Impact of Advancing Age on Frequency Tuning of Ocular Vestibular Evoked Myogenic Potential

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Abstract

Normal aging is mostly associated with global decline in almost all aspects. While aging affects the 500 Hz tone-burst evoked ocular vestibular evoked myogenic potentials (oVEMP) by reducing the amplitudes and prolonging the latencies, its interaction with oVEMP responses at other frequencies has sparingly been explored. Therefore the present study aimed at investigating the impact of advancing age on the frequency tuning of oVEMP. The oVEMPs were recorded from 50 healthy individuals divided under five age groups (20-30, 30-40, 40-50, 50-60, &> 60 years) for tone-burst frequencies of 250, 500, 750, 1000, 1500 and 2000 Hz. The results revealed significantly lower response rates for age groups above 50 years than all the other groups at almost all the frequencies (p< 0.05). Although there was a trend towards lower peak-to-peak amplitudes in age groups above 50 years, the differences were not statistically significant (p> 0.05). Further, the frequency tuning was obtained at 500 Hz or 750 Hz in majority of individuals below 60 years and at e" 1000 Hz in most of the individualsabove 60 years. The proportion of ears showing frequency tuning at e" 1000 Hz was significantly higher in the above 60 years age groups than below it (p <0.05). Thus, there was a significant shift in frequency tuning of oVEMP from 500 Hz or 750 Hz in the younger and the middle-aged adults to e" 1000 Hz in older adults, especially above 60 years of age. Since the shift in frequency tuning to e" 1000 Hz is popularly used for identification of Meniere's disease, it is suggested that age-related correction be used for diagnosis of Meniere's disease when using frequency tuning of oVEMP for doing so.

Key Words: *Age, Frequency tuning, Meniere's disease, Ocular vestibularevoked myogenic potential.*

Introduction

Human being are blessed with extraordinarily tuned senses. This makes it possible for the human kind to cope effectively with the environment by utilizing the capability in processing the incoming sensory input. Unfortunately, advancing age brings a systematic reduction in the effectiveness of this capability of unstrained processing of the sensory systems (Harman, 2003; Crane, Devries, Safdar, Hamadeh, &Tarnopolsky, 2010).

Normal aging is mostly associated with global decline in almost all aspects. Human aging has been shown to involve changes at molecular, biochemical and also cellular level across all the body systems (Harman, 2003). Changes in the skin, epithelial membrane, skeletal muscles, bones, joints and tissues have also been reported (Harman, 2003; Crane, Devries, Safdar, Hamadeh, & Tarnopolsky, 2010). Our system of balance is a congregation of a number of the above mentioned parts and therefore would be likewise affected by the aging process.

As in any organ, age-related degenerative changes are also shown in the vestibular system. These changes have been identified from the end organs of the vestibular system to its central nuclei (Bergstrom, 1973). Previous studies have shown steady decline in the vestibular hair cell counts and densities with advancing age (Merchant, Velazquez-Villasenor, Tsuji, Glynn, Wall, & Rauch, 2000). Similar data was also presented for Scarpa's ganglion neuronal counts (Merchant, et al, 2000) and vestibular neurons in the vestibular brainstem (Tang, Lopez, & Baloh, 2001-2002).

The advances in technology provided the clinicians an opportunity to assess function of semicircular canals and the otolith organs. However, only a few tests help in the evaluation of the functional integrity of the otolith organs. One such test is vestibular evoked myogenic potential (VEMP). VEMP is a clinical tool that helps explore the functional integrity of the otolith organs and reflexes that involve them as a of their primary components (Colebatch & Halmagyi, 1992; Colebatch, Halmagyi, & Skuse, 1994).

VEMP is a biphasic potential which can be evoked by air-conducted (Honaker, & Samy, 2007; Mudduwa, Kara, Whelan, & Banerjee, 2010), bone-conducted (Tseng, Wang, & Young, 2012) or galvanic stimulation (Cunha, Labanca, Tavares, & Goncalves, 2014). This myogenic potential may be recorded from various locations in the body and based on the recording site, the target generation site differs. The primary recording site is the

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sternocleidomastoid (SCM) muscle along the cervical spine (Colebatch & Halmagyi, 1992). The VEMP elicited from the SCM muscle is called Cervical VEMP (cVEMP). VEMP can also be recorded from the inferior extraocular muscles of the eye (Rosengren, Todd, & Clebatch, 2005), in which case it is referred as Ocular VEMP (oVEMP). In addition to being easier to perform and being less taxing on the patient than cVEMP, oVEMP also provides information regarding the vestibulo-ocular reflex (VOR) pathway (Rosengren et al., 2005; Todd, Rosengren, Aw, & Colebatch, 2007; Welgampola, Migliaccio, Myrie, Minor, & Carey, 2009), which makes it a complementary test rather than supplementary test to cVEMP. Since oVEMP has contributions from most of the afore mentioned areas that are affected by the process of aging, it is likely that oVEMP could also be affected by the process of aging and hence diagnosis needs to be made only after comparisons with the age-related norms.

