**Introduction**

Normal hearing ability is the main source for development of speech and language skills. Any impairment to hearing during childhood may lead to delay in the speech and language skills, making the communication process more difficult. However, early identification and rehabilitation if given can work for the benefit of the children. Therefore in neonates, it is recommended to carry out a complete hearing examination before moving out from the hospital setup post delivery. In addition, it is necessary to focus on the test battery used for testing the hearing of neonates. In accordance with the literature, there still persists a debate on using 226Hz probe tone and 1000Hz probe tone for testing the neonatal hearing.

Tympanometry is an umbrella term encompassing impedance, admittance, and their components. It is defined as mechanical analysis of the auditory system in response to acoustic stimulation. Acoustic immittance measures the acoustic energy transfer that occurs when sound pressure is applied to the tympanic membrane causing its movement. Thus it helps to evaluate the ease or opposition to this sound energy flow within the auditory system. Today, its contribution to clinical diagnosis has aided to better middle ear status diagnosis and has now become a routine part of the audiological test battery approach ranging towards neonates to geriatrics.

In the early years of life up to two years, normal ear tympanic-ossicular system acts differently as mass is the dominating physical feature of the ear. After this age, reaching the adult stage, there is a change in behavior which is controlled by stiffness and is better evaluated using probe frequency of 226 Hz [1]. Thus carrying out conventional tympanometry using 226Hz probe tone at early years of life may not serve of much help. Therefore, condition of the middle year in the early stages of life can be evaluated more expeditiously using high frequency probe tones such as 678 Hz and...
1,000 Hz. Conventional tympanometry using 226Hz probe tone in neonates and infants has been used by many audiologists [1-4], although the literature shows that the use of a single frequency is not sensitive enough to detect all cases of middle ear pathology, which hinders accurate diagnosis [5-8].

Tympanometry using high frequencies help to clear up the false-positive screening results that pass off due to pathology in the middle ear or the presence of secretion. Adequate middle ear assessments in the neonatal period results in suitable medical and audiological referrals and can thereby improve the efficacy of newborn hearing screening programs [9]. Evidence from previous literature suggests that the use of a single frequency probe is not highly sensitive enough to identify all cases of middle ear alterations, thereby making diagnosis difficult. Therefore, the purpose of this study was to characterize tympanometry measurements in neonates between 0-17 days of age using 2 probe tones.

Materials and Method

The study was undertaken with the approval of ethical committee formed at Nitte Institute of Speech and Hearing, Mangalore. To conduct the study, following inclusion criteria was considered for the participants of the study:

A. Neonates of 1 to 17 days,

B. Neonates administered and screened for high risk register using Joint Committee on Infant Hearing [10] to rule out possible presence of hearing impairment,

C. Ear canal screened to examine the possible obstacles,

D. Neonates with bilateral Distortion Product Otoacoustic Emissions (DPOAE) present.

A Term of Free and Informed Consent form was furnished to the Parents/caregivers which contained information about the study in very clear and uncomplicated language. Once the parental consent was signed and obtained the study was continued. The study incorporated 60 neonates: 21(35%) female and 39 (65%) . The age ranged between from 1day to 17 days, with an average age of 2.9 days of life. A total of 120 years were evaluated right (100%) and left (100%).

Audiological interview was carried out to begin with the screening assessment, which was followed by visual inspection of the pinna and ear canal, Distortion Product Oto-acoustic Emission testing and tympanometry measurements. Audiological interview in collaboration with information from hospital files was carried out with the parents/caregivers using leading questions, in simple and uncomplicated language. The purpose was to serve the information regarding neonatal hearing, presence of any upper respiratory tract infection, and high risk indicators for hearing impairment.

Visual inspection of the pinna, ear canal and tympanic membrane was carried out by an experienced clinical audiologist to rule out any disturbances in testing of tympanometry. If any disturbances were encountered the neonate was referred to the Oto-rhino-laryngologist for the further evaluation.

To acquire the tympanometry measurements, Interacoustics AT 235h impedance audiometer was used. Following the specifications of the equipment manual, tympanometry was applied using 226 Hz and 1000Hz frequencies, with the pressure ranging from + 200 to -400 daPa and a pressure change rate of 600/200 daPa per second. The intensity of probe tone was adjusted at 85 dB SPL for 226Hz and 1,000 Hz.

Two forty tympanograms were obtained: 114 (95%) with a 226 Hz probe tone, 109 (90.8%) with a 1,000 Hz probe tone. The occlusion encountered during the testing procedure lead to unequal distribution of the tympanograms. In the consequence of occlusion with a 226 Hz and 1000Hz probe tone, the probe was removed from ear and repositioned to restart the testing.

