

Immittance Findings in Infants Using Different Probe Tone Frequencies

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Abstract

The study aimed at providing tympanometric measures such as tympanometric peak pressure (TPP), Peak compensated static acoustic admittance compensated with +ve and -ve tail, (Y_{+400} & Y_{-600}), ear canal volume (ECV) using 226 Hz, 678 Hz and 1000 Hz probe tone frequencies and ipsilateral acoustic reflex thresholds (ARTs) at 500 Hz, 2000 Hz and 4000 Hz using 226 Hz and 1000 Hz probe tones in infants of 2 – 12 months age range. Seventy normal hearing infants who were divided into 4 subgroups with 25 infants in 2 – 4 months age group, 15 infants each in the age groups of 4 – 6 months, 6 – 8 months and 8 – 12 months respectively participated in the study. The results are analyzed qualitatively and quantitatively. The qualitative analysis for tympanograms showed that % of ears with single peaked tympanogram increases and double peaked tympanogram decreases with age, for 226 Hz and 678 Hz probe tones. On the other hand, for 1000 Hz probe tone, the % of ears with single peaked tympanogram decreases and % of ears with double peaked tympanogram increases with age. The ARTs at 500 Hz and 2000 Hz were present in all infants. But ARTs were present in 83.6% and 86.4% of ears when 226 Hz and 1000 Hz probe tones were used. Quantitative analysis was done using appropriate statistical tools which showed significant effect of age and probe tone frequency on tympanometric and ART measures.

Keywords: Tympanometric peak pressure, peak compensated static acoustic admittance, acoustic reflex threshold, ear canal volume

Introduction

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 assessing 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, 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).

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.

Tympanometric 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 provide information related to the function of the middle ear and the sensory, neural and motor pathways associated with acoustic reflex arc.

Tympanometry

The most commonly used probe tone frequency for tympanometry 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, Petrak & Prigge, 2004). Peak compensated static acoustic admittance, peak pressure and ear canal volume are used routinely in interpreting standard low frequency tympanograms.

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 older children, due to poor sensitivity in identifying middle ear effusion in infants. For instance, Paradise, Smith & Bluestone (1976) reported type A tympanograms (normal static admittance and normal middle ear pressure) based on

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Liden / Jerger classification system (Liden, 1969; Jerger, 1970), coexisting with confirmed middle ear effusion in that age group. 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, Margolis, Hirsch & Napp, 1999). 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, Cavanaugh & Margolis, 1991; Keith 1975; McKinley, Grose & Roush, 1997; Keith, 1973; Sprague, Wiley, & Goldstein, 1985; Holte et al., 1991). This makes it difficult to apply the traditional type A, B, C tympanogram classification scheme in infants.

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 & Donaldson, 1981), rapid increases in all dimensions of the external auditory meatus during the first 2 years of life (Keefe, Fitzpatrick, Liu, Sanford & Gorga, 1993), increase in the size of the middle-ear cavity and mastoid with temporal bone growth and increase in the size of the antral and mastoid sinuses throughout childhood (Eby & Nadol, 1986; Anson & Donaldson, 1981), fusion of the tympanic annulus with the temporal bone (Anson, Bast & Richany, 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, Lino, Kakizaki, & Murakami, 1999).

The resonant frequency of the neonatal tympanic membrane is also reported to be much lower than in adults (Weatherby & Bennett, 1980; Holte, 1991). Meyer, Jardine and Deverson (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 study done by Baldwin (2006) where it was reported that tympanometry using 226 Hz is invalid below 21 weeks of age in infants.

Many studies on using high frequency probe tones for tympanometry have been explored over the past 30 years focusing on the use of a higher frequency probe tone such as 660 or 678 Hz (Himelfarb, Popelka and Shanon, 1979; Marchant, McMillan, Shurin, Johnson, Turczyk, Feinstein & Panek, 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 (1986). Paradise et al. (1976), Marchant et al., (1986) and Shurin, Pelton and Finklestein (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 susceptibility 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, Purdy and Barbar (1995) and Rhodes et al. (1999), Margolis, Bass-Ringdahl, Hanks, Holte and Zapala (2003) who found that the use of a 1000-Hz probe tone yielded more accurate diagnosis of middle-ear pathology than 678 Hz.