Age-related changes for oVEMP are well-documented in the literature. Piker, Jacobson, McCaslin and Hood (2011) reported absent oVEMPs in 25% of otologically and neurologically intact normal subjects over the age of 60 years. Similar alterations to various oVEMP parameters were also reported after the age of 60 years in other studies (Nguyen, Welgampola, & Carey, 2010. Therefore, aging plays an important role in the determination of the norms to which a pathological finding should be compared.

oVEMPs were shown to be clinically useful in the diagnosis of several peripheral vestibular pathologies like Meniere's disease (Lin, Wang, & Young, 2013; Jerin, Berman, Krause, Ertl-Wagner, & Gurkav 2014), superior canal dehiscence (Rosengren, Halmagyi, & Colebatch, 2008; Janky, Nguyen, Welgampola, Zuniga, , & Carey, 2013), and vestibular neuronitis (Taylor, Bradshaw, Halmagyi, & Welgampola, 2011; Adamec et al., 2013). Meniere's disease is one among the most explored pathologies using oVEMP. The parameters like latency, amplitude and assymetry ratio have been shown to be useful in diagnosis of Meniere's disease by the above studies. However, the finding in these parameters do not apply only to Meniere's disease, rather they are common to several other pathologies like benign paroxysmal positional vertigo (BPPV) (Akkuzu, Akkuzu, & Ozluoglu, 2006; Yang, Kim, Lee, & Lee, 2008; Korres, Gkoritsa, Giannakakou-Razelou, Yiotakis, Riga, & Nikolpoulos, 2010; Singh, & Barman, 2015), vestibular neuritis (Taylor et al., 2011; Adamec et al, 2013)and labyrinthitis (Murofushi, Halmagyi , Yavor , & Colebatch, 1996; Moon, Lee, Park, & Lee, 2012). Frequency tuning is the most recent addition to this list of parameters that are reported to be useful in the diagnosis of Meniere's disease.

Frequency tuning or the tuned frequency refers to the frequency at which largest oVEMP responses are obtained (Sandhu, Low, Rea, & Saunders, 2012). Healthy individuals have shown the presence of frequency tuning at 500 Hz (Singh & Barman, 2013, 2014), 1000 Hz (Taylor et al., 2011) or between 400 and 800 Hz (Todd, Cody, & Banks, 2000). The frequency tuning is found to be shifted to higher frequencies in patients with Meniere's disease and the best frequency for such patients was reported to be 1000 Hz or higher frequencies (Sandhu et al., 2012). This shift in frequency tuning from 500 Hz in normals to 1000 Hz or higher frequencies in Meniere's disease was explained on the basis of changes in the stiffness characteristics of the utricular membrane causing a change in the resonance frequency of the utricle (Sandhu et al., 2012).

The prevalence of Meniere's disease is known to increase with increasing age (Alexander, & Harris, 2010). The disease was reported to be most commonly affecting the individuals in their fifth and sixth decade of life (Alexander, & Harris, 2010). Infact, the mean age of individuals with Meniere's disease was found to be above 50 years in a number of studies (Alexander & Harris, 2010). Likewise, the age range above 50 years have also been shown to involve age related degenerative changes in the vestibular system (Walther and Westhofen, 2007). These studies reporded neural degeneration as well as reduction in volume and number of otoconia in the utricular macula. Reduction in the number of otoconia and the consequent reduction in the volume of macula would potentially affect the resonant frequency by virtue of altering the balance between mass and stiffness characteristics within the utricle. Therefore there would be a likelihood of a change in resonant frequency and a consequent change in frequency tuning could take place due to aging. Nonetheless, such a phenomenon has sparingly been explored previously in healthy individuals.

Piker, Jacobson, Burkard, McCaslin and Hood (2013) explored the effect of advancing age on frequency tuning of oVEMP. They considered 39 individuals, who were divided into three groups of 13 subjects each as young adults (18-39 years), middle aged adults (40-59 years) and old adults (e"60 years group). They reported a significant shift in the frequency tuning towards higher frequencies with increasing age. Although they showed significant effect of age on frequency tuning of oVEMP, they did so using a small sample size which would give only a few data points under each of the age groups. Additionally, the study also included wider and unevenly distributed age ranges which may result in erroneous conclusions. This substantiates the need for more research for evaluating the effect of advancing age on frequency tuning of oVEMP by including larger participant number, spacing the groups evenly and using smaller age spans for each group. Thus, the present study was aimed to investigate the effect of advancing age on frequency tuning of oVEMP in healthy individuals.

Method

Participants

Fifty healthy volunteers (25 males & 25 females) in the age range of 20-80 years were selected as participants. They were equally divided into 5 age groups [Group I: 20-30 years (mean age = 25.6, standard deviation = 2.9), Group II: 30-40 years (mean age = 34.7, standard deviation = 2.1), Group III: 40-50 years (mean age = 45.6, standard deviation = 3.5) , Group IV:50-60 years (mean age = 56.7, standard deviation = 3.1), Group V: >60 years (mean age = 70.5, standard deviation = 6.2)], each covering a span of 10 years except for the fifth group which covered a span of 20 years. Care was taken to include equal number of participants above and below the mid-point of each of these groups in order to have even spread of age within each group. Also, there were equal number of male and female participants within each age group in order to overcome the gender effect, if any. Prior to the testing, each participant was explained regarding the experiment and subsequently a written consent for participation was obtained. None of the participants were paid for their participation in this study.

Pure-tone audiometry, speech audiometry, immittance evaluation, and auditory brainstem response testing were done to rule out conductive pathology and any retro-cochlear pathology. All the participants showed a fair-to-good agreement between pure-tone average and speech recognition thresholds. Those showing only 'A' type tympanograms were included in the study. The subjects with complaint or history of vertigo, dizziness, light headedness, and/or imbalance were excluded from the study. The study also excluded those with high blood pressure and diabetes, as they were shown to have deleterious impact on the oVEMP responses (Ghosh & Sinha, 2012). All the participants had UCL of 100 dB HL or more.The vestibular evaluation included subjective vestibular assessments like Romberg test, Fukuda stepping test, Tandem gate test and Past pointing test (Finger-to-nose test) in order to screen out any vestibular pathology.

