CHAPTER - 5

Discussion

The present study aimed at characterizing the frequency tuning of ocular vestibular evoked myogenic potentials (oVEMP) in healthy individuals and those with Meniere’s disease (MD), benign paroxysmal positional vertigo (BPPV) and auditory neuropathy spectrum disorders (ANSD). Additionally the present study also aimed to investigate the existence of a difference, if any, in frequency tuning properties between the groups of the present study. For the fulfilment of these aims, the oVEMP responses were recorded from a total of 108 individuals with Meniere’s disease, BPPV and ANSD, with 36 participants in each of these groups. The oVEMP responses were also obtained from 113 healthy individuals with normal auditory and vestibular functioning. The findings of the study are discussed below for different parameters of oVEMP under the respective groups.

5.1 oVEMP in healthy individuals

Ocular VEMPs were recorded across the frequencies from both ears of 113 healthy individuals. Separate ear-wise and frequency-wise analysis was done for response rates, peak-to-peak amplitude and oVEMP threshold. Frequency tuning was also analyzed separately for the right and left ears. Further, test-retest reliability was examined on randomly selected 10% of the individuals from this group. The results obtained are discussed below:

5.1.1 Response rate of oVEMP in healthy individuals.

The largest response rates were obtained at 750 Hz and 1000 Hz in both the ears; however the response rate at these frequencies did not differ significantly from that
observed at 500 Hz. The response rates decreased significantly for frequencies above and below these three frequencies. Thus, the largest number of ears of healthy individuals produced oVEMP in the low-to-mid frequency region. These findings are in consonance with those reported previously for healthy individuals (Piker 2012; Piker et al., 2013). Both these studies reported almost 100% response rates at 500 Hz, 750 Hz and 1000 Hz. However, Sandhu et al (2012) reported 100% response rate of oVEMP only at 500 Hz, with 750 Hz and 1000 Hz showing response rates of 88% and 69% respectively. The differences in the findings of response rate between the present study and that of Sandhu et al (2012) could be attributed to the differences in the stimulus presentation level. Sandhu et al (2012) used a stimulus intensity of 120 dB peSPL, which is relatively lesser than the levels used in the present study and those in Piker (2012) and Piker et al (2013) who used levels of 125 dB peSPL or higher to elicit oVEMP. Murnane et al (2011) reported reduction in response rate of oVEMP with decreasing stimulus levels.

The finding of larger response rate at 500 Hz, 750 Hz and 1000 Hz compared to the other frequencies in the present study might be attributed to the elastic and inertial properties of the utricle, the mechanical resonance of the hair cells’ stereocilia within the utricle and/or the electrical tuning of the utricular hair cells, which were hypothesized as the reasons behind frequency selective responsiveness of the otolith organs including utricle (Fernandez & Goldberg, 1976; Young, Fernandez, & Goldberg, 1977; Holton & Hudspeth, 1983; Fettiplace & Fuchs, 1999; Welgampola & Colebatch, 2001). All these three properties probably work in unison to contribute to the best responsivity of the utricle in the above mentioned frequency range. A similar reason was attributed to the frequency selective responsiveness of the otolith organs by previous studies who had also found similar results to that of the present study (Piker, 2012; Piker et al., 2013).
The results of the present study further revealed no significant difference in the response rate of oVEMP between the two ears of healthy individuals at any frequency. The ears though could not be compared at 750 Hz and 1000 Hz because the left ears demonstrated response prevalence in 100% of the individuals which resulted in the non-dichotomous responses, dichotomy of responses being a pre-requisite for McNemar test (McNemar, 1947; Eliasziw & Donner, 1991; Fagerland et al., 2013). The previous studies evaluating the effect of stimulus frequency on oVEMP response rates did not analyze the response rates by comparing between the ears across frequencies (Piker, 2012; Sandhu et al., 2012; Piker et al., 2013), possibly because they assumed relative symmetry between the ears in healthy individuals. In fact, this might be the reason behind no significant difference in the response rate at any of the frequencies between the two ears of the healthy individuals group could be observed.

5.1.2 Peak-to-peak amplitude of oVEMP in healthy individuals

The peak-to-peak amplitude of oVEMP was compared between the frequencies within each ear and the results revealed the largest peak-to-peak amplitude at 500 Hz and 750 Hz in both the ears of healthy individuals, with no significant difference in amplitude between these two frequencies. This is in accordance with those reported previously in healthy individuals (Chihara et al., 2009; Park et al., 2010; Zhang Govender, & Colebatch, 2011; Piker 2012; Sandhu et al., 2012; Piker et al., 2013). However, there was a slight disagreement of these findings with some of other studies who reported largest amplitude of oVEMP at only one of these frequencies (Murnane et al., 2011; Singh & Barman, 2013, 2014) or at an entirely different frequency (Lewis et al., 2010; Taylor et al., 2012). Todd et al (2009b) reported largest oVEMP amplitude in the frequency range of 400-800 Hz, Lewis et al (2010) and Taylor et al (2012) observed this at 1000 Hz in more than 50% of
the healthy individuals whereas Murnane et al (2011) and Singh and Barman (2013, 2014) found largest peak-to-peak amplitude at 500 Hz.

The disparity in findings of the present study from those of Todd et al (2009b), who reported largest amplitude of air-conduction tone-bursts evoked oVEMP between 400 Hz and 800 Hz, might be credited to the differences in the frequencies used for obtaining oVEMP responses. They did not specifically record the responses at 500 Hz. Nonetheless, the finding of having the largest peak-to-peak amplitude at 500 Hz or 750 Hz in the current study is well within the range of frequencies reported by Todd et al (2009b).

The differences in the findings between the present study and the study by Murnane et al (2011), who reported 500 Hz as the frequency that produced largest peak-to-peak amplitude, could be accounted by their non-use of 750 Hz as a stimulus frequency. Nonetheless, the studies that have used 750 Hz as a stimulus in addition to the other frequencies (Piker, 2012; Piker et al., 2013) have shown an aggregation of largest amplitude at 500 Hz, 750 Hz and 1000 Hz, with proportions varying with age. Therefore, there could a possibility that the use of 750 Hz also in the study by Murnane et al (2011) might have yielded similar results to the findings of the present study.

The differences in the findings of the present study from Singh and Barman (2013 & 2014) could be attributed to the differences in the age range of the participants used in these studies. In the present study, the participants’ age ranged from 15 to 50 years (mean age = 32.4 years). In comparison, the age of the participants used in Singh and Barman (2013 & 2014) ranged from 18-30 years (mean age of approximately 25 years in both the studies). Piker et al (2013) observed the presence of largest peak-to-peak amplitude at 500 Hz in higher proportion of individuals in the young adults group (20-39 years) compared to the middle aged adults group (40-59 years) which had almost an equal distribution of
largest peak-to-peak amplitude at 500 Hz, 750 Hz and 1000 Hz. Therefore the proportion of individuals with occurrence of largest amplitude at frequencies beyond 500 Hz was found to increase with advancing age. Considering that the present study had several individuals between 40 and 50 years of age, the contribution of largest amplitude to 750 Hz would have been more in the present study than the above mentioned studies by Singh and Barman (2013 & 2014).

The results of the present study are entirely different from that reported by Lewis et al (2010). They observed the largest amplitude at 1000 Hz in the majority of their healthy subjects. The differences might be attributed to the use of 10 ms plateau time and 1-ms rise/fall time by these authors as opposed to 0-ms plateau and 2-ms rise/fall time in the present study. The studies assessing the effect of rise/fall and plateau time on oVEMP amplitude have shown mixed results (Cheng, Wu, & Lee, 2012; Joshi, 2013). While Joshi (2013) demonstrated a significant effect of variation in rise/fall and plateau times on amplitude and threshold of oVEMP, no significant effect of variations in these parameters on amplitude was shown by Cheng et al (2012). However the plateau times that Cheng et al (2012) utilized were very limited (2 ms & 4 ms) and therefore may not explain the differences observed. Joshi (2013) showed that the amplitude was smaller for smaller rise/fall and plateau time combinations and increased subsequently with increasing the values of these parameters. The increased rise/fall and plateau time would correspond to a greater increase in the number of cycles at 1000 Hz compared to 500 Hz. In the present study the difference in the number of cycles between 500 Hz and 1000 Hz was only 4 cycles as opposed to 12 cycles in Lewis et al (2010). Such a large difference in the number of cycles between 500 Hz and 1000 Hz in their study might have caused larger differences in energy between the two sets of stimuli in Lewis et al (2010) than the present study. This large difference in the number of cycles (12 cycles) might have resulted in higher
amplitude corresponding to 1000 Hz than 500 Hz in the study by Lewis et al (2010). In the present study, the difference was relatively smaller (4 cycles) between the two sets of stimuli which probably did not augment 1000 Hz much. The difference in the findings between the present study and the study by Lewis et al (2010) might be further contributed by the large difference in the sample size used (12 participants in their study as against 113 subjects in the present study). The finding of largest amplitude at 1000 Hz in about 50% of the 12 subjects in Lewis et al (2010) could be put to the chance result as some of the healthy individuals in the present study also demonstrated the largest amplitude at 1000 Hz.