The obtained Tympanogram shapes were categorized as either a single-peaked tympanogram (SP) with maximum tympanometric peak pressure (TPP), a double peaked tympanogram (DP) with two-peak TPP, an inverted peaked tympanogram (IP) with an inverted TPP, an asymmetric tympanogram (A) with a gradual decline of TPP ranging from +200 to -200 daPa and, or a flat peaked tympanogram (F) with no TPP. Similarly, compensated static peak acoustic admittance (Ymt) mentions the maximum peak admittance, where the pressures of the external and middle ear are equal. The maximum admittance peak pressure is expressed in daPa.

With the previous literature [10-12], using 226Hz probe tone SP or DP tympanograms were classified as normal. For 1,000Hz frequency, tympanograms were classified using the Sutton Protocol [13], used for infants upto the age of 4 months. Shadowing this protocol, tympanograms with Ymt<0 or TPP>200 daPa were considered as normal. Tympanograms with Ymt<0 or TPP<-200 daPa were considered as abnormal. On classification of tympanograms as either normal or abnormal, the tympanograms with the two different probe tones were compared. In addition, to the literature, single-peaked and double-peaked tympanograms are considered normal, while asymmetric, inverted, and flat tympanograms are considered abnormal [13-16].

Results

Tympanometry was carried out in 60 healthy neonates using 226Hz and 1000Hz probe tone. The tympanometry measures were compared in accordance with type of tympanogram, ECV, Ymt, TPP. The obtained measures for 226Hz and 1000Hz were compared to know the efficacy of the probe tones. Sampled paired t test using SPSS version: 16 was used to obtain a statistically significant difference between the two probe tones.

On comparing the type of tympanograms, 226Hz elicited 71 Single Peaked tympanogram with maximum tympanometric peak pressure and 49 flat peaked tympanogram (F) with no
TPP. However, 1000Hz elicited 83 Single peak tympanogram with maximum tympanometric peak pressure, 26 double peak tympanograms with two-peak TPP and 11 flat tympanogram with no TPP (Figure 1).

Using sampled paired t test it is evident that 1000Hz probe tone is more compatible for neonatal hearing testing then 226Hz probe tone with respect to ECV, Ymt and TPP. However there is a significant difference between the ECV and Ymt. Table 1 shows the statistical differences between the three parameters (Table 1).

**Table 1: Comparison between 226Hz and 1000Hz probe tone.**

| Parameters | 226Hz Probe Tone | 1000Hz Probe Tone | T value | P value |
|------------|------------------|------------------|---------|---------|
| ECV        | 0.74cm^3         | 0.71cm^3         | 0.94    | <0.05   |
| Ymt        | 0.33cm^3         | 0.45cm^3         | -3.56   | >0.05   |
| TPP        | -31.33dapa       | -12.07dapa       | -3.08   | >0.05   |

Using the 226Hz and 1000Hz probe tone, the ECV values were almost the same. There was less variation observed using 226Hz and 1000Hz probe tone frequencies. The sampled paired t test revealed no significant difference for the same. However, using 226Hz and 1000Hz probe tone Ymt and TPP were not the same. Sampled Pair t test revealed a significant difference between Ymt and TPP using 226Hz and 1000Hz probe tone in neonates.

A significant difference was observed, when the 226Hz and 1000Hz probe tone frequencies were compared. This indicates that the 1000Hz probe tone surpassed the limitations of 226Hz probe tone in the mass dominating middle ear system of the neonates with better measurements.

**Discussion**

The results were earned by grouping the right and left ears to facilitate better statistical analysis, as there was no significant difference found between the ears in the previous studies. In the present study a statistically significant difference across two subsections was seen.

It determines compensated static acoustic admittance. For 226 Hz probe tone, the normal range of ECV is between 0.3 and 1.0 mL [17-19]. Literature reports a mean of about 1.5 mm [6,11]. GRASON-STADLER [20] reports, ECV values acquired with a frequency of 678 Hz are 3 times larger than the ECV values obtained at 226 Hz, and that at 1,000 Hz probe tone. This difference can be up to 4.4 times larger. The current study reports that the ECV values obtained using high frequencies are greater with 1000Hz than values obtained with 226 Hz. Consistent with this statement, a significant difference between the results of the 3 frequencies was found, with the mean ECV value at 1,000 Hz greater than the mean values at 678 Hz and 226 Hz [14].