Procedure

oVEMPs were recorded from both ears of all the participants. For recording oVEMP, participants were instructed to sit in an upright position. A commercially available skin preparing gel was used to scrub the electrode sites and gold plated electrodes (wire length = 1.5 m) were placed with the help of a commercially available conduction paste and surgical plaster. The non-inverting electrode was placed 1 cm below the centre of the lower eye lid, the inverting electrode 2 cm below the non-inverting electrode and ground electrode on the lower forehead. This electrode placement is similar to those used previously (Rosengren et al, 2005; Singh & Barman, 2013, 2014, 2015). The absolute and inter-electrode impedance were maintained below 5 k&! and 2 k&! respectively. The contralateral stimulation was achieved through the use of default Etymotic ER-3A insert earphones of the Biologic Navigator Pro evoked potential system. During the recording, the participants were required to fix their gaze at a point kept constant for every participant at an angle of 30°. Further, the participants were also instructed to avoid any movements of head, neck or jaw to avoid adulteration of responses through muscle artifacts.

The ocular VEMPs were recorded using monaural mode of stimulation using alternating polarity short tone-bursts of 250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz and 2000 Hz. The intensity was kept constant at 125 dB SPL. The stimuli were ramped using Blackman gating with rise/fall and plateau times of 2 ms and 1 ms respectively and were presented at a repetition rate of 5.1 Hz (Singh et al., 2013). The responses were band-pass filtered between 1 Hz and 1000 Hz (Wang, Jaw, & Young, 2013) and amplified by a factor of 30000. An epoch time of 70 ms, inclusive of pre-stimulus recording of 10 ms, was used and 200 sweeps were averaged per recording. Monoaural stimulation with contralateral eye recordings was employed for recording of oVEMPs. The number of subjects tested with ascending and descending order of frequencies was counterbalanced in order to avoid order effect. Further equal number of participants were tested with right ear first and left ear first in order to overcome the order effects for the ears, if any.

Two independent experienced audiologists analyzed the responses. The oVEMPs were analyzed along the major parameters of peak-to-peak amplitude and response rate. A 'present oVEMP' was defined as an initial negative peak occurring at about 10 ms (8-12 ms) with a subsequent positive peak occurring at about 15 ms (14-18 ms). Conversely, oVEMPs were deemed absent when the biphasic waveform was not observed. The frequency with the largest peak-to-peak amplitude of oVEMP was termnd as "best frequency". This way of deciding frequency tuning (best frequency) is similar to all the previous studies (Piker et al., 2013; Singh & Barman, 2013).

Statistical analysis

A free public domain software namely SSP (Smith's Statistical Package) was used for Equality of tests of proportion. The descriptive statistical analyses, subsequent statistical procedures and plotting of graphs was done using a commercially available statistical tool- Statistical Package for Social Sciences (SPSS) version 17.0. Shapiro Wilk's test of normality was used to check whether the data is normally distributed.

Due to non-normal distribution of the data in several age groups and at several frequencies, appropriate non-parametric statistical procedures were subsequently used.

Results

The present study was designed with an aim to investigate the effect of age on frequency tuning properties of oVEMP. In order to achieve the aim, oVEMP responses were acquired from both ears of 50 individuals, with 10 individuals in each of the 5 age groups (Group I: 20- 30 years, Group II: 30-40 years, Group III: 40-50 years, Group IV: 50 to 60 years & Group V: > 60 years). Figure 4.1 shows the representative waveforms of oVEMP across the octave and mid-octave frequencies from 250 Hz to 2000 Hz from one individual in each age group.

The acquired responses were analysed for response rates, peak-to-peak amplitude and frequency tuning. In order to test whether the data is normally distributed, Shapiro-Wilk's test of normality was used and the results showed non-normal distribution for all the frequencies in all the age groups. Therefore non-parametric statistical procedures were used for further statistical analyses. The results of the study are discussed below under the above mentioned parameters.

The ear differences in response rate of oVEMP

Response rate was defined as the percentage of ears in which the responses were present at a particular frequency.. The response rates were compared between the ears in the same group (within group) using McNemar test. The results revealed no significant difference in the response rates between the ears at any frequency in any of the age groups.

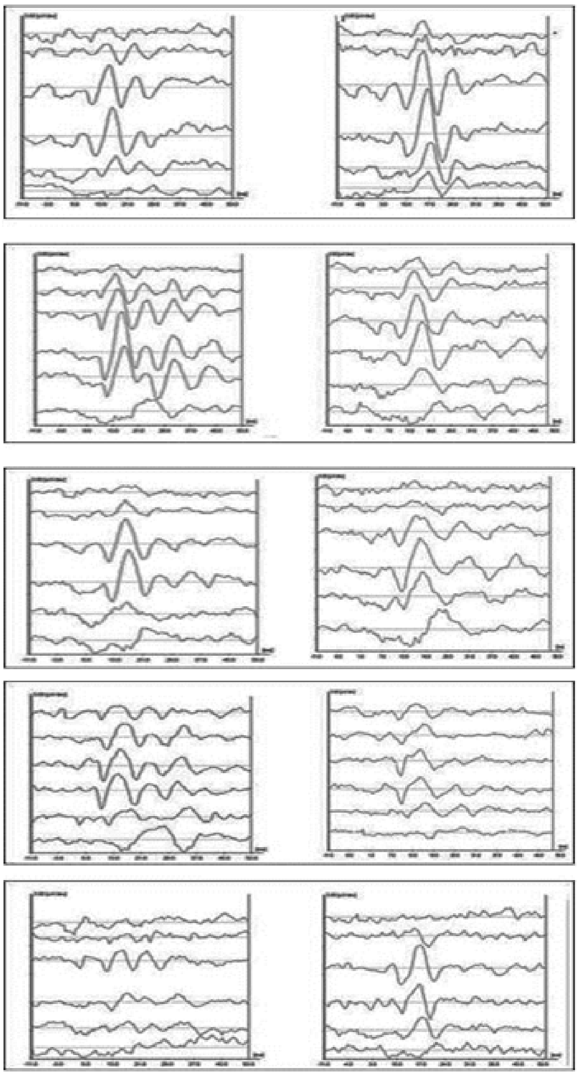

Figure 1: *The representative oVEMPwaveforms of obtained at octave and mid-octave frequencies from both ears of one individual in each of the above mentioned age groups with Group I (20-30 years) displayed in the top-most panel and Group V (> 60 years) displayed in the lowermost panel.*

At certain frequencies, the dichotomy of data was not found due to presence of responses in 100% of individuals at least in one ear. Since dichotomous data is a major assumption of McNemar test (McNemar, 1947), the between ears comparison at these frequencies could not be performed.