The distinction in the findings of the present study from those of Taylor et al (2012), who also reported the finding of the largest amplitude at 1000 Hz in the majority of healthy subjects of their study, might be attributed to the use of stimuli calibrated in different units. While Taylor et al (2012) used decibel normalized hearing level (dB nHL), the present study made use of decibel peak sound pressure level (dB peSPL). When using dB nHL across the frequencies, the middle ear resonance properties are included in the calibration resulting in increased sound pressure level at mid frequencies reaching to the inner ear, whereas this does not happen in the use of dB peSPL. This might lead to differences in the energy levels among the frequencies while using dB nHL than using dB peSPL. Since the energy level (intensity) has been reported to be an important factor affecting the amplitude of oVEMP (Murnane et al., 2011), the differences between the studies might be related to this difference in the effective stimulus intensity reaching the utricle.

The finding of largest amplitude at 500 Hz or 750 Hz in most of the healthy individuals can be explained on the basis of (1) electrical resonance of the utricular hair
cells (2) mechanical resonance of the stereocilia of the utricular hair cells and (3) mechanical resonance of the utricle. Welgampola and Colebatch (2001, 2005) posited that the reason behind higher amplitude in the low frequencies (around 500 Hz) was the electrical resonance of the hair cells and the mechanical resonance of the stereocilia of the hair cells. However, this reason failed to explain the shift in the largest amplitude from about 500 Hz in healthy subjects to 1000 Hz or higher frequencies in pathologies like Meniere’s disease (Sandhu et al., 2012) and superior semicircular canal dehiscence (Taylor et al., 2012). Therefore the theory proposed by Todd et al (2000), which attributes this to the mass-spring damping properties of the otolith organs, has received wider acceptance. The latter explanation appears more practical and has drawn support from the studies on pathologies which cause changes in the location of peak in the amplitude plots across the frequencies (Kim-Lee, Ahn, Kim, & Yoon, 2009; Sandhu et al., 2012; Taylor et al., 2012; Winters et al., 2012; Jerin et al., 2014).

The anatomical and structural reports in literature have indicated towards a lower mechanical resonance for the otolith organs (Rosenhall, 1972; Uzun-Coruhlu, Curthoys, & Jones, 2007). Todd et al (2000) suggested that the largest amplitude of VEMP at 500 Hz or the frequencies in its vicinity in the healthy human adults could be successfully modelled by a 2nd-order mechanical system that contains the elements of mass and stiffness. For the otolith organs, the inertial force could arrive from the mass of the otoconia, which boasts of density of about 2.71 g/cm$^3$ (Carlstrom, 1963). The stiffness component could be arising out of the coupling between the sensory hair cells and the overlying visco-elastic mesh-gel layer, which along with the membranous labyrinth, are relatively inelastic in nature (Rabbit, Damiano, & Grant, 2004). The mass and the stiffness components of the otolith system work against each other and vary in their properties across the frequencies. While mass tend to limit the responses to high-frequency stimuli, the stiffness component
counters the low frequencies. This causes enhancement in amplitude at a frequency where neither of the two components are dominant by virtue of cancelling each other. Todd et al (2000, 2009b) posited that this interaction between stiffness and mass components of the utricle might be occurring in the frequency range of 400 to 800 Hz, which gets reflected in the finding of the largest amplitude in this frequency range in majority of the healthy individuals. In the healthy individuals of the present study, this might have been occurring at 500 Hz in some individuals and 750 Hz in others. This was reflected in the finding of the largest amplitude at one of these frequencies in all of these individuals. The sporadic incidence of larger peak-to-peak amplitude at 1000 Hz could be due to the effects of age, as was observed by Piker et al (2013).

The between ears comparison of the peak-to-peak amplitude in the healthy individuals’ group revealed no significant difference in the peak-to-peak amplitude between the ears at any of the frequencies. Like the response rate, the previous studies did not analyze ear differences in peak-to-peak amplitude of oVEMP across frequencies in healthy individuals (Piker, 2012; Sandhu et al., 2012; Piker et al., 2013). However, Singh and Barman (2013, 2014) reported no main effect of the ear on peak-to-peak amplitude across frequencies in healthy individuals. Therefore the findings of the current study are in agreement with those by Singh and Barman (2013, 2014). This indicates towards relative symmetry in the utricle mediated vestibulo-ocular reflex in healthy individuals.

5.1.3 Threshold of oVEMP in healthy individuals.

The thresholds of oVEMP were compared among the frequencies and the results revealed significantly better (lower) thresholds at 500 Hz and 750 Hz in both the ears of healthy individuals. There was no significant difference in the oVEMP threshold between 500 Hz and 750 Hz. Therefore best oVEMP thresholds corresponded to the frequencies of
500 Hz and 750 Hz. These findings are in agreement with the findings of the previous studies (Taylor et al., 2012; Winters et al., 2012). While Taylor et al (2012) reported similar mean values of oVEMP threshold at 500 Hz and 1000 Hz (99.3 dB nHL & 98.5 dB nHL respectively, without accomplishing any statistical comparison between these threshold values), Winters et al (2012) reported lowest thresholds only at 500 Hz. However, both these studies did not specifically record oVEMP at 750 Hz.

The finding of the best oVEMP threshold at 500 Hz and 750 Hz could be attributed to better responsivity of the utricle in this frequency region as was demonstrated by the finding of higher amplitude and best response rates at these frequencies by previous studies (Todd et al., 2009b; Piker, 2012; Piker et al., 2013). This was attributed to the mechanical resonance of the stereocilia of utricular hair cells, electrical resonance of the hair cells of the utricle and/or the mechanical resonance of the utricle itself (Fernandez & Goldberg, 1976; Young et al., 1977; Holton & Hudspeth, 1983; Fettiplace & Fuchs, 1999; Todd et al., 2009a,b). All these factors in unison could have enhanced the responsivity of the utricle around these frequencies which was reflected in better oVEMP thresholds at these frequencies than the other frequencies used in the study.

The results also revealed no ear difference in the oVEMP threshold at any of the frequencies. None of the previous studies on the effect of stimulus frequency on oVEMP threshold have obtained such a comparison, possibly due to a presumption of bilateral symmetry in the response threshold in the healthy individuals. Although vestibular system is not a completely symmetrical system, as shown by presence of some value of asymmetry ratio for cVEMP (Singh, Sinha, Govindaswamy, & Apeksha, 2013) and oVEMP (Singh & Barman, 2014), the asymmetry is small enough to be disguised from statistical differences.
5.1.4 Frequency tuning of oVEMP in healthy individuals.

In the healthy individuals, frequency tuning was found to be at 500 Hz or 750 Hz in almost 99% of the ears. Several previous investigations have examined the frequency tuning properties of oVEMP in healthy individuals (Todd et al., 2009b; Park et al., 2010; Lewis et al., 2010; Murnane et al., 2011; Zhang et al., 2011; Piker 2012; Sandhu et al., 2012; Winters et al., 2012; Taylor et al., 2012; Piker et al., 2013; Singh & Barman, 2013, 2014). While the findings of the present study appear to be in agreement with those of Piker (2012) and Piker et al (2013), the observations in the remaining reports in literature seem to be in slight dissonance with the present study’s findings (Todd et al., 2009b; Park et al., 2010; Lewis et al., 2010; Murnane et al., 2011; Zhang et al., 2011; Sandhu et al., 2012; Winters et al., 2012; Taylor et al., 2012; Singh & Barman, 2013, 2014). Among these, most of the studies demonstrated frequency tuning at 500 Hz only (Park et al., 2010; Murnane et al., 2011; Zhang et al., 2011; Sandhu et al., 2012; Winters et al., 2012; Singh & Barman, 2013, 2014), whereas others agreed over frequency tuning at 1000 Hz in the majority of healthy individuals (Lewis et al., 2010; Taylor et al., 2012). Todd et al (2009b) obtained frequency tuning between 400 Hz and 800 Hz for air-conduction tone-burst evoked oVEMP. These differences could be explained on the basis of differences in the stimulus and subject related parameters used in each of these studies compared to the present study.

