infants were further divided into 4 subgroups based on their age with 25 infants in the age group of 2 – 4 mnths, 15 infants each in the age groups of 4 – 6 mnths, 6 – 8 mnths and 8 – 12 mnths respectively.

To accomplish the aim, tympanometric measures such as TPP, Y_{+400} and ECV using 226 Hz probe tone frequency, TPP, Y_{+400} and Y_{-600} using 678 Hz and 1000 Hz probe tone frequencies were obtained across different age groups from 2 – 12 mnths. Ipsilateral acoustic reflex thresholds at 500 Hz, 2000 Hz and 4000 Hz were obtained using 226 Hz and 1000 Hz probe tone frequencies across different age groups from 2 – 12 mnths

The results of the present study are analysed and described both qualitatively as well as quantitatively. Qualitative analysis was done based on simple visual inspection system for tympanograms obtained for 226 Hz, 678 Hz and 1000 Hz probe tones. The quantitative analysis was done by obtaining the mean, standard deviation and range for tympanometric peak pressure (TPP), peak compensated static acoustic admittance compensated at + tail (Y_{+400}), peak compensated static acoustic admittance compensated at –ve tail (Y_{-600}), ear canal volume (ECV), ipsilateral acoustic reflex thresholds (ARTs) at 500 Hz, 2000 Hz and 4000 Hz.

Results of the quanlitative analysis for tympanograms showed that as age increases, % of ears with single peaked tympanogram increases and % of ears with double peaked tympanogram decreases when 226 Hz and 678 Hz probe tones were used.

On the other hand, when 1000 Hz probe tone was used it was seen that as age increases, % of ears with single peaked tympanogram decreases and % of ears with double peaked tympanogram increases. The frequency of occurrence of ARTs was also analyzed and the results show that all infants had presence of ARTs at 500 Hz and 2000 Hz when 226 Hz and 1000 Hz probe tones were used. But however ARTs were present in 83.6 % and 86. 4 % of ears when 226 Hz and 1000 Hz probe tones were used.

Results were analyzed quantitatively using appropriate statistical tools like descriptive statistics, one way ANOVA, paired sample t - test, Duncan post hoc test. The result of the quantitative analysis shows the effect of age and probe tone frequency on each tympanometric parameters and ARTs.

Effect of age on TPP shows

Significant difference in TPP ($p < 0.05$) across age groups for 226 Hz probe tone and 678 Hz probe tone and no significant difference ($p > 0.05$) across age groups for 1000 Hz probe tone

- When 226 Hz probe tone was used, there was significant difference ($p < 0.05$) between younger (2 – 4 mnths & 4 – 6 mnths) and older (6 – 8 mnths & 8 – 12 mnths) infants.
- When 678 Hz probe tone was used, there was significant difference ($p < 0.05$) between infants of 2 – 4 mnths age group and other age groups.

Effect of probe tone frequency on TPP shows

- Significant difference in TPP ($p < 0.05$) between 226 Hz & 1000 Hz and 678 Hz &1000 Hz at 8 - 12 months and no significant difference ($p > 0.05$) between probe tone frequencies were seen at 2 - 4 mnths, 4 - 6 mnths and 6 - 8 mnths

Effect of age on Y_{+400} shows

Significant difference in Y_{+400} ($p < 0.05$) across age groups for 226 Hz, 678 Hz and 1000 Hz probe tone

- When 226 Hz probe tone was used, Y_{+400} values in infants in the age range of 6 – 8 months, differed significantly ($p < 0.05$) from other age groups (2 - 4 mnths, 4 - 6 mnths & 8 – 12 mnths).
- when 678 Hz and 1000 Hz probe tones were used, Y_{+400} values in infants in the age range of 2 - 4 mnths differed significantly ($p < 0.05$) from older age groups (4 – 6 mnths , 6 – 8 mnths & 8 - 12 mnths).

Effect of probe tone frequency on Y_{+400} shows

- Significant difference in Y_{+400} between 226 Hz & 1000 Hz ($p < 0.05$) at 2 - 4 mnths, 6 – 8 mnths and 8 – 12 mnths and significant difference between 678 Hz &1000 Hz ($p < 0.05$) at 2 – 4 mnths, 4 – 6 mnths and 6 – 8 mnths and significant difference in Y_{+400} between 226 Hz & 678 Hz ($p < 0.05$) at 8 - 12 months.

Effect of age on Y_{-600} shows

Significant difference in Y_{-600} ($p < 0.05$) across age groups for 1000 Hz probe tone and no significant difference in Y_{-600} ($p > 0.05$) across age groups for 678 Hz probe tone

- When 1000 Hz probe tone was used, there was significant difference ($p < 0.05$) between 2 - 4 mnths and 8 - 12 mnths for Y_{-600} values

Effect of probe tone frequency on Y_{-600} shows

- Significant difference between 678 Hz & 1000 Hz ($p < 0.05$) at 2 - 4 mnths, 4 - 6mnths and 6 - 8 mnths and no significant difference between 678 Hz &1000 Hz ($p > 0.05$) at 8 - 12 mnths

Effect of age on ECV show

- significant difference in ECV ($p < 0.05$) across age groups for 226 Hz probe tone

Effect of age on ART at 500 Hz, 2000 Hz and 4000 Hz show

- Significant difference between 226 Hz &1000 Hz ($p < 0.05$) at all age groups for all the 3 reflex activating signals.

Effect of probe tone frequency on ART at 500 Hz, 2000 Hz and 4000 Hz shows

- No significant difference on ART at 500 Hz, 2000 Hz and 4000 Hz across age groups for 226 Hz and 1000 Hz ($p > 0.05$) probe tone frequencies.

Limitations of the study:

- The study was not conducted on clinical population for validation purposes.
- Number of subjects considered were not equal across age groups

Future research and directions:

- The results from the present study can be used on clinical population for validating the findings
- The findings of the present study can be used clinically to evaluate middle ear function in infants
- The findings of the present study can be used to study the incidence and prevalence of middle ear disorders in infants

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