The effect of frequency of tone-burst on response rates of oVEMP

The above statistical analyses (McNemar test) for comparison of repetition rates between the ears revealed no significant ear effect on response rate of oVEMP at any frequency in any age group. Therefore the data of response rates for the ears were combined in each group and are shown in Figure 2.

In order to investigate the effect of frequency on response rate of oVEMP, within group between frequencies comparison of response rates was done using McNemar test. The results revealed that response rates at 1500 Hz and 2000 Hz were significantly lower than those at 500 Hz and 750 Hz in all the age groups, with few exceptions. The results of McNemar test for within group between frequencies comparisons are shown in Figure 2.

Effect of age on response rate of oVEMP

Equality of test for proportions was used for between groups comparison of response rates of oVEMP. The results revealed that the age groups upto 50 years demonstrated significantly higher response rates across the frequencies than the age groups above 50 years of age ($p < 0.05$). Further, there was no significant difference in response rates between the age groups of 50-60 years and > 60 years ($p>0.05$).

Also, there was no significant difference in response rates between the groups till 50 years (20-30 years, 30-40 years & 40-50 years) ($p>0.05$). Figure 3 displays the response rates across different age groups for different stimulus frequencies. Figure 4show the outcomes of the Equality of test for proportions for proportions between groups comparison of response rates at 250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz and 2000 Hz.

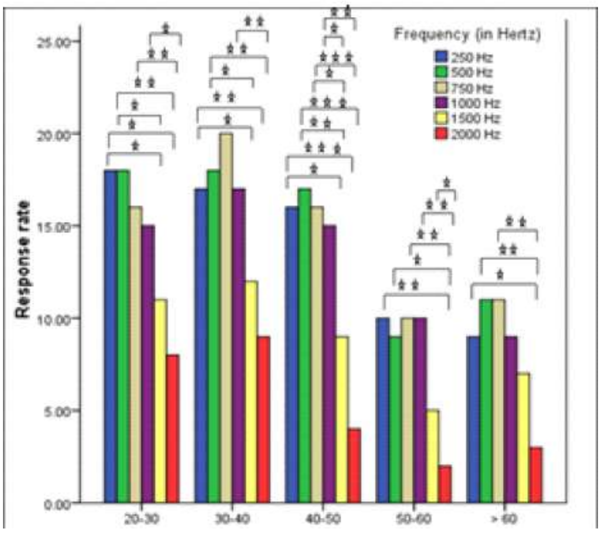

Figure 2:*Response rates of oVEMP across frequencies in each of the age groups*

Note: *'*'- p < 0.05; '**'- p < 0.01 & '***'-p< 0.001.*

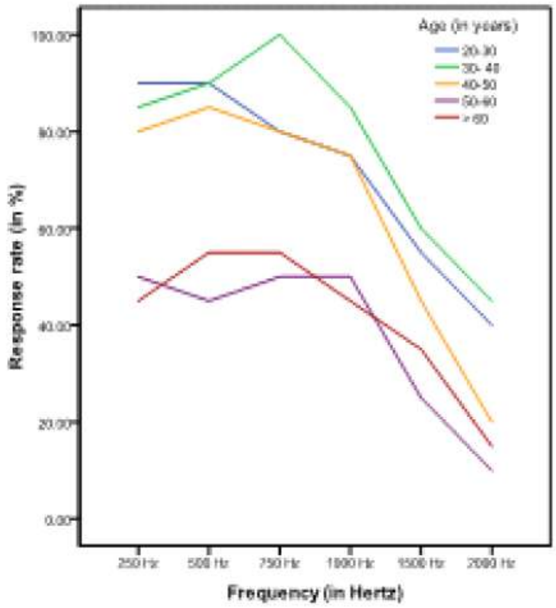

Figure 3: Response rates of oVEMP across frequencies in different age groups.

The ear differences in peak-to-peak amplitude of oVEMP

The peak-to-peak amplitude of oVEMP of the right and left ear were obtained from all the participants and the data was subjected to descriptive statistics. Both ears appeared to portray similar values of peak-to-peak amplitude. In order to examine the The results revealed no significant difference between the two ears at any frequency in any age group. Therefore the data for the ears wee combined for further statistical evaluation. A Wilcoxon signed rank test was done to investigate the ear differences in the peak-to-peak amplitude of oVEMP.

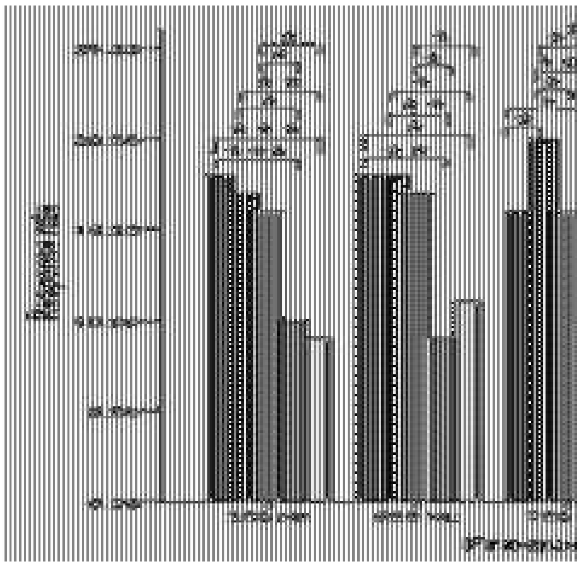

Figure 4:Outcome of Equality of test for proportions for between groups comparison of response rates at all the frequency

The effect of frequency of tone-burst on peak-to-peak amplitude of oVEMP

The above statistical analyses revealed no significant ear effect on peak-to-peak amplitude of oVEMP at any frequency in any age group. Therefore the data of peak-to-peak amplitude for the ears were combined in each group.