Todd et al (2009b) obtained oVEMP corresponding to octave frequencies from 100 Hz to 3200 Hz and reported the frequency tuning of oVEMP to air-conducted sound in the frequency range of 400-800 Hz. However, Todd et al (2009b) did not record the responses specifically at 500 Hz or 750 Hz. Nonetheless, the finding of frequency tuning in healthy individuals at 500 Hz or 750 Hz in the present study is well within the range of frequencies.
reported by them. The disparity in the findings of the present study from those of Murnane et al (2011) and Winters et al (2012), both of whom found frequency tuning at 500 Hz alone, could also be similarly explained on the basis of their non-use of 750 Hz as a stimulus frequency.

Lewis et al (2010) observed frequency tuning of oVEMP at 1000 Hz as against 500 Hz or 750 Hz in majority of the healthy individuals in the present study. The differences might be attributed to the use of 10-ms plateau time and 1-ms rise/fall time by these authors as opposed to 1-ms plateau and 2-ms rise/fall time in the present study. The amplitude of oVEMP was reported to be significantly impacted by the changes in these parameters (Joshi, 2013).

The dissimilarity also existed between the findings of the present study and those of Taylor et al (2012), who reported frequency tuning to 1000 Hz in healthy individuals. This might be credited to the utilization of dB nHL by them as against dB peSPL in the present study, as explained earlier.

The observations in the present study were also different from the twin studies by Singh and Barman (2013 & 2014), both of which reported frequency tuning at 500 Hz in most of their subjects and this could be attributed to the differences in the age range of the participants used in these studies, as explained earlier. Piker et al (2013) reported a more skewed frequency tuning towards 500 Hz in the young adults group (20-39 years) than the middle aged group (40-59 years) which had an almost equal distribution of individuals showing frequency tuning at 500 Hz, 750 Hz and 1000 Hz.

The reasons behind frequency tuning of oVEMP exclusively to low frequencies (500 Hz and 750 Hz in the present study) in healthy individuals with normal vestibular function could be explained on the basis of mass-spring model proposed by Todd et al
(2000, 2009b). They suggested that the interaction between the stiffness and the mass components of the utricle might be occurring between 400 Hz and 800 Hz, which gets reflected in the finding of the frequency tuning at 500 Hz or 750 Hz in majority of the healthy individuals. In addition to this, the role of the middle ear transfer function might be believed to override the frequency tuning of oVEMP as the middle ear resonance has been shown to occur between 600 Hz and 1340 Hz (Colletti, 1977; Shanks, 1984; Valvik et al., 1994; Carvall, 1997). However, this was not accounted for in the preset study as Piker et al (2013) had previously shown no significant relationship between the middle ear power reflectance measures and the occurrence of largest amplitude of oVEMP. The sporadic incidence of frequency tuning at 1000 Hz in healthy individuals could be attributed to the effects of age on frequency tuning of oVEMP, which was demonstrated by Piker et al (2013).

5.1.5 Test-retest reliability of oVEMP in healthy individuals.

The results of test-retest reliability of peak-to-peak amplitude, oVEMP threshold and frequency tuning revealed an excellent test-retest reliability of all these parameters, except peak-to-peak amplitude at 4000 Hz which demonstrated fair/moderate test-retest reliability. An across frequency analysis of test-retest reliability of peak-to-peak amplitude and thresholds of oVEMP has not been reported previously. The previous studies used only 500 Hz as the stimulus frequency and reported excellent test-retest reliability of the latencies and amplitude of oVEMP (Nguyen et al., 2010; Singh et al., 2011). In the present study also, the peak-to-peak amplitude at 500 Hz demonstrated excellent test-retest reliability which shows an agreement between the findings of the present study and those reported in literature previously. Both these studies (Nguyen et al., 2010; Singh et al., 2011) did not obtain threshold of oVEMP. Obtaining lower value of the Chronbach ‘$\alpha$’
coefficient at 4000 Hz could be attributed to drastically lower amplitude at this particular frequency compared to all the other frequencies. Lower amplitude leaves the response predisposed to being affected even by the slightest amount of noisiness in the response curve, and probably resulted in lower coefficient value at 4000 Hz.

Therefore to conclude, response rates, peak-to-peak amplitude as well as threshold of oVEMP varies as a function of frequency. The frequency tuning is observed at 500 Hz or 750 Hz in almost all the healthy individuals. The different parameters of oVEMP like amplitude, threshold and frequency tuning show excellent test-retest reliability.

5.2 oVEMP in individuals with Meniere’s disease

The Meniere’s disease group consisted of 36 participants with unilateral definite Meniere’s disease. In order to compare the findings of oVEMP in them, 36 age- and gender-matched healthy individuals were used as comparison group. The responses obtained were analyzed for response rate, peak-to-peak amplitude, threshold and frequency tuning. The findings under each of these parameters are discussed below:

5.2.1 Response rate of oVEMP in individuals with Meniere’s disease.

In the present study, the highest response rate observed at any frequency was around 70% in the affected ears of individuals with Meniere’s disease. This is in consonance with those reported by Sandhu et al (2012) who reported the highest response rate of 75% in the ears affected with definite Meniere’s disease. However this response rate is higher than those reported by Winters et al (2012) who reported response rates of 55% or less across the range of frequencies. Higher response rates in the present study than Winters et al (2012) could be attributed to the differences in the stimulus level used between the studies. While Winters et al (2012) used a maximum stimulus level of 120 dB
peSPL, the present study obtained responses at a maximum stimulus intensity of 125 dB peSPL. Murnane et al (2011) reported significantly smaller response rate at 120 dB peSPL than 125 dB peSPL. They reported a drop in the response rate from almost 100% to nearly 66% in the healthy adults when the stimulus level was reduced from 125 dB peSPL to 120 dB peSPL. Therefore, the use of a lower maximum intensity level could explain lower response rates across frequencies in the previous studies than the present study.

The results of the present study revealed significantly larger response rates at 500 Hz, 750 Hz and 1000 Hz than all other frequencies in the affected ears of individuals with Meniere’s disease. However there was no significant difference in the response rates between these three frequencies. Similar highest response prevalence at low-to-mid frequencies was reported previously by Sandhu et al (2012) and Winters et al (2012). The finding of larger response rate in this low-to-mid frequency range compared to the other frequencies could be attributed to the resonance of the otolith organs, majorly utricle in the case of oVEMP. Several previous investigations have reported the resonance frequency of the utricl in the above mentioned frequency region (Fernandez & Goldberg, 1976; Young et al., 1977; Holton & Hudspeth, 1983; Fettiplace & Fuchs, 1999; Welgampola & Colebatch, 2001).

In the present study, the response rates were found to be significantly smaller in the affected ears than the unaffected ears of individuals with Meniere’s disease at the stimulus frequencies of 500 Hz, 750 Hz and 1000 Hz. The response rates were found to be significantly smaller in the affected ears of Meniere’s disease group than the matched ears of healthy controls at all the frequencies. This is in agreement with the findings of Sandhu et al (2012), who also reported lower response rates across the frequencies in the affected ears of individuals with Meniere’s disease than their unaffected ears and also the ears of
healthy controls. The finding of lower response rates in the affected ears of individuals with Meniere’s disease than their unaffected ears and the ears of healthy controls could be attributed to a combination of two factors which were reasoned to best explain the pathophysiology and symptoms of Meniere’s disease by Paparella (1991). These factors include the reduction in response caused by the hydrostatic changes caused by the mechanical deformation of the otolith organs (Tonndorf, 1983; Brown, Chihara, & Wang, 2013) and the ionic disturbances within the labyrinth on the affected side (Vosteen & Morgenstern, 1986).

### 5.2.2 Peak-to-peak amplitude of oVEMP in individuals with Meniere’s disease.

The peak-to-peak amplitude of oVEMP was found to be smaller across the frequencies in the affected ears of individuals with Meniere’s disease than their unaffected ears; however the statistically significant ear difference in the amplitude was evident only at 500 Hz, 750 Hz and 1000 Hz. This is in consonance with the findings of Winters et al (2012), who obtained oVEMP responses only at 250 Hz, 500 Hz and 1000 Hz and found significantly reduced oVEMP amplitude at 500 Hz and 1000 Hz but not at 250 Hz. Although the other studies obtained oVEMP only at 500 Hz, they reported significantly lower amplitude of oVEMP in the affected than the unaffected ears of individuals with Meniere’s disease (Khali & Kabarity, 2011; Winters et al., 2011). Therefore, the findings of the present study are also in agreement with these studies.