Tympanometry using 1000 Hz probe tone seems to be the most recommended (JCIH 2007; ASHA, 2004; Kei et al., 2003; Margolis et al., 2003; Baldwin, 2006). There are evidences to support the application of 1000 Hz probe tone frequency in children from birth to 4 months (Baldwin, 2006).

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. 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, Bennett, Marchant & Shurin, 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). 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. McMillan, Bennett, Marchant & Shurin (1985) 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) reported 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, Werner, Hugo, Louw, Owen and Swanepoel (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. Mazlan, Kei, Hickson, Stapleton, Grant, Lim and Gavranich (2007) suggested the need to have separate sets of normative data for infants at birth and 6 -7 weeks.

Despite numerous studies indicating the relevance of

high frequency tympanometry, limited normative data are available for 1000 Hz tympanometry. 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 (226 Hz) and high frequency (668 Hz & 1000 Hz) 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 as 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.

Hence, the present study was taken to establish various tympanometric measures in infants using 1000 Hz probe tone and to know how the tympanometric measures differ in infants for 226 Hz, 668 Hz and 1000 Hz probe tone frequency by documenting the changes in tympanometric findings across different age groups in infants and also to 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.

Method

Participants

Seventy infants (140 ears) in the age range of 2 to 12 months participated in the present study. Seventy infants were further divided into 4 subgroups based on their age with 25 infants in the age group of 2 – 4 months, 15 infants each in the age groups of 4 – 6 months, 6 – 8 months and 8 – 12 months respectively. All the infants had normal otoscopic examination indicating absence of external and middle ear pathology. They were healthy with no symptoms of cold or ear discharge at the time of assessment. They had no complaints and prior histories of any high risk factors or neurological symptoms. All the infants had age appropriate minimum response levels in behavioral observation audiometry, normal outer hair cell functioning ensured by recording TEOAEs and normal hearing sensitivity ensured by recording ABR

Instrumentation

An otoscope was used 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 was used to present stimuli for behavioral observation audiometry (BOA). A calibrated otoacoustic emission system ILO - V6 was used 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 was used to record auditory brainstem responses (ABR). A calibrated Grason Stadler Inc. - tymptstar middle ear analyzer (version 2) was used to carry out tympanometry and acoustic reflexometry.

Test Environment

All the tests were 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.

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 infants 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 and 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. 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 overall SNR of greater than or equal to +3 dB and the reproducibility of greater than 50% were considered (Dijk & Wit, 1987) for the presence of otoacoustic emissions to determine normal outer hair cell functioning.

Auditory brainstem response (ABR): Single channel click evoked ABR was recorded in infants using IHS Smart EP system with insert ear phone (ER- 3A) using repetition rate of 11.1/sec and filter setting of 30-3000 Hz with analysis time of 15 ms. Initially

electrode sites were cleaned with the help of skin preparing gel. Vertical electrode montage was used where the non inverting electrode is placed on high forehead, inverting on the test ear mastoid and common on the non test ear mastoid. 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 k Ω and inter electrode impedance was within 2 k Ω . The subjects were considered to have normal hearing sensitivity if the ABR wave V was clearly seen at 30 dB nHL.

Tympanogram measurements: Tympanometry was carried out by placing appropriate sized probe tip that comfortably fits into the ear canal of the infant 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 and 1000 Hz. The probe intensity used was 85 dB SPL for 226 Hz and 678 Hz probe tones and 75 dB SPL for 1000 Hz probe tone frequency. The 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 months. 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:

Tympanometric peak pressure (TPP): Tympanometric peak pressure was obtained by moving the cursor 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.

Peak compensated static acoustic admittance compensated with +ve tail (Y_{+400}): 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).