A Friedman's test was done to compare the peak-to-peak amplitude among frequencies. The results revealed significant differences among frequencies [÷^{2}(3) = 0.00, p< 0.05]. The frequency of 250 Hz and 2000 Hz were not used due to low response rates (N d" 5) at these frequencies. Since there was a significant difference among the frequencies, further pair-wise analysis using Wilcoxon signed rank test was done.

The results revealed that the amplitude at 1500 Hz was significantly smaller than the amplitude at 750 Hz in all the age groups, whereas it was significantly smaller than those at 1000 Hz in the age groups upto 40 years. The results of Wilcoxon signed rank test for within group between frequencies comparison are shown in the Figure 5 for the age groups I, II, III, IV and V.

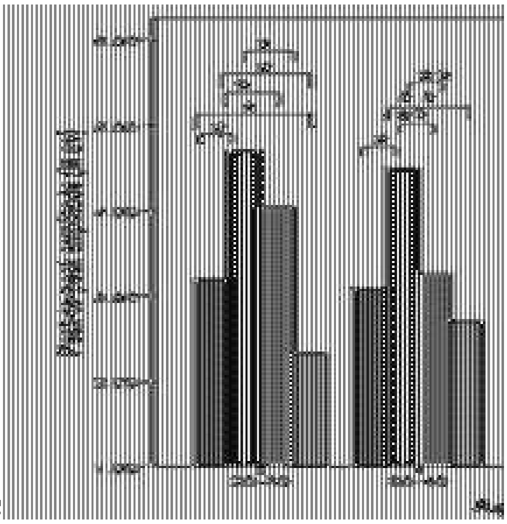

Fig for within group between frequencies comparison of peak-to-peak amplitude in all the age groups.

Note: '*'- p<0.05, '**'- p<0.01 & '***'-p<0.001.

The effect of age on peak-to-peak amplitude of oVEMP

The peak-to-peak amplitude was observed to be largest for younger age groups and reduced subsequently with advancing age. In order to examine the statistical significance of these observations, a Kruskal Wallis test was done for comparison of peak-to-peak amplitude among age groups. The results revealed no significant difference in the peak-to-peak amplitude among the age groups at 250 Hz [÷^{2}(4) = 4.42, p> 0.05], 500 Hz [÷^{2}(4) = 3.00, p> 0.05], 750 Hz [÷^{2}(4) = 2.32, p> 0.05], 1000 Hz [÷^{2}(4) = 8.43, p> 0.05], 1500 Hz [÷^{2}(4) = 1.12, p> 0.05] and 2000 Hz [÷^{2}(4) = 1.50, p> 0.05]. Since there was no significant difference in peak-to-peak amplitude of oVEMP among the age groups at any frequency, further pair-wise analysis (Mann- Whitney U test) was not done.

The effect of age on the frequency tuning of oVEMP

The frequency resulting in the largest response amplitude was termed as "best frequency" or frequency tuning. Figure 6 provides the percentage of ears with frequency tuning at various frequencies in each age group. The best frequency was observed mainly at 500 Hz, 750 Hz and 1000 Hz, with occasional prevalence of frequency tuning at frequencies above 1000 Hz. There was no individual who demonstrated frequency tuning at 250 Hz. The largest percentage of ears in each group showed frequency tuning at 750 Hz, except the age group of > 60 years. In this age group, the largest percentage of ears demonstrated frequency tuning at 1000 Hz or higher frequencies. Figure 6displays the percentage of ears with frequency tuning at each frequency. It should be noted that the number of ears showing presence of response was different among age groups. Since frequency tuning was obtained only from those ears in whom responses were present, the percentages for some of the groups are similar despite the differences in the number of ears with frequency tuning at a particular frequency.

As can be seen from Table 1, the percentage of individuals with frequency tuning at different frequencies was different. In order to examine if these differences were statistically significant, Equality of test for proportions was used for between groups comparison of these proportions at each frequency. The proportion of ears with frequency tuning at 1000 Hz or higher frequencies was significantly higher in the age group > 60 years than all the other age groups (p< 0.05). There was no significant difference in proportion of ears with frequency tuning at any other frequency between the groups. The results of Equality of test for proportions are shown in Figure 7 for between groups comparison of proportion of ears with frequency tuning at 500 Hz, 750 Hz and 1000 Hz respectively.

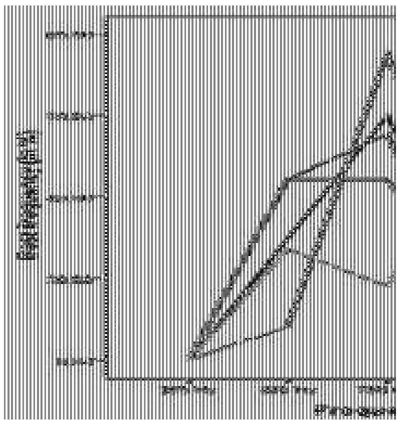

Figure 6: Proportion of ears in each age group showing frequency tuning (best frequency) at various frequencies.