The finding of smaller peak-to-peak amplitude of oVEMP in the affected ears than the unaffected ears of individuals with Meniere’s disease could be attributed to the nature of the pathology itself. Meniere’s disease is well documented to be mostly a unilateral pathology. The endolymphatic hydrops, which is largely accepted as the reason behind the symptoms in Meniere’s disease (Hallpike & Cairns, 1938; Yamakawa, 1938; Monsell et
al., 1995), has been reported to not only mechanically suppress the function of the vestibular hair cells but also cause ionic disturbances which results in reduced responses from the affected side (Tonndorf, 1983; Vosteen & Morgenstern, 1986; Paparella, 1991; Brown et al., 2013). This therefore transpires into a reduced response from the affected side. Since Meniere’s disease not only affects saccule and cochlea but also utricle (Okuno & Sando, 1987), the reduced response amplitude of oVEMP on the affected side with Meniere’s disease could be understood.

The between groups comparison in the present study revealed significantly lower peak-to-peak amplitude of oVEMP in the affected ears with Meniere’s disease than the matched ears of the healthy controls at 500 Hz and 750 Hz. These findings are in accordance with the findings of the previous studies (Khalil & Kabarity, 2011; Winters et al., 2011, 2012; Sandhu et al., 2012). They also reported smaller amplitudes in the Meniere’s affected ears than the ears of the healthy controls. The reason behind such a result could be the reduction in response caused by the hydrostatic changes caused by the mechanical deformation of the otolith organs (Tonndorf, 1983; Brown et al., 2013) and the ionic disturbances within the labyrinth on the affected side (Vosteen & Morgenstern, 1986).

Further, the between groups comparison revealed no significant difference in peak-to-peak amplitude at any of the frequencies between the unaffected ears of Meniere’s disease group and the matched ears of the healthy controls. This is in agreement with the findings of the previous studies (Khalil & Kabarity, 2011; Winters et al., 2011, 2012), who also reported no significant difference in the peak-to-peak amplitude between the unaffected ears of individuals with Meniere’s disease and the ears of the healthy controls. However, this is not in agreement with those reported by Sandhu et al (2012). They
observed significantly lower amplitude of oVEMP in the unaffected ears of individuals with Meniere’s disease than the controls’ ears. The differences between the findings of the present study and those of Sandhu et al (2012) could be attributed to non-consideration of age as an important factor in their study. While the participants in the healthy controls group were age- and gender-matched to the participants in the Meniere’s disease group in the present study, Sandhu et al (2012) had a large difference in the age range of the participants between the clinical and the control groups. In their study, the participants in the clinical group were in the age range of 30-75 years (mean age = 53 years) whereas those in the control group had an age range of 25-45 years (mean age = 31 years). The study on the effect of age on peak-to-peak amplitude across the frequencies demonstrated significant reductions in the amplitudes of oVEMP at all the frequencies with advancing age, even in the middle-aged group (Piker et al., 2013). Additionally, the contribution to the difference between the two set of studies might also have come from the relatively small sample size (N = 12 in the clinical group) in Sandhu et al (2012) as opposed to considerably larger sample size (N = 36 in the clinical group) in the present study’s clinical group.

An intriguing finding in the present study was the occurrence of the largest peak-to-peak amplitude at 1000 Hz in the affected ears of Meniere’s disease as against 500 Hz and 750 Hz in the unaffected ears of individuals with Meniere’s disease and both ears of the healthy controls. This is in agreement with the results of the previous studies which also demonstrated largest amplitude at 1000 Hz in the affected ears of individuals with Meniere’s disease and at 500 Hz and 750 Hz in the unaffected ears as well as the ears of the healthy controls (Sandhu et al., 2012; Winters et al., 2012; Jerin et al., 2014). This difference in the location of largest peak-to-peak amplitude in the pathological ears (affected ears) of individuals with Meniere’s disease from their unaffected ears and the
ears of healthy individuals could be caused by the increased stiffness of the utricular membrane. The histopathological studies have shown the presence of significantly high amount of endolymphatic accumulation in the Meniere’s ears than the non-Meniere’s ears (Hallpike & Cairns, 1938; Yamakawa, 1938; Fraysse, Alonsio, & House, 1980; Schuknecht & Gulya, 1983; Okuno & Sando, 1987). This excessive accumulation of the endolymph within the otolith organs was shown to cause increased stiffness of their membranes by causing distension (Okuno & Sando, 1987; Young, Wu, & Wu, 2002; Merchant et al., 2005; Morita et al., 2009). Since the resonant frequency of a mechanical system is directly proportional to the square root of its stiffness and inversely proportional to the square root of its mass (Vanhuysse, Creten, & Van Camp, 1975; Popelka & Hunter, 2013), the increase in the stiffness within a system results in the corresponding increase in the resonance frequency of the system. In the present study’s context, the change in resonance frequency of the utricle that is caused by the distension of the utricular membrane would have caused a shift in largest peak-to-peak amplitude from 500 Hz and 750 Hz in the non-Meniere’s ears to 1000 Hz in the Meniere’s disease affected ears.

5.2.3 Threshold of oVEMP in individuals with Meniere’s disease.

The threshold of oVEMP was assessed across the frequencies and the results revealed higher (elevated) oVEMP threshold in the affected ears of individuals with Meniere’s disease than their unaffected ears, though statistically significant difference was observed only at 750 Hz. These findings are in consonance with those reported previously by Winters et al (2011, 2012). The finding of higher thresholds in the affected ears of individuals with Meniere’s disease than their unaffected ears could be attributed to the reduction in response caused by the hydrostatic changes caused by the mechanical deformation of the otolith organs (Tonndorf, 1983; Brown et al., 2013) and the ionic
disturbances within the labyrinth on the affected side (Vosteen & Morgenstern, 1986). Thus, the finding of elevated threshold of oVEMP in the affected ears compared to their unaffected ears could be justified.

The results of the between groups comparisons revealed higher oVEMP thresholds in the affected ears of individuals with Meniere’s disease than the matched ears of the comparison group; however the statistically significant difference was obtained only at 500 Hz and 750 Hz. This is in agreement with the results of Winters et al (2011, 2012). This could again be attributed to the mechanical and ionic disturbances caused by the excessive accumulation of endolymph within the endolymphatic space (Tonndorf, 1983; Vosteen & Morgenstern, 1986; Paparella, 1991; Brown et al., 2013) which would have lead to less responsiveness of the utricle, resulting in increased threshold.

A captivating finding in the present study was that of elevated thresholds in the unaffected (asymptomatic) ears of individuals with clinically unilateral Meniere’s disease than the matched ears of the healthy controls, although statistically significant difference was found only at 500 Hz and 750 Hz. Although the previous studies in this regard (Winters et al., 2011, 2012) found similar results of elevated thresholds in the unaffected ears of individuals with Meniere’s disease than the control data, but failed to observe statistically significant difference at any of the frequencies. However, the finding of relatively higher oVEMP threshold in the unaffected ears of individuals with Meniere’s disease compared to the healthy controls tends to demonstrate agreement between these studies.

The finding of elevated oVEMP threshold even in the unaffected ears of individuals with Meniere’s disease compared to the controls data could be attributed to the ‘occult’ or ‘latent’ Meniere’s disease in the unaffected ears which has been described in
several previous investigations using other kinds of tests like cVEMP (Ribeiro, Almeida, Cavoilla, Gananca, 2005; Lin et al., 2006; Fouly, Minawi, & Dessouki, 2012), electrocochleography (Visu & Singh, 2012) and oto-acoustic emissions (Magliulo, Cianfrone, Gagliardi, Cuiuli, & D’Amico, 2004). The words like ‘occult’ or ‘latent’ Meniere’s disease refers to the possibility of a bilateral involvement at a later stage although one of the ears is asymptomatic at the moment (Tsuji, Velazquez-Villasenor, Rauch, Glynn, Wall, & Merchant, 2000; Morita et al., 2009). The post-mortem studies that were performed on the temporal bones of the individuals with unilateral Meniere’s disease showed the existence of hydrops in nearly 35-38% of the asymptomatic ears (Tsuji et al., 2000), which further substantiates the findings of the present study.