Peak compensated static acoustic admittance compensated with -ve tail (Y_{-600}): 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 Peak admittance (Y), static acoustic admittance at the +ve tail (Y_1) and static acoustic admittance at the -ve tail (Y_2) values were recorded. Peak admittance (Y) 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). Static acoustic admittance at the +ve tail (Y_1) 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 and 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 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 starting 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 frequency of acoustic reflex thresholds. Quantitative analysis was done by

obtaining TPP, Y_{+400} , Y_{-600} , ECV values with ipsilateral acoustic reflex thresholds at 500 Hz, 2000 Hz and 4000 Hz for different probe tone frequencies across age groups. The data obtained for various tympanometric measures and ART for each probe tone frequency across each age group was compared using appropriate statistical analysis.

Results and Discussion

Qualitative analysis: The qualitative analysis of tympanograms using 226 Hz probe tone showed single peaked tympanogram in 36 ears (72%), a double peaked tympanograms in 14 ears (28%) in 2-4 months age group. Single peaked tympanogram in 28 ears (93.3 %), a double peaked tympanograms in 2 ears (6.6 %) were obtained in 4-6 months age group and single peaked tympanogram in all 30 ears were obtained in 6-8 months and 8-12 months age group respectively. The qualitative analysis of tympanograms using 678 Hz probe tone showed single peaked tympanogram in 17 ears (34 %), a double peaked tympanograms in 28 ears (56%), inverted/ V shaped tympanogram in 3 ears (6%) and more than two peaks in 2 ears (4%) in 2-4 months 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 months age group. Single peaked tympanogram in 22 ears (73%), a double peaked tympanogram in 8 ears (27%) were obtained in 6-8 months age group. Single peaked tympanogram in 23 ears (76.6%), a double peaked tympanogram in 7 ears (23.3%) were obtained in 8-12 months age group.

When 226 Hz and 678 Hz probe tones were used, the percentage 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, the percentage 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 and 678 Hz probe tones were used. The percentage 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 months and 4 – 6 months) 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 using 1000 Hz probe tones showed single peaked tympanogram in 44 ears (88%), a double peaked tympanograms in 6 ears (12%) in 2–4 months age group. Single peaked tympanogram in 18 ears (60%), a double peaked tympanograms in 12 ears (40%) were obtained in 4 – 6 months age group. Single peaked tympanogram in 13 ears (43.3%), a double peaked tympanogram in 17 ears (56.6%) were obtained in 6–8 months age group. Single peaked tympanogram in 13 ears (43.3%), a double peaked tympanogram in 17 ears (56.6%) were obtained in 8–12 months age group. When 1000 Hz probe tone was used, the percentage of ears with single peaked tympanogram decreases as the age increases. On the other hand, the percentage 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 months and 8–12 months) 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.

The frequency of occurrence of ipsilateral acoustic reflex threshold from 140 healthy infant ears using 226 Hz and 1000 Hz probe tone revealed that 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. But 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).

Quantitative analysis: 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, Mirinda and Polka (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, Baldwin and Brooks (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.

Effect of age and probe tone frequency on TPP: Descriptive statistics was done to obtain mean and standard deviation of TPP for each age range and each probe tone frequency separately and is shown in Table 1.

The mean values of TPP is more positive for infants of younger age groups (2–4 months & 4–6 months) and more negative for infants of older age groups (6–8 months & 8–12 months). However among the younger age groups, the mean TPP is more for 2–4 months than 4–6 months infants. Since during the testing infants of 2–4 and 4–6 months were sleeping in the present study, this slight positive mean TPP observed in the infants of 2–4 months and 4–6 months can be attributed to occurrence of positive middle ear pressure while sleeping. It has been reported in adults by Hergil 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 other age groups.