Figure 7:The outcome of Equality of test for

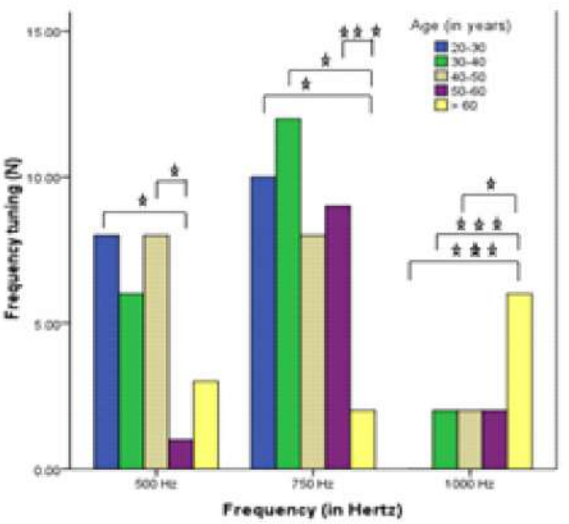

*Figure 7:*The outcome of Equality of test for proportions for between groups comparison of proportion of ears with frequency tuning at 500 Hz

Overall, the results indicate that there is a reduction in the response rate at all the frequencies in the age groups above 50 years. There was also a trend towards reduction in the peak-to-peak amplitude of oVEMP with age, however it was statistically not significant. Frequency tuning was found at 500 Hz or 750 Hz in majority of individuals below 60 years of age and was shifted to 1000 Hz or higher frequencies in majority of individuals above 60 years of age.

Discussion

The purpose of this study was to investigate the effect of advancing age on frequency tuning of oVEMP in healthy individuals. To fulfil the purpose of the present study, the contralateral oVEMPs were recorded from 50 individuals with normal audio-vestibular system. Response rates, peak-to-peak amplitude and the frequency tuning of oVEMPs were considered as the major parameters across which the ear differences, effect of frequency and aging effect of were assessed.

Ear differences in response rate and peak-to-peak amplitude of oVEMP

The results of within group between ears comparison of response rate and peak-to-peak amplitude of oVEMP revealed no significant ear effect on both these parameters of oVEMP at any frequency in any age group. These findings could be attributed to the relative symmetry between the ears which has been demonstrated by the findings of d” 20 % asymmetry ratio in healthy individuals by a number of studies (Piker et al., 2011; Singh & Barman, 2014, 2015).

Effect of frequency of tone-burst on response rate of oVEMP

The response rates at each of the frequencies were compared against those at other frequencies in each age group. The results revealed that response rates at 1500 Hz and 2000 Hz were significantly lower than those at 500 Hz and 750 Hz in most of the age groups. This is in agreement with the previous studies which also reported reduction in response prevalence for higher frequencies (Murnane, Akin, Kelly, & Byrd 2011; Sandhu et al., 2012). Higher response rates for low to mid frequencies might be attributed to low frequency resonance of the otolith organs (Todd et al., 2007). Additionally, some amount of contribution to this could also be made by the middle ear resonance which is likely to enhance the energy between 600 Hz to 1340 Hz (Colletti, 1977; Valvik, Johnsen & Laukli, 1994). Since the higher energy is associated with higher amplitude of oVEMP (Murnane et al., 2011), the higher amplitude could make some of the low amplitude responses that are hidden within the EMG noise more visible and thereby result in higher response rate.

Effect of age on response rate of oVEMP

When the effect of age on response rate of oVEMP was analysed, the results revealed that the age groups upto 50 years of age had significantly higher response rates across the frequencies than the age groups above 50 years of age. Further, there was no difference in response rates between the age groups of 50-60 years and > 60 years. Also there was no significant difference in response rates between the age groups till 50 years of age (20-30 years, 30-40 years, & 40-50 years). These results are in agreement with those of previous studies (Piker et al., 2013; Asal, 2014). Asal (2014) reported that oVEMPs were present contralaterally in 88% of the healthy participants (44 of 50 ears) and the percentage of individuals with presence of oVEMP decreased with age to 60% in the oldest age group (>55 years).

Table 1:*Percentage of ears with frequency tuning at a particular frequency in each age group*

Age (in years)	N	500 Hz		750 Hz		= 1000 Hz	
		N_1	N^1%	N,	N^1%	N'	N^1%
20-30	18	8	44.44 %	10	55.55. %	0	**0%**
30-40	20	6	30%	12	60%	2	**10%**
40-50	18	8	44.44 %	8	44.44 %	2	**11.11 %**
50-60	12	1	8.33 %	9	75%	2	**16.66 %**
>60	11	3	27.27 %	2	18.18 %	6	**54.54 %**

Note: 'N'- number of ears with presence of oVEMPatleast at one frequency; 'N[1]' – number of ears with frequency tuning at a particular frequency; 'N[1]%' – proportion (percentage) of ears with frequency tuning at a particular frequency out of the 'N'.

The reduction in the response rate with advancing age could be attributed to the age related degenerative changes which have been identified from the end organs of the vestibular system to its central nuclei (Bergstrom, 1973). Previous studies have shown steady decline in the vestibular hair cell counts and densities with advancing age (Merchant et al., 2000). Similar data was also presented for Scarpa's ganglion neuronal counts (Merchant et al., 2000) and vestibular neurons in the vestibular brainstem (Tang et al., 2001-2002). Thus, the well-documented neuro-anatomical and physiological changes due to aging in the peripheral and central vestibular system may explain the commonly reported decrease in the response rate of responses acquired from the vestibular system (Rosenhall, 1973), oVEMP in the case of the present study.

Effect of frequency of peak-to-peak amplitude of oVEMP

In each of the age groups, the peak-to-peak amplitude were compared between the frequencies. The comparison of peak-to-peak amplitude between the frequencies revealed maximum amplitude at 500 Hz and 750 Hz and a subsequent reduction in the amplitudes on either side of these frequencies thereafter. These findings conform with the findings of the previous studies (Todd et al, 2007; Park, Lee, Shin, Lee, & Park, 2010; Sandhu et al, 2012), who also reported reduction in the peak-to-peak amplitude of oVEMP with increasing frequencies above 1000 Hz and decreasing below 500 Hz. The findings of the present study are however in disagreement with some of the other studies reported previously in this context (Taylor et al., 2011; Zhang, Govender, & Colebatch, 2012), all of which reported largest amplitude at 1000 Hz for oVEMP in majority of their subjects.