Like the peak-to-peak amplitude of oVEMP, the best (lowest) oVEMP threshold was also associated with the stimulus frequency of 1000 Hz in the affected ears of individuals with Meniere’s disease. In their unaffected ears and both the ears of the healthy controls however, the best thresholds were obtained at 500 Hz and 750 Hz. This is in agreement with the findings of Winters et al (2012), who also demonstrated best thresholds of oVEMP at 1000 Hz in the affected ears of individuals with Meniere’s disease and 500 Hz in the unaffected ears of these individuals as well as the ears of the healthy controls.

The differences in the finding of frequency corresponding to best threshold in the affected ears of individuals with Meniere’s disease than their unaffected ears and the ears of the healthy controls could be attributed to the increase in the stiffness of the utricular membrane evidenced in the histopathological studies on Meniere’s disease (Okuno & Sando, 1987; Young et al., 2002; Merchant et al., 2005; Morita et al., 2009). The increase in the stiffness of utricular membrane could have caused an increase in the resonance
frequency of the system and therefore the reduction in the oVEMP threshold in the region of the resonance frequency in the affected ears with Meniere’s disease.

5.2.4 Frequency tuning of oVEMP in individuals with Meniere’s disease.

The results of frequency tuning in the present study demonstrated the existence of frequency tuning at 1000 Hz in a significantly higher proportion of the affected ear of individuals with Meniere’s disease than the matched ears of the healthy controls. Nearly 68% of the affected ears of the Meniere’s disease group demonstrated frequency tuning at 1000 Hz or higher frequencies as against almost 99% of healthy control ears showing frequency tuning at 500 Hz or 750 Hz. These findings pertaining to the frequency tuning in the affected ears of Meniere’s disease group are in agreement with the previously reported investigations (Sandhu et al., 2012; Winters et al., 2012; Jerin et al., 2014). However, the tuned frequency in these studies was decided based on the highest group average amplitude data across the frequencies whereas the tuned frequency was obtained for each individual and then a percentage value (proportion of ears) was calculated in the current study.

The shift in the frequency tuning from 500 Hz or 750 Hz in the healthy control ears to 1000 Hz or higher frequencies in the affected ears of individuals with Meniere’s disease could be explained on the basis of the changes in the stiffness properties of the utricle due to excessive accumulation of endolymph within the labyrinth. The histopathological evidence has shown the presence of significantly high amount of endolymph within the membraneous labyrinth (Hallpike & Cairns, 1938; Yamakawa, 1938; Fraysse et al., 1980; Schuknecht & Gulya, 1983; Okuno & Sando, 1987) which was shown to result in corresponding increase in the stiffness of the utricular membrane (Okuno & Sando, 1987; Young et al., 2002; Merchant et al., 2005; Morita et al., 2009). The occurrence of this
change in stiffness characteristics within the utricle could have therefore caused a shift in the largest peak-to-peak amplitude from 500 Hz or 750 Hz (since resonance frequency is directly proportional to square root of a system’s stiffness) in the ears of the healthy individuals to 1000 Hz in the ears affected by Meniere’s disease.

The results of the present study also demonstrated the existence of frequency tuning at 1000 Hz in a significantly higher proportion of unaffected ears (asymptomatic ears) of the individuals with Meniere’s disease than the matched ears of the healthy controls, a vast majority of which revealed frequency tuning to 500 Hz or 750 Hz. The previous studies in this regard did not attempt to compare the individual subjects’ frequency tuning data between the groups (Sandhu et al., 2012; Winters et al., 2012; Jerin et al., 2014). Nonetheless, the finding of higher proportion of unaffected ears showing frequency tuning at 1000 Hz than the healthy controls could be explained on the basis of the existence of the ‘occult’ or ‘latent’ endolymphatic hydrops in the unaffected ear which was shown previously through the use of tests like cVEMP (Ribeiro et al., 2005; Lin et al., 2006; Fouly et al., 2012), electrocochleography (Visu & Singh, 2012), oto-acoustic emissions (Magliulo et al., 2004) and post-mortem studies (Tsuji et al., 2000).

The analysis using the ROC curves in the present study demonstrated the best sensitivity of 68% and specificity of 100% for detecting Meniere’s disease if a criterion point of frequency tuning at 875 Hz is used. None of the previous studies have attempted to find the sensitivity and specificity of the frequency tuning measure in identifying Meniere’s disease. Nonetheless, the values obtained in the present study appear to be encouraging enough to recommend the clinical use of this measure to identify Meniere’s disease. In the opinions of McNeil and Hanley (1984), if the area under the curve of a ROC curve falls between 0.7 and 0.9, it represents a ‘good test’. The area under the curve
of the ROC curve obtained in the present test was found to be 0.931. Therefore, Frequency tuning of oVEMP is a ‘good test’ for the diagnosis of Meniere’s disease.

Therefore to conclude, the affected ears of individuals with Meniere’s disease produce lower response rates, smaller peak-to-peak amplitude and elevated thresholds compared to their unaffected ears and ears of healthy controls. Majority of the affected ears of individuals with Meniere’s disease demonstrate frequency tuning to 1000 Hz or above as against 500 Hz or 750 Hz in the healthy controls. Using criteria of 875 Hz for frequency tuning yields sensitivity and specificity of 68% and 100% respectively for identifying Meniere’s disease. Therefore frequency tuning properties in the affected ears of individuals with Meniere’s disease are significantly different from the healthy individuals.

5.3 oVEMP in individuals with BPPV

The BPPV group consisted of 36 participants with unilateral BPPV. The oVEMP results in them were compared between their two ears (the within group comparisons at each frequency) and also against 36 age- and gender-matched healthy controls. The results of the statistical analysis are discussed below:

5.3.1 Response rate of oVEMP in individuals with BPPV.

Out of the 36 individuals with unilateral BPPV, the oVEMP responses were present in 32 affected ears and 34 unaffected ears, at least at one frequency. Thus, oVEMP were absent across the frequencies in 4 affected ears (about 11%) and 2 unaffected ears (almost 6%) in the individuals with BPPV. The finding of absent oVEMP on the affected side or binaurally is consistent with those in the existing literature (Nakahara et al., 2013; Seo et al., 2013; Singh & Barman, 2015). Nonetheless the proportions appear to be differing between the studies. While Nakahara et al (2013) observed absence of oVEMP
on the affected side in 8 (nearly 67%) and on the unaffected side in 5 (nearly 42%) out of
the 12 subjects with posterior canal BPPV, Seo et al (2013) reported absent oVEMP in 3
(almost 19%) out of 16 subjects bilaterally. Singh and Barman (2015) found absence of
oVEMP on the affected side in 5 (close to 17%) out of 30 subjects with posterior canal
BPPV. However they did not specifically mention whether the responses were also absent
on the unaffected side in these individuals. While Nakahara et al (2013) and Singh and
Barman (2015) obtained oVEMP for a stimulus frequency of 500 Hz, Seo et al (2013) used
a 750 Hz frequency to elicit oVEMPs. When the response prevalence between the present
study and that by Seo et al (2013) was compared at 750 Hz itself (this being a common
stimulus frequency between the two studies), the results revealed no significant difference
between the proportions between the studies [Z = 0.74, p = 0.45; equality of tests for
proportions]. A similar comparison at 500 Hz between the present study and the study by
Singh and Barman (2015) revealed no significant difference between the studies [Z = 0.29,
p = 0.77]. However this comparison between the present study and that by Nakahara et al
(2013) revealed significantly lower response rates at 500 Hz in the effected ears [Z = 3.05,
p = 0.002] as well as the unaffected ears [Z = 3.06, p = 0.002] than the present study.

The differences form the Nakahara et al (2013) but not from Seo et al (2013) and
Singh and Barman (2014) could be accounted for by the differences in the use of filter
setting between the studies. While the band-pass filter used in the present study and that by
Singh and Barman (2015) was 1-1000 Hz and that in the study by Seo et al (2013) was 5-
500 Hz, the filter setting used in Nakahara et al (2013) was 20-2000 Hz. The changes in
the high-pass filter setting were shown to significantly reduce the response rate of oVEMP
previously (Wang et al., 2013), mainly due to the dominance of low frequencies in the
oVEMP response waveform.
The affected ears of the individuals with BPPV demonstrated lower response rates than the matched ears of healthy controls; nonetheless the statistical difference between the groups was obtained only at 1000 Hz, 1500 Hz and 2000 Hz. The finding of lower response rate in the affected ears of individuals with BPPV compared to the matched ears of the healthy controls is in agreement with those reported previously (Nakahara et al., 2013; Seo et al., 2013; Singh & Barman, 2015), although they obtained oVEMP for only one tone-burst frequency.