In the present study 226Hz probe tone obtained single and flat peaked tympanograms however; 1000Hz probe tone lead to single, double and flat peaked tympanograms. The possible tympanometry measures leading to differences in the type of peaks could be attributed to the possible presence of cerumen in the ear canal, placement of the probe in the infant ear, or differences in the middle ear system of infants [20]. Similarly, the presence of unequal distribution in number of tympanograms could be due to the presence of occlusion effect. Infants younger than 4 months old have higher chance of obtaining occlusion effect during the testing [11] and a low occurrence of flat tympanograms, indicate possible presence of fluid in the middle ear.

Similarly, literature reports, occurrence of single-peaked tympanograms to be predominant [11,20], while other studies indicate a preponderance of double-peaked tympanograms in neonates and infants using 1000Hz probe tone [12,21]. In addition, a higher incidence of single-peaked tympanograms [11,14], followed by flat tympanograms is also quoted [14]. Single-peaked and double-peaked tympanograms are considered normal, while asymmetric, inverted, and flat tympanograms are considered abnormal [13-16]. Literature reports different findings with 226Hz and 1000 Hz probe tones. The uncommon characteristics of the tympanograms obtained from newborns and babies, may be attributed to the physiological differences concerning the ears from newborns and adults [22]. Literature reports high frequency tympanometry provides more detailed information about the state of the mechanics and acoustics of the ear, especially for changes related to the mass factor [23-24], as well as the possibility of middle ear pathology at the initial or final stage. To conclude, 1000Hz probe tone reduces prevalence of flat tympanograms in newborns and 1000 Hz probe tone has also been identified as having greater sensitiveness to identify mild middle ear disorders [25].

The differences observed in obtaining tympanometric curves in neonates can be attributed mainly to normal variations in the subject population and also by the differences in the age of neonates. During growth there are many changes that occur in the ears of neonates, which have an impact on the mechanical properties of the auditory canal. Among the physical changes,
differences are seen in the external ear and in the middle ear growth such as enlargement of the external ear, mastoid and middle ear cavity, changes in tympanic membrane orientation and tympanic annulus fusion. Some other variations such as changes in bone density and mesenchymal loss occurring during development could also play a major role. These changes could be related to the acoustic changes influencing the recording of the tympanogram with compensated static peak acoustic admittance [6, 16, 26, 27].

In addition, mass components are larger in high frequency and lower in the low frequency tympanometry [6, 22]. The normal middle ear is primarily dominated by the stiffness of low frequency sounds (226 Hz). In a higher frequency (for instance: 1000 Hz), the relative participation of each anatomical structure is changed and the acoustic admittance measured at the middle ear inlet becomes more predominated by the mass [12].

The admittance measurement in tympanometry indicates changes in the middle ear, literature reports higher mean Ymt values at 1,000 Hz compared to 226 Hz [11,13]. Mean Ymt values equal to 1.06 mmho when evaluating neonates [28]. The results of the current study are in accordance with the literature. On statistical analysis 1000Hz probe tone obtained higher values of Ymt in comparison to 226Hz probe tone. Low frequency tympanometry in newborns and babies younger than six months has low sensitivity, in other words, the high level of false-negatives.

While comparing the tympanometric peak pressure (TPP) obtained with 226 Hz and 1000Hz. The current study indicated a higher TPP value with 1000 Hz than with 226 Hz. Similar findings have been reported in the literature [3, 6]. Although there were statistically significant differences between the frequencies with respect to TPP, none of the differences were clinically significant. That is, the results for all 2 frequencies were within the normal range [13].

In addition, testing neonates using tympanometry, tympanograms with probe tone of 1000 Hz are reliable, easier to interpret than 226Hz probe tone. It is easy to interpret and more reliable (91%) using 1000 Hz than the 226 Hz (35%) probe tone, which shows a significantly better result in the assessment of the middle ear system. Similarly, in infants younger than 3 months of age, 226 Hz probe tone resulted in 58% of false-positive results [25].

The results of the current study are in accordance with the findings in the literature. These findings provide evidence for further obtaining normative values at different ages to adapt clinical practice to the use of high frequency probe tones with young children.

Conclusion

The current study aimed at comparing the two probe tones and its measurements in tympanometry. The neonates were considered as the subjects of the study. The difficulties met by 226Hz probe tone were seen to be surpassed by 1000Hz probe tone in neonatal tympanometry acoustic measurements. Neonates can be well diagnosed using a 1000HZ probe tone using tympanometry. However, further is warranted as the sample size used in current is small.

Informed consent

Informed oral consent was obtained from all individual participants included in the study.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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