The differences from the findings of Lewis, Mustain, Xu, Eby, & Zhou (2010) might be attributed to the use of 10 ms plateau time and 1 ms rise/fall time by these authors as against the use of 0 ms plateau time and 2 ms rise/fall time in the present study. Longer plateau time in Lewis et al (2010) would have caused greater difference in the energy between 500 Hz and 1000 Hz in their study than the present study, thereby showing larger amplitudes at 1000 Hz than 500 Hz in their study as opposed to the other way round in the present study. In addition to the differences in stimulus parameters observed between the two set of studies, the differences might also have been brought about by the large difference in sample size [12 subjects in Lewis et al (2010) as against 50 subjects in the present study].

The differences from Taylor et al (2011) might be attributed to the use of a different unit of intensity level of the stimulus [dB nHL in the study by Taylor et al (2011) as opposed to dB peSPL in the present study]. The dB nHL values across different frequencies considers the middle ear properties in the calibration which results in an increased sound pressure level at mid frequencies. This does not happen when the unit is dB peSPL. These differen

ces would result in variation in the total energy reaching the inner ear for dB nHL than when dB peSPL is used. Energy reaching the inner ear is an important variable which affects the utricular response (Murnane, et al, 2011) and therefore Taylor et al (2011) obtained largest amplitude at 1000 Hz.

The differences from the findings of Zhang, Govender, & Colebatch, (2012) could be attributed to the differences in the mode of stimulation. They studied the effect of frequencies on the peak-to-peak amplitude through bone-conduction mode of stimulation as against the use of air-conduction mode in the present study. For a bone-conduction mode, the stimulus bypasses the external ear and middle ear and directly stimulates the inner ear (Bekesy, 1954), whereas in an air-conduction mode the stimulus travels through the external ear via the middle ear to the inner ear (Zwislocki, 1975). The resonance characteristics, of especially the middle ear, are concentrated in the low to mid frequencies which might also play a role in enhancing the energy at these frequencies and thereby the amplitude when using air-conduction mode of stimulus presentation.

The findings of maximum amplitude at 500 Hz or 750 Hz have been explained previously on the basis of electrical resonance of the otolothicsteriocilia (Welgampola & Colebatch, 2005) and mechanical resonance caused by mass-spring properties within the otolith organs (Todd et al 2000, 2007). The latter explanation has received more support from the studies on the pathologies which cause changes in the finding of largest amplitude from 500 Hz in normals to 1000 Hz in these pathologies (Sandhu et al., 2012). The resonance frequency for human otolith organs has been reported to be in the vicinity of 500 Hz (Goldberg & Fernandes, 1975).Therefore the oVEMPs show the finding of maximum amplitude at 500 Hz with slight variations in some of the individuals probably causing it to be present at 750 Hz in these healthy individuals.

Effect of age on peak-to-peak amplitude of oVEMP

The results of the present study revealed no significant effect of age on peak-to-peak amplitude of oVEMP, although the age groups above 50 years demonstrated a trend towards lower amplitude than those below 50 years. The finding of no significant difference in peak-to-peak amplitude of oVEMP between the age groups is in disagreement with most of the previous studies who showed significant reduction in amplitude and signiûcant increase in threshold due to aging (Nguyen et al., 2010; Tseng, Chou, & Young, 2012 2010; Piker et al., 2013). The finding of no significant effect of age on the peak-to-peak amplitude of oVEMP could be attributed to high variability which is proved by the presence of high values of standard deviation, which was more than 50% of the mean (occasionally almost equal to the mean) and also high values of variance in the obtained data across the age groups. A larger data pool might have been effective in reducing the variance in peak-to-peak amplitude in each of the age groups which might have yielded a clearer picture of the age effects on this parameter. This might be looked at in the future studies.

Although the present study did not reveal any significant difference in the peak-to-peak amplitude between the age groups at any of the frequencies, there was a trend towards higher amplitude in the younger age groups (d" 50 years) than the older (> 50 years). This could be attributed to the changes in the labyrinthine neural epithelia with advancing age (Rosenhall 1973; Richter 1980) and also age related degenerative changes which has been identified from the end organs of the vestibular system to its central nuclei (Bergstrom, 1973). The studies have shown a steady decline in the vestibular hair cell counts and densities with advancing age (Merchant et al., 2000). Similar data was also presented for Scarpa's ganglion neuronal counts (Merchant et al., 2000) and vestibular neurons in the vestibular brainstem (Tang et al., 2001-2002). However, these changes probably were to a lesser extent which prevented the differences from being significant.

Frequency tuning of oVEMP

The results of the present study showed that frequency tuning was obtained at 500 Hz or 750 Hz in majority of the individuals in all the age groups until 60 years of age. This is in consonance with those reported previously in this regard (Todd et al., 2007; Sandhu et al.,2012; Singh & Barman, 2013, 2014). However, these findings are in dissonance with the findings of some of the other studies (Lewis et al., 2010; Taylor et al., 2011; Zhang et al., 2012). The differences from these studies could be attributed to the use of longer plateau times and smaller sample size in the study by Lewis et al (2010), use of dB nHL rather than dB peSPL for stimulus calibration in the study by Taylor et al (2011) and use of bone-conduction mode of stimulation rather than air-conduction mode in the study by Zhang et al (2012), as explained above.