Lower response rates in the affected ears could be attributed to the histopathological changes in the utricle. The source of the otoconia debris was shown to be mainly the utricle in the affected ears of individuals with BPPV (Parnes & McClure 1992; Buckingham, 1999; von Brevern et al., 2006). Therefore, the loss of otoconia from the utricular macula could cause a utricular impairment in the ears with BPPV. The utricular impairment in BPPV was also demonstrated through the use of other techniques meant for the measurement of utricular function like ocular counter-rolling test (Markham, Diamond, & Ito, 1987), eccentric rotation (Takeda, Nishiike, Kitahara, Kubo, Ogino, & Koizuka, 1997; Hong et al., 2008), sinusoidal off-vertical axis rotation method (Sugita-Kitajima et al., 2007) and subjective visual vertical test (von Brevern et al., 2006; Faralli et al., 2009). Thus the loss of otoconia from the utricle could have resulted in absent oVEMP in some thus resulting in reduced response rates in the affected ears with BPPV.

The response rates obtained at 500 Hz, 750 Hz and 1000 Hz were significantly higher than the response rates corresponding to other frequencies in both the ears of the BPPV group. However there was no significant difference in the response rates among these three frequencies. There are no previous attempts of assessing response rates of oVEMP across the frequencies in individuals with BPPV and therefore the findings of the
present might be considered the first in this direction. The finding of highest response rates at 500 Hz, 750 Hz and 1000 Hz could be due to the resonance frequency of utricle which is around 750 Hz and probably would have lead to increased response rate around these frequencies.

5.3.2 Peak-to-peak amplitude of oVEMP in individuals with BPPV.

The BPPV group revealed significantly smaller peak-to-peak amplitude in the affected ears than the unaffected ears at each of the frequencies except 2000 Hz. Although all the previous studies exploring oVEMP in BPPV obtained oVEMP only at one frequency (500 Hz or 750 Hz), most of them reported the finding of smaller peak-to-peak amplitude in the majority of ears with BPPV than the unaffected ears of these individuals (Nakahara et al., 2013; Seo et al., 2013; Singh & Barman, 2015). While Nakahara et al (2013) reported only reduced oVEMP amplitudes in the ears with BPPV compared to the controls in all the individuals with BPPV, Seo et al (2013) and Singh and Barman (2015) also reported augmented oVEMP amplitudes in 5 out of 13 ears and 5 out of 25 ears respectively. A close scrutiny of the peak-to-peak amplitude values in the present study also revealed larger peak-to-peak amplitude at 500 Hz, 750 Hz, 1000 Hz and 1500 Hz in the affected ears than the unaffected ears in 6 individuals with BPPV.

The augmented nature of oVEMP in these 6 participants as opposed to the findings of reduced amplitude among the others in the BPPV group could be attributed to the possibility of a hyper reaction in the affected ears in these participants (Seo et al., 2013). The possible causes for finding such a hyper reactive oVEMP in the affected ears of these individuals is yet to be clearly understood. Nonetheless in an effort to uncover the basis of such a finding, Seo et al (2013) proposed the use of a simplified mass-spring-damper model for utricle that was put forth by Todd et al (2009) for elucidating the frequency
tuning of oVEMP to low-frequency. As per this model, utricle is a dynamic body with the mass positioned at the top in the form of the calcium carbonate crystal forming the mesh-gel layer. In a normally functioning utricle, the depolarization of the hair cells is caused by the sound-induced deviation of the stereocilia. In case of BPPV there is loss of otoconia from the utricular macula, which is largely accepted as the source for free-floating otoconia particles that are found in the semicircular canals of the affected ears with BPPV (Parnes & McClure, 1992; Buckingham, 1999; Von Brevern et al., 2006). This loss of otoconia from the utricle could cause reduction of the mass that is present at the top in the normal utricle. This could predispose the system to hypermobility by virtue of reducing moment of inertia in them. The higher mobility of the stereocilia would therefore produce enhancement of oVEMP amplitude when compared to the unaffected ears that has an intact utricle. The foundation of this assumption comes from the investigations on cVEMP that were administered under the conditions of microgravity (Shokuja et al., 2008). They obtained significantly augmented amplitudes of cVEMP under the temporary microgravity conditions observed during the parabolic flight than under the normal gravity conditions. They assumed that the temporary detachment of the otoconia from the otolithic membrane during the parabolic flight (weightless conditions) was the main reason behind such a augmentation of cVEMP. Nevertheless, there is dearth of evidence based studies that could prove that the otoconial detachment from the otolithic membrane indeed is the reason behind augmented saccular and utricular responses. Therefore, more research evidence is needed before this hypothesis could be accepted with any degree of conviction.

*The affected ears of the BPPV group further demonstrated significantly smaller peak-to-peak amplitude compared to the controls at frequencies ranging from 250 Hz to 1000 Hz.* Although all the previous studies exploring oVEMP in BPPV obtained oVEMP only at one frequency (500 Hz or 750 Hz), most of them reported the findings of smaller
peak-to-peak amplitude in the majority of ears with BPPV than the control ears (Nakahara et al., 2013; Seo et al., 2013; Singh & Barman, 2015). The reduced peak-to-peak amplitude in the BPPV ears compared to the control ears could be attributed to the existence of the utricular pathology because of the loss of otoconia particles from the utricle in the BPPV ears which was shown previously through histopathological evidences (Parnes & McClure, 1992; Buckingham, 1999; von Brevern et al., 2006) and findings of the other tests meant for the measurement of utricular function (Markham et al. 1987; Takeda et al., 1997; Sugita-Kitajima et al., 2007; Hong et al., 2008; Faralli et al., 2009).

The peak-to-peak amplitude obtained at 750 Hz was found to be significantly larger than the peak-to-peak amplitude at other frequencies in both the ears of the BPPV group and the age- and gender matched healthy controls. The occurrence of largest peak-to-peak amplitude at 750 Hz could be attributed to the resonance frequency of the utricle which is around this frequency. Also, the mean age of the individuals in the BPPV group and the matched healthy controls was 40.1 years which is on the higher side. This would have also caused the largest amplitude at 750 Hz in the clinical and the control groups, as was shown by Piker et al (2013). They had observed an increase in the finding of largest peak-to-peak amplitude at 750 Hz and decrease in largest peak-to-peak amplitude at 500 Hz in the middle age group compared to the young adults group. Since a number of individuals in the BPPV group and the age- and gender-matched healthy controls were above the age of 40 years (middle age as per Piker et al., 2013), the finding of largest peak-to-peak amplitude at 750 Hz could be justified.
5.3.3 Threshold of oVEMP in individuals with BPPV.

The oVEMP threshold obtained at 750 Hz was significantly lower (better) than the thresholds at other frequencies in both the ears of the BPPV group as well as the age- and gender matched healthy controls. The lowest oVEMP threshold at 750 Hz rather than 500 Hz could be attributed to the effects of age. Although Piker et al (2013) did not measure the oVEMP thresholds, they observed largest peak-to-peak amplitude at 500 Hz in majority of the young adults. They further found that the proportion of individuals with largest peak-to-peak amplitude at 500 Hz decreased and was almost equal to ears with largest peak-to-peak amplitude at 750 Hz in the middle aged individuals. A similar age effect might have caused the finding of better threshold at 750 Hz rather than 500 Hz in the BPPV group as well as the age- and gender-matched healthy controls.

The threshold of oVEMP was found to be higher (elevated) in the affected ears of individuals with BPPV than their unaffected ears and the ears age- and gender-matched healthy controls. This is in consonance with a previous study exploring the threshold of oVEMP in individuals with posterior canal BPPV (Masoom et al., 2014). This could be attributed to the utricular pathology in the affected ears of these individuals that was shown previously through the histopathological evidences (Parnes & McClure, 1992; Buckingham, 1999; von Brevern et al., 2006) and other tests of utricular function (Markham et al., 1987; Takeda et al., 1997; Sugita-Kitajima et al., 2007; Hong et al., 2008; Faralli et al., 2009). The loss of otoconia in the utricle would have decreased the overall response amplitude and thus elevated the threshold of oVEMP.