To see the effect of age on TPP, one way analysis of variance (ANOVA) was done at each probe tone frequencies. The results showed that there was significant difference in TPP across age groups for 226

Table 1: Mean and Standard Deviation (S.D) for TPP in daPa across age groups for different probe tone frequencies

Probe tone frequencies		226 Hz TPP (in daPa)		678 Hz TPP (in daPa)			1000 Hz TPP (in daPa)		
Age groups	N	Mean	S.D	N	Mean	S.D	N	Mean	S.D
2 - 4 months	36	20.69	28.38	17	31.76	28.55	44	7.27	48.84
4 - 6 months	28	10.35	32.74	13	4.61	34.42	18	2.36	60.58
6 - 8 months	30	-19.33	59.43	22	-15.90	62.88	13	-16.92	50.97
8 – 12 months	30	-14.50	53.58	23	-12.82	66.39	13	8.07	77.25

Hz probe tone [$F(3, 120)=5.91$, $p < 0.05$] and 678 Hz probe tone [$F(3, 71)=3.05$, $p < 0.05$].

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 showed that when 1000 Hz probe tone was used there was no age effect seen on TPP. When 226 Hz probe tone was used, there was significant age effect seen between younger (2 – 4 months & 4 – 6 months) and older (6 – 8 months & 8–12 months) infants. When 678 Hz probe tone was used, there was significant age effect seen between infants of 2–4 months age group and other age groups.

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 showed that when 1000 Hz probe tone was used there was no age effect seen on TPP. When 226 Hz probe tone was used, there was significant age effect seen between younger (2–4 months & 4–6 months) and older (6–8 months & 8–12 months) infants. When 678 Hz probe tone was used, there was significant age effect seen between infants of 2–4 months age group and other age groups.

These results of the present study are in accordance with the previous study done by Mazlan et al., (2007) where they compared TPP measures between neonates at birth and at 6 weeks of age using 1000 Hz probe tone and reported 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, Luts and Wouters (2007) where they reported no significant differences between the 3 to 9 month and the 9 to 32 months age groups with regard to TPP with 1000 Hz probe tone.

To see the effect of probe tone frequency on TPP, Paired sample t - test was done at each age group. 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 and 1000 Hz [$t(12)=-3.13$, $p<0.05$] and, 678 Hz and 1000 Hz [$t(10)=-4.25$, $p<0.05$] at 8 to 12 months. However no significant difference for TPP between probe tone frequencies were seen at 2 - 4 months, 4-6 months and 6-8 months

This difference in TPP between 226 Hz and 1000 Hz and, 678 Hz and 1000 Hz probe tone frequencies at 8-12 months 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 and 1000 Hz and, 678 Hz and 1000 Hz at 8–12 months 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.

Effect of age and probe tone frequency on Y_{+400} : Descriptive statistics was done to obtain mean and standard deviation of Y_{+400} , for each age range and each probe tone frequency separately as given in Table 2. As shown in Table 2, the mean values of Y_{+400} increased as probe tone frequency increased for infants of older age groups (6–8 months & 8–12 months). However among the younger age groups (2–4 months & 4–6 months) there was no specific trend of increase or decrease in admittance seen with increase in probe tone frequency.

Table 2: Mean and Standard Deviation (S.D) 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)			Y_{+400} (in mmho)			Y_{+400} (in mmho)		
Age groups	N	Mean	S.D	N	Mean	S.D	N	Mean	S.D
2 – 4 months	36	0.55	0.64	17	0.47	0.24	44	1.23	0.46
4 - 6 months	28	0.50	0.29	13	0.85	0.55	18	0.82	0.37
6 – 8 months	30	0.69	0.34	22	0.85	0.44	13	1.01	0.37
8 - 12 months	30	0.44	0.19	23	0.82	0.44	13	0.88	0.46

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 that there was significant difference in Y_{+400} across age groups for 226 Hz [$F(3, 120)=4.24, p<0.05$], 678 Hz [$F(3, 71) = 3.30, p<0.05$] and 1000 Hz [$F(3, 84)=4.89, p > 0.05$] probe tones. 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 the Duncan post hoc analysis show that, there was significant difference seen between infants in the age range of 2-4 months and older age groups (4–6 months, 6–8 months & 8-12 months) 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 months, 4-6 months & 8–12 months).