The finding of frequency tuning at 500 Hz or 750 Hz in majority of individuals could be explained on the basis of the second order mechanical system that consists of the components of mass and stiffness which was proposed to explain a similar finding for cVEMP by Todd et al (2000) and later for oVEMP in 2009 by the same authors. The utricle could be considered a second order mechanical system with the mass being contributed by the utricular macula and stiffness by the otolithic membrane of the utricle. As in any mechanical system, the interaction between the mass and stiffness components tends to nullify each other's impact which causes the structure to resonate at this frequency (Vanhuyse, Creten & Van Camp, 1975; Popelka & Winter, 2013). This characteristic frequency for the otolith organs has been reported to lie in the low-to-mid frequency region (Goldberg & Fernandes, 1975), in the vicinity of 500 Hz. This could therefore be contributing to the finding of peak in the frequency tuning curve of oVEMP at 500 Hz or 750 Hz in these individuals. Further, there could also be a contribution from the resonance properties of the middle ear which could be altering the energy reaching the utricle in the range of frequencies between 600 Hz and 1340 Hz (Colletti, 1977; Valvik et al., 1994), when using air conduction mode of stimulation. Therefore probably a combination of resonance properties of the middle ear and the otolith organs might be attributed to cause the peak of the frequency tuning curve at 500 Hz or 750 Hz in the majority of the individuals in the present study.

Effect of age on frequency tuning of oVEMP

The effect of aging on frequency tuning of oVEMP was also explored in the present study. Out of all the frequencies used in the present study, 500 Hz, 750 Hz and 1000 Hz had larger response amplitude than the rest of the frequencies in each individual participant. In the age groups upto 60 years, the frequency tuning was found to be at 500 Hz or 750 Hz in 91.1% of the individuals and at 1000 Hz in 8.82 % of individuals. The frequency tuning at 1000 Hz was in a significantly higher proportion of individuals in the age group of > 60 years (54.5%) than the age groups d" 60 years (8.82%). The only previous study, to the best of our knowledge, compared frequency tuning between only three age groups [young adults (18-39 years), middle-aged adults (40-59 years), & older adults (e" 60 years)]. Although they did report of higher incidence of frequency tuning at 1000 Hz in the older adults, a direct comparison with the present study would be difficult owing to the differences in the way the groups were formed in the present study and that by Piker et al (2013). If the data of the present study is reallocated in the way it was considered by Piker et al (2013), the results of the present are in consonance with their's study. They found frequency tuning in the older age group to be around 62% at 1000 Hz which was nearly similar to the 54.5% in the present study. The best frequencies according to Piker et al (2013) for the middle age group were evenly split between 500 Hz (in 31%), 750 Hz (in 38%), and 1000 Hz (in 31%). The findings in the present study are also in agreement with their study for this age range, with slight differences in the proportion of ears with frequency tuning at 750 Hz and 1000 Hz. In the current study, the best frequencies for the middle age group was 500 Hz (in 30%), 750 Hz (in 56.6%), and 1000 Hz (in 13.3%). These slight differences might be attributed to the differences in the sample size between the studies [13 in Piker et al (2013) as against 40 in the middle-aged group of the present study]. For the young adults, the majority of participants (nearly 92%) showed the best amplitudes at either 500 Hz or 750 Hz with only one participant showing the best frequency at 1000 Hz in the study by Piker et al (2013). In the present study also, the frequency tuning for the young adults was either at 500 Hz or 750 Hz in majority of individuals (nearly 96%) with only two participants showing the best frequency at 1000 Hz. Thus in general, there seems to be agreement in the findings of age and frequency interaction between present study and those of Piker et al (2013).

The frequency tuning of the vestibular end organs has been attributed to the inertial and elastic properties of these end organs, the mechanical resonance of thestereocilia, and/or the electrical tuning of the hair cells (Fernandez & Goldberg, 1976; Fettiplace & Fuchs, 1999). However as stated earlier, the utility of the second order mechanical model in explaining frequency tuning of oVEMP has received wider acceptance (Todd et al., 20002009). The resonant frequency is a function of the mass and stiffness characteristics of a system (Vanhuyse et al., 1975; Popelka& Hunter, 2013), which arise out of the utricular macula and the utricular membrane, respectively in case of utricle. The resonant frequency is directly proportional to the square root of stiffness and inversely proportional to the square root of mass (Vanhuyse et al., 1975; Popelka& Hunter, 2013)This means that smaller the mass of the macula and/or higher the stiffness of the utricular membrane, higher will be the resonance frequency. This perspective, along with the understanding of age related changes in the utricle, could be helpful in understanding why the frequency tuning shifts to higher frequencies in older adults. Previous studies have shown that the mass of the utricular macula reduces as a result of degeneration associated with aging (Walther &Westhofen, 2007). Since the resonance frequency is inversely proportional to the square root of mass (Vanhuyse et al., 1975; Popelka& Hunter, 2013), the reduction in the mass of the utricular macula would cause an increase in the resonance frequency of the utricle, which is largely accepted as the generator end organ for oVEMP. This would therefore enhance the amplitude in the higher frequencies in older adults and reduce the amplitude in the lower frequencies where the resonance was occurring in the younger age groups. Thus, the changes in aging-associated anatomy in the utricular macula could be attributed to the shift the frequency tuning from 500 Hz or 750 Hz in individuals below 60 years of age to e" 1000 Hz in individuals > 60 years of age.

Conclusion

The response rates and peak-to-peak amplitude were independent of the ear being stimulated. Stimulus frequency of tone-burst had an effect on the response rates and peak-to-peak amplitude of oVEMP, with highest response rates and largest amplitudes coinciding at 500 Hz or 750 Hz. There was a significant decline in the response rate across the frequencies with advance of age beyond 50 years. The peak-to-peak amplitude of oVEMP showed a trend towards decrement with increase in age beyond 50 years, although it was statistically not significant. Aging had a significant effect on frequency tuning of oVEMP, with frequency tuning shifting to 1000 Hz or higher frequencies in majority of individuals over 60 years of age as opposed to 500 Hz or 750 Hz below it.

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