5.3.4 Frequency tuning of oVEMP in individuals with BPPV.

Nearly 60% of both the ears of BPPV group showed frequency tuning at 750 Hz whereas almost 40% of both their ears showed frequency tuning at 500 Hz. There was no
difference in the proportion of ears demonstrating frequency at any frequency between the BPPV group and the age- and gender-matched healthy controls. All the previous studies exploring oVEMP in BPPV have recorded oVEMP using only one frequency. Thus, none of the previous studies actually tried to explore the frequency tuning property of oVEMP in individuals with BPPV.

The histopathological evidences have shown loss of otoconia particles from the utricular macula in ears with BPPV which confirms the presence of utricular pathology in the BPPV ears (Parnes & McClure, 1992; Buckingham, 1999; von Brevern et al., 2006). This should mean a reduction in the mass component when the second order mechanical model of mass and stiffness is applied to explain the frequency tuning. This imbalance between the mass and stiffness should therefore cause a shift in the frequency tuning towards higher frequencies as mass has been reported to be inversely proportional to the resonance frequency (Vanhuyse et al., 1975; Popelka & Hunter, 2013). However, the findings of the present study did not show a significant difference between the frequency tuning in BPPV ears and the matched ears of the healthy controls. This possibly means that the reduction in mass caused by the loss of otoconia particles in the BPPV ears is not large enough to sufficiently shift the peak to an expected higher frequency in the frequency tuning curve.

To conclude, the affected ears of individuals with BPPV are characterized by reduced response rates, lowered amplitudes and elevated thresholds of oVEMP. They further produce similar frequency tuning to those observed in the age- and gender matched healthy controls. Therefore, frequency tuning properties of oVEMP is not an efficient parameter to identify the presence of BPPV. Nonetheless, the threshold and peak-to-peak amplitude measures seem to be promising in identification of BPPV.
5.4 oVEMP in individuals with ANSD

The ANSD group consisted of 36 participants having bilateral ANSD. The findings of response rate, peak-to-peak amplitude, oVEMP threshold and frequency tuning oVEMP in this group were compared against those of the 36 age- and gender-matched healthy controls. The findings are discussed below:

5.4.1 Response rate of oVEMP in individuals with ANSD.

The ANSD group revealed presence of oVEMP at least at one frequency in 13 out of 36 individuals in this group. Of these 13, oVEMP were present bilaterally in 6 individuals and unilaterally in 7 individuals. In total, 11 (30.55%) right ears and 8 (22.22%) left ears demonstrated the presence of oVEMP at least at one frequency. The comparison between the groups revealed significantly smaller response rates across the frequencies in individuals with ANSD than the age- and gender-matched healthy controls. The findings pertaining to the response rate in the individuals with ANSD is in disagreement with those reported by Sinha, Shankar et al (2013). They reported complete absence of 500 Hz air-conduction tone-burst evoked oVEMP in all the 22 ears of 11 individuals with ANSD. The differences in the findings between the present study and that by Sinha, Shankar et al (2013) could be accounted by the use of a solitary frequency (500 Hz) in their study as against the use of multiple frequencies to elicit oVEMP in the present study. The recording of oVEMP at multiple frequencies is likely to increase the chance of obtaining the responses at least at one frequency. This assumption is further supported by the finding of larger response rates at 750 Hz and 1000 Hz than 500 Hz in the healthy individuals in the present study. The difference in the findings of present study from those of Sinha, Shankar et al (2013) could also be caused by the use of a smaller sample size in their study (N = 11) than the present study (N = 36) and the inherent diversity in the
disorder itself (Fuzikawa & Starr, 2000; Ismail et al., 2014). Both these studies reported presence of caloric responses in some of the individuals with ANSD and absence in others. Like oVEMP, the responses for caloric test are also mediated by the vestibulo-ocular reflex (VOR) (Chen & Young, 2003). Hence, the presence of abnormal VOR in some individuals with ANSD and not in others could be concluded. The presence of oVEMP in some individuals could possibly indicate lesser degree of dys-synchrony in them than those with absence of oVEMP at all frequencies.

The absence of responses at all the frequencies in a large majority of the individuals with ANSD could be an indicator of pathology in the utriculo-ocular reflex pathway. The anatomical alterations such as reduced neuronal population between the utricle and the scarpa’s ganglion, irregular beaded appearance of the nerve fibres, fragmentation of the myelin layer with gaps as large as the diameter of the nerve fibers and/or a completely distorted vestibular nerve was found in the individuals with ANSD (Starr et al., 2003). This supports the finding of existence of vestibular pathology in majority of these individuals which was also demonstrated by the results of tests like electronystagmography (Fuzikawa & Starr, 2000; Sheykholeslami et al., 2005), videonystagmography (Ismail et al., 2014) and cVEMP (Wu et al., 2004; Sheykholeslami et al., 2005; Kumar et al., 2007; Akdogan et al., 2008; Sazgar et al., 2010; Ismail et al., 2014).

Among the individuals with ANSD who showed presence of oVEMP, the responses were present only up to 1000 Hz. While recording responses at frequencies above 1000 Hz, the frequency is further shifted away from the resonance frequency of the utricle. As shown for healthy individuals and those with vestibular pathologies, the frequencies further away from the resonance frequency produce smaller amplitude of oVEMP (Sandhu
et al., 2012) and would require larger intensity to elicit oVEMP. This makes oVEMP at these frequencies (above 1000 Hz) more vulnerable to being absent, especially in individuals having ANSD.

Further, the findings of the present study revealed highest response rates at 500 Hz and 750 Hz which were significantly higher than those at 250 Hz and 1000 Hz in individuals with ANSD. This was similar to the findings in the age- and gender-matched comparison group. The finding of largest response rates at 500 Hz and 750 Hz could be attributed to the resonance frequency of the utricle which is around these frequencies (Todd et al., 2000, 2009b; Sandhu et al., 2012; Winters et al., 2012; Singh & Barman, 2013). The pathology in individuals with ANSD is mainly believed to be neural in nature (Starr et al., 2003) whereas the best responsivity is mainly a labyrinthine phenomenon (Todd et al., 2009b) with minimal contributions from the central mechanisms. Since the utricle is not affected by the pathology in ANSD, it is likely to result in similar best frequency (500 Hz or 750 Hz) to those observed in healthy individuals.

5.4.2 Peak-to-peak amplitude of oVEMP in individuals with ANSD.

The peak-to-peak amplitude was found to be higher at 500 Hz and 750 Hz than all the frequencies in individuals with ANSD; however a statistically significant difference in the peak-to-peak amplitude between the frequencies was observed only in the right ears. There are no known previous studies exploring the effect of frequency of stimulus on the oVEMP amplitudes in individuals with ANSD. Nonetheless, the results of the study indicate that the effects of frequency on the peak-to-peak amplitude across the frequencies in individuals with ANSD follows a similar trend to that in the healthy controls, at least among the individuals in whom the responses were present. Thus in a similar way to the
healthy individuals, the finding of highest amplitudes at 500 Hz and 750 Hz in the individuals with ANSD could be attributed to the resonance properties of the utricle.

*The peak-to-peak amplitudes were significantly smaller across the frequencies in both the ears of the individuals with ANSD compared to the ears of the age- and gender-matched healthy controls.* A synchronous neural activity was shown to produce robust amplitude of the compound action potential which can be recorded from surface electrodes as large amplitude potentials (Starr, Picton, & Kim, 2001). In contrast if the neural firing is dys-synchronous, the amplitude of the compound action potential that is recorded from the surface electrodes would reduce (Starr et al., 2001). Since the studies on individuals with ANSD have shown affected neural reflexes involving the superior vestibular nerve (Fuzikawa & Starr, 2000; Sheykholeslami et al., 2005; Ismail et al., 2014), which is also responsible for oVEMP production, the finding of reduced amplitude could be explained on the basis of the presence of dys-synchronous firing of the neurons of the superior vestibular nerve.

### 5.4.3 Threshold of oVEMP in individuals with ANSD.

*The oVEMP thresholds were found to be significantly higher (elevated) across the frequencies in both the ears of the individuals with ANSD compared to the ears of the age- and gender-matched healthy controls.* Sinha et al (2013) observed absence of oVEMP in both ears of the individuals with ANSD even at maximum intensity (95 dB nHL). This means that the thresholds in their study could have been beyond 95 dB nHL which is way above the thresholds in healthy individuals. Therefore there seems agreement between the present study and the study by Sinha, Shankar et al (2013). The finding of significantly elevated thresholds of oVEMP in individuals with ANSD than healthy controls could be attributed to the presence of dys-synchrony caused by the pathology of the nerve fibers.
within the superior vestibular nerve in the individuals with auditory neuropathy which was shown by the results of the tests that evaluate the functioning of the superior vestibular nerve (Fuzikawa & Starr, 2000; Sheykholeslami et al., 2005; Ismail et al., 2014). The dys-synchrony in the firing of the superior vestibular nerve fibers could cause smaller compound action potentials than normal fibers (Starr et al., 2001). This could have caused absence of responses at lower levels thereby resulting in higher oVEMP thresholds.