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 mmhos and over the 6-week period, it increased to 1.01 mmhos when 1000 Hz 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 1000Hz 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.

To see the effect of probe tone frequency on Y_{+400} , Paired sample t - test was done at each age group. At 2-4 months, 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 4-6 months there was significant difference between 678 Hz & 1000 Hz [$t(9)=-4.06, p<0.05$]. At 6-8 months, 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 8-12 months there was significant difference between 226 Hz & 678 Hz [$t(22)=-3.90, p<0.05$] and 226 Hz & 1000 Hz [$t(12)=-3.58, p<0.05$].

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 months 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.

Effect of age and probe tone frequency on Y_{-600} : Descriptive statistics was done to obtain mean and standard deviation of Y_{-600} for each age range and each probe tone frequency separately as given in Table 3.

Table 3: Mean and Standard Deviation (S.D) for Y_{-600} in mmho across age groups for different probe tone frequencies

Probe tone frequencies	678 Hz			1000 Hz		
	Y_{-600} (in mmhos)			Y_{-600} (in mmhos)		
Age groups	N	Mean	S.D	N	Mean	S.D
2 - 4 months	17	0.98	0.40	43	1.77	0.61
4 - 6 months	13	1.22	0.70	18	1.40	0.47
6 - 8 months	22	1.21	0.43	13	1.49	0.43
8 - 12 months	23	1.07	0.46	13	1.25	0.53

Table 4: Mean and Standard Deviation (S.D) for ECV across age groups

Age groups (months)	226 Hz		
	N	Mean (ml)	S.D
2 - 4 months	50	0.43	0.10
4 - 6 months	30	0.52	0.15
6 - 8 months	30	0.59	0.12
8 - 12 months	30	0.66	0.13

N: number of ears

As it is shown in Table 3, 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. The mean values showed that Y_{-600} values are more for when 1000 Hz probe tone was used than when 678 Hz probe tone was used.

To see the effect of age on Y_{-600} , one - way ANOVA was done at each probe tone frequency. The results showed that there was significant difference in Y_{-600} across age groups for 1000 Hz probe tone [$F(3, 83)=3.84, p<0.05$]. 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 1000 Hz 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 showed that there was significant difference between 2-4 months and 8-12 months 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 months middle ear becomes more stiffness dominated and at 2-4 months 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.

To see the effect of probe tone frequency on Y_{-600} , Paired sample t test was done at each age group. The results showed that there was significant difference between 678 Hz & 1000 Hz at 2-4 months [$t(16) = -6.57, p<0.05$], 4-6 months [$t(9) = -7.35, p<0.05$] at 6-8 months [$t(12) = -3.59, p < 0.05$]. However, there was no significant difference between 678 Hz & 1000 Hz [$t(10) = -0.39, p>0.05$] at 8-12 months.

The results of the present study 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 months, 4-6 months & 6 - 8 months) 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} .

Effect of age on ECV: Descriptive statistics was done to obtain mean and standard deviation of ECV values for each age range and each probe tone frequency separately as given in Table 4. There was general trend of increasing ECV values with age when 226 Hz probe tone was used.

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 showed that ECV values differ significantly between all the age groups.

The mean ear canal volume values clearly increased from 0.43 ml to 0.66 ml as age increased from 2–12 months in the present study. The mean ECV values in the present study are in agreement with previous studies done by Margolis & Heller (1987) where ECV values were reported to be 0.3 ml to 1 ml between children of 6 weeks to 7 years. The increase in ECV 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 & Lilly, 1981). Such developmental changes include the development of the osseous external auditory meatus during the first 12 months of life (Anson & Donaldson, 1981), rapid increase in all dimensions of the external auditory meatus during the first 2 years of life (Keefe et al., 1993).

Effect of age and probe tone frequency on ART at 500 Hz: Descriptive statistics was done to obtain mean and standard deviation of ART at 500 Hz for each age group and each probe tone frequency separately as given in Table 5. As it is shown in Table 5, the mean ART at 500 Hz inferred no specific trend with respect to increase / decrease in ARTs across age groups and also 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.