The results of the present study further demonstrated lowest oVEMP thresholds at 500 Hz in both the ears of individuals with ANSD; however this was significantly lower than other frequencies only in the right ears. There are no previous studies on effect of frequency on oVEMP thresholds in individuals with ANSD. The lack of difference in thresholds could be explained on the basis of widening of the tuning curves as has been shown previously for the auditory system using the behavioral measures like the psychophysical tuning curves (Vinay & Moore, 2007). Alternatively, it could be explained by slight procedural deficiency in the present study. The present study used a 10 dB step-size to arrive at oVEMP threshold which would obscure the finer differences in the threshold between the frequencies that might have been exposed by use of smaller step-sizes. However, the use of smaller step sizes could impose severe discomfort and cause fatigue, which would in turn affect the responses. Further, it would have also exposed the ears to loud sounds for much longer duration, which appears to be a growing concern after a recent publication reported of deleterious impact of sound levels used for recording VEMP (Krause et al., 2013). Therefore such small step-sizes were not incorporated in the present study.
5.4.4 Frequency tuning of oVEMP in individuals with ANSD.

Over 60% of the ANSD ears demonstrated frequency tuning at 500 Hz and the remaining at 750 Hz amongst the ears which has oVEMP response. There was no group difference in the proportion of ears with frequency tuning at any of the frequencies between the ANSD group and the healthy controls. None of the previous studies have looked at exploring the frequency tuning properties of oVEMP in individuals with ANSD. The existence of frequency tuning at 500 Hz or 750 Hz in almost all the clinical and the control ears could be attributed to the resonance frequency of the utricle. Further, the similarity in the findings of the frequency tuning between the individuals with ANSD and the healthy controls is a testimony to the fact that the contribution to frequency tuning mainly arises from the utricle and that there is minimal, if any, contribution of neural system in frequency tuning of oVEMP. A similar view-point about frequency tuning of air-conduction tone-burst evoked oVEMP was proposed by Todd et al (2009a, b), who believed the frequency tuning to be essentially caused by the peripheral mechanisms rather than the neural ones.

Thus ANSD is characterized by low response rates of oVEMP across frequencies. Among those with presence of oVEMP, the responses are found only up to 1000 Hz. These are characterized by reduced amplitudes and elevated thresholds. Nonetheless the frequency tuning of oVEMP, which is found to be at 500 Hz or 750 Hz in these individuals, is similar to those found in the age- and gender-matched healthy controls.

5.5 Comparison of frequency tuning of oVEMP among the pathological groups

The results of comparison of frequency tuning between the pathological ears of all the groups revealed significantly higher proportion of ears with frequency tuning at 1000 Hz and significantly lower proportion of ears with frequency tuning at 500 Hz in
Meniere’s disease group compared to the BPPV group and the ANSD group. There was no significant difference in the proportion of ears with frequency tuning at any frequency between the BPPV group and the ANSD group. None of the previous studies have looked at exploring the possibility of such a distinction in frequency tuning between the pathologies. The differences in frequency tuning in affected ears of individuals with Meniere’s disease from those of the affected ears with BPPV and the ears of ANSD could be attributed to a combination of two factors- the inherent differences in the pathophysiological aspects between these pathologies and the physiology involved in producing the frequency tuning.

In the Meniere’s ears, the excessive accumulation of the endolymph within the utricle causes increase in the stiffness of the utricular membrane (Okuno & Sando, 1987; Young et al., 2002; Merchant et al., 2005; Morita et al., 2009). Since the resonance frequency is directly proportional to the stiffness (Vanhuyse et al., 1975; Popelka & Hunter, 2013), increase in the stiffness of utricular membrane would have caused a consequent increase in the resonance frequency which gets reflected in the shift of the peak of the frequency tuning from 500 Hz or 750 Hz in the healthy individuals to 1000 Hz or higher frequencies in the affected ears of individuals with Meniere’s disease. In the ANSD ears, there is no known change in the mechanics within the utricle rather there is pathology involving the superior vestibular nerve (Starr et al., 2003). Todd et al (2009a, b) proposed that the peak in the frequency tuning of air-conduction tone-burst evoked oVEMP is a consequence of the mechanics within the labyrinthine system rather than having any neural influence. Therefore, the ANSD ears demonstrated significant difference in the location of frequency tuning peak from the Meniere’s ears. In the affected ears of BPPV, the loss of otoconia particles from the utricle has been well documented (Parnes & McClure, 1992; Buckingham, 1999; von Brevern et al., 2006). This should
theoretically alter the mass of the macula and therefore the resonance frequency should increase. Nonetheless, the results of the present study show otherwise. This possibly means that the reduction in mass caused by the loss of otoconia particles in the BPPV ears is not large enough to sufficiently shift the peak of the frequency tuning curve to a relatively higher frequency. Thus the findings of the present study show that frequency tuning could differentiate the affected ears with Meniere’s disease from the affected ears with BPPV and those with ANSD.

5.6 Comparison of frequency tuning of oVEMP among the comparison groups used for the three clinical groups

An interesting observation noted in the present study is the presence of higher proportion of ears with frequency tuning at 750 Hz than 500 Hz in the control group for BPPV as opposed to higher proportion of ears with frequency tuning at 500 Hz than 750 Hz in the control groups for Meniere’s disease and ANSD. The control group used for BPPV group demonstrated frequency tuning to 500 Hz in nearly 44% of the ears and at 750 Hz in almost 55% ears (both ears included). Almost 57% of the ears of the control group for Meniere’s disease group and nearly 54% of the ears of the control group for ANSD group demonstrated frequency tuning at 500 Hz as opposed to 43% of both these control groups ears showing frequency tuning at 750 Hz. However when analyzed statistically, there was no significant difference in the proportion of ears with frequency tuning at these two frequencies between these control groups ($p > 0.05$; Equality of test for proportions). Although the difference in the proportion of ears with frequency tuning at any particular frequency was statistically not significant, the observation of higher proportion of the ears of the control group for BPPV demonstrating frequency tuning at 750 Hz when compared to larger proportion of the ears of the other two control groups
showing frequency tuning at 500 Hz. This could be explained by the effects of age (Piker et al., 2013). In a study exploring the effects of age on frequency tuning of oVEMP, Piker et al (2013) observed a decline in proportion of individuals exhibiting frequency tuning at 500 Hz and increase in the proportion of individuals demonstrating frequency tuning at 750 Hz with advancing age. In their study, the young adults (18-39 years) demonstrated dominance of frequency tuning at 500 Hz when compared to almost equal proportions of individuals showing frequency tuning at 500 Hz and 750 Hz in the middle aged adults (40-59 years). In the present study, the individuals in the control group for BPPV group consisted of 22 individuals falling in the middle aged group (40-50 years) as against 12 and 2 individuals in this age range in the control groups for Meniere’s disease group and ANSD group respectively. Therefore the presence of larger number of middle aged individuals in the control group for BPPV than the other two control groups could explain the finding of larger proportion of ears of control group for BPPV group exhibiting frequency tuning at 750 Hz and lesser at 500 Hz than the other two control groups.

Therefore to conclude, the results of the present study revealed significantly reduced response rates, smaller peak-to-peak amplitudes and poorer (higher) thresholds in the affected ears of the individuals with Meniere’s disease, BPPV and ANSD compared to their respective age- and gender-matched healthy controls. The frequency tuning was obtained at 500 Hz or 750 Hz in almost 99% of the ears of healthy individuals. Similar results were also obtained for both ears of the individuals with BPPV and those with ANSD. Nonetheless, a significantly high proportion of affected ears and unaffected ears of individuals with Meniere’s disease demonstrated frequency tuning at 1000 Hz or higher frequencies than any of the other three groups. Using a criterion point of 875 Hz for finding the peak in the frequency tuning curve, the sensitivity and specificity for identifying Meniere’s disease was found to be 68% and 100% respectively. Thus the
results of the present study indicate that shift in frequency tuning is an efficient parameter for not only discriminating Meniere’s disease from healthy individuals but also distinguishing it from other vestibular pathologies like BPPV and ANSD. Therefore, frequency tuning is recommended as test parameter for identification of Meniere’s disease.