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 of the paired sample t test showed that there was significant difference in ART at 500 Hz between 226 Hz and 1000 Hz probe tone frequency at 2–4 months $[t(49) = 11.22, p < 0.05]$, at 4–6 months $[t(29) = 11.17, p < 0.05]$, at 6–8 months $[t(29) = 11.14, p < 0.05]$ and at 8–12 months $[t(29) = 6.01, p < 0.05]$.

Effect of age and probe tone frequency on ART at 2000 Hz: Descriptive statistics was done to obtain mean and standard deviation of ART at 2000 Hz for each age range and each probe tone frequency separately as given in Table 6. The mean values show 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.

Table 5: Mean and Standard Deviation (S.D) of ART at 500 Hz across age groups for different probe tone frequencies

Probe tone frequencies		226 Hz (dBHL)		1000 Hz (dBHL)		
Age groups	N	Mean	S.D	N	Mean	S.D
2 - 4 months	50	92.10	6.31	50	81.30	6.68
4 - 6 months	30	92.66	6.91	30	82.16	7.50
6 - 8 months	30	92.66	6.39	30	82.66	5.68
8 -12 months	30	89.33	5.37	30	82.50	6.12

N: number of ears

Table 6: Mean and Standard Deviation (S.D) of ART at 2000 Hz across age groups for different probe tone frequencies

Probe tone frequencies		226 Hz (dBHL)		1000 Hz (dBHL)		
Age groups	N	Mean	S.D	N	Mean	S.D
2 – 4 months	50	93.60	6.85	49	81.02	6.45
4 – 6 months	30	94.83	7.36	30	84.00	7.70
6 – 8 months	30	95.00	6.82	30	83.50	7.44
8 -12 months	30	91.83	6.62	30	82.66	7.03

N: number of ears

Table 7: Mean and Standard Deviation (S.D) of ART at 4000 Hz across age groups for 226 Hz and 1000 Hz probe tone frequencies

Probe tone frequencies		226 Hz (dBHL)		1000 Hz		
Age groups	N	Mean	S.D	N	Mean	S.D
2 – 4 months	45	96.22	5.65	42	83.09	7.95
4 – 6 months	23	94.56	6.72	25	84.60	8.40
6 -8 months	23	96.08	4.99	24	84.58	8.32
8 -12 months	26	95.00	6.32	30	83.50	7.32

N: number of ears

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.

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 of the paired sample t test showed that there was significant difference in ART at 2000 Hz between 226 Hz and 1000 Hz probe tones at 2–4 months [$t(48)=13.7, p<0.05$], at 4–6 months [$t(29)=9.02, p<0.05$], at 6–8 months [$t(29)=9.20, p<0.05$] and at 8–12 months [$t(29)=7.09, p<0.05$].

Effect of age and probe tone frequency on ART at 4000 Hz: Descriptive statistics was done to obtain mean and standard deviation of ART at 4000 Hz for each age range and each probe tone frequency separately as given in Table 7. As it shown in Table 7, 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.

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. 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 of pared sample t test showed that there was significant difference between ART at 4000 Hz between 226 Hz and 1000 Hz probe tones at 2–4

months [$t(40)=13.48, p<0.05$], at 4 - 6 months [$t(22)=6.48, p<0.05$], at 6 - 8 months [$t(22)=7.24, p<0.05$] and at 8-12 months. Hz [$t(25)=8.63, p<0.05$].

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.

Conclusions

Acoustic immittance testing represents a powerful tool in the clinician's armamentarium, by providing information regarding the presence of even a mild conductive pathology. The present study aimed at providing tympanometry findings and acoustic reflex thresholds in normal hearing infants in the age of 2 – 12 months of age. Results showed that immittance measurements using 1000 Hz probe tone frequency gives more accurate results till the age of 4 months in infants.

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 and also can be used to study the incidence and prevalence of middle ear disorders in infants

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