Altered Frequency Tuning of VEMP: Could it be a Diagnostic Tool to Identify Endolymphatic Hydrops?

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List of Figures

List of Tables

List of Abbreviations

AAO-HNS	American Academy of Otolaryngology – Head and Neck Surgery
ABR	Auditory Brainstem Response
ANSI	American National Standards Institute
CHAMP	Cochlear Hydrops Analysis Masking Procedure
cVEMP	Cervical Vestibular Myogenic Potentials
ENG	Electronystagmography
MD	Meniere's disease
ROC	Receiver operating characteristic
SCM	Sternocleidomastoid Muscle
SD	Standard Deviation
VEMP	Vestibular Evoked Myogenic Potentials

Abstract

Several procedures have been employed for accurate diagnosis of hydrops resulting in Meniere's disease; however most of them are marred by either low sensitivity and/or specificity.. Cervical vestibular evoked myogenic potential (cVEMP), through the use of large inter-aural amplitude asymmetry, has recently emerged as a clinical tool for the diagnosis of Meniere's disease. Unfortunately, large inter-aural amplitude asymmetry is a common finding among several other peripheral pathologies thereby showing lack of specificity. More recently, frequency tuning of cVEMP has been showing promise in the specific diagnosis owing to changes in mechanical properties of cochlea which is associated only with Meniere's disease. Hence, the present study aimed at investigating the usefulness of the frequency tuning of cVEMP in the identification of Meniere's disease. For this, 22 participants with unilateral Meniere's disease in the age range of 18-50 years along with 22 age and gender matched healthy individuals underwent cVEMP recording at all octave and mid-octave frequencies from 250 to 4000 Hz using Biologic Navigator Pro evoked potential system. The order of frequencies was counter-balanced in order to avoid fatigue affecting the recordings. The results revealed a significant shift in frequency tuning of cVEMP from ≤750 Hz in healthy individuals to ≥750 Hz among ears affected with Meniere's disease. A large proportion of unaffected ears of individuals with Meniere's disease also demonstrated a tendency for a similar shift in frequency tuning towards higher frequencies. This might be useful in predicting a future progression to bilateral condition from a monaural onset, The sensitivity and specificity for the diagnosis of Meniere's disease through the use of shift in frequency tuning were found to be 100% and 63.64% respectively for a criterion cut-off frequency of ≥750 Hz. Thus, frequency tuning characteristic could be is a useful technique for the diagnosis of endolymphatic hydrops in ears affected with Meniere's disease.

Key words: Endolymphatic hydrops, Meniere's disease, frequency tuning, cVEMP

Introduction

Endolymphatic hydrops is pathology brought about by the build up of condition wherein there is a accumulation of excessive fluid in the inner ear (cochlea and/or vestibule). It could result from a number of pathologies like Meniere's disease, Syphilis or auto-immune diseases (Schuknecht, 1976; Lin, Timmer, Oriel, Zhou, Guinan, et al, 2006; Miller, Makary, Lopez & Ishiyama, 2010). Irrespective of the causative agent, the presenting symptoms have been found to be alike. These usually involve fluctuating hearing loss, true vertigo, tinnitus, nausea, vomiting and aural fullness. Any of these or all of these may be present in a single person with Endolymphatic hydrops. Due to an extremely high rate of co-existence, several literature reports use Endolymphatic hydrops and Meniere's disease synonymously. The term 'Meniere's disease' will be used as a synonymous term to Endolymphatic hydrops throughout the course of the present study as well.

The attempts at the diagnosis of Meniere's disease have resulted in mushrooming of a number of tests. These involve electronystagmography, videonystagmography, dehydration techniques like glycerol test and furosemide loading, electrocochleography, oto-acoustic emission and cochlear hydrops analysis masking procedure, vestibular evoked myogenic potential (VEMP). Any of these tests, though may assist in the diagnosis, are not complete in terms of the sensitivity and specificity in detecting Meniere's disease.

VEMP is one of the upcoming procedures and is showing promise in the diagnosis of the vestibular pathologies. It is an inhibitory potential which can be recorded from the tonically contracted ipsilateral Sternocleidomastoid muscle (SCM muscle) in response to loud monaural clicks or tone-bursts. It is characterized by a biphasic response with a positive peak namely P13 at a latency of about 13 ms and a negative peak namely N23 at a latency of about 23ms (Colebatch, Halmagyi, & Skuse, 1994; Bath, Harris, & Yardley, 1998; Wu, Young, & Murofushi, 1999; Wu & Murofushi, 1999; Welgampola & Colebatch, 2001; Ochi, Ohashi, &

Nishino, 2001). The research in the area suggests that VEMP is vestibulocollic reflex whose ascending fibers arise from the cells in the saccule and carry the signal forward via the inferior vestibular nerve to the vestibular nuclei (Bickford, Jacobson, and Cody, 1964; Colebatch & Halmagyi, 1992; Colebatch et al., 1994). This makes it a viable test for checking the functional integrity of saccule as well as the inferior vestibular nerve. Since VEMP can also be recorded from several other muscles of the body, when recording from the SCM muscle, it has more recently been referred specifically as Cervical VEMP (cVEMP).

A number of studies have shown the involvement of Saccule in a high proportion of cases with Meniere's disease (Rauch et al., 1989; de Waele, Huy, Diard, Freyss, & Vidal, 1999; Yamane, Sunami, Iguchi, Sakamoto, Imoto & Rask-Andersen, 2012; Yamane & Takayama, 2011). This being the case, cVEMP appears promising for the detection of existing Saccular pathology in cases with Meniere's disease.

Need for the study

Most studies comparing clicks and tone-burst-evoked cVEMP have suggested a lower threshold and higher amplitude for the later compared to the former (Welgampola & Colebatch, 2001; Wu, Shiao, Yang & Lee, 2007). However these findings are true only for 500 Hz tone-burst as cVEMP evoked by tone-burst has been shown to be highly dependent on the frequency of the evoking stimuli. The average best frequency has been reported to range from a low frequency extreme of 300 Hz to 350 Hz (Todd, Cody, & Banks, 2000) to up to a high frequency extreme of 750 Hz (Welgampola & Colebatch, 2001) among healthy individuals. Many other studies have shown the frequency tuning of cVEMP at 500 Hz specifically (Rauch, Zhou, Kujawa, Guinan, & Herrmann, 2004; Timmer, Zhou, Guinan, Kujawa, Herrmann, & Rauch, 2006; Janky & Shepard, 2009; Piker 2012).

The individuals with Meniere's disease have been shown to have Cochleo-Saccular hydrops (Rauch, Merchant, & Thedinger, 1989). Since cVEMP also arise from the Saccular afferents, it is only logical to presume that an altered motion mechanism of a distended Saccule may cause corresponding alteration of cVEMP in such individuals. Murofushi, Shimizu, Takegoshi and Cheng (2001) reported an absence of cVEMP in 51% subjects with Meniere's disease compared to only 12% or lesser (12% absence for tone-burst evoked and 2% absence for click- evoked cVEMP) absence of cVEMP in subjects with normal audio-vestibular functions (Cheng, Huang, & Young, 2003). Most studies have shown abnormal or absent cVEMP (Katayama, Yamamoto, Teranishi, Naganawa, Nakata, Sone, & Nakashima, 2010), higher asymmetry ratio (Katayama et al., 2010; Osei-Lah, Ceranic, & Luxon, 2008), or changes in cVEMP threshold following dehydration technique (Magliulo, Cianfrone, Gagliardi, Cuiuli, & D'Amico, 2004) in cases with Meniere's disease.

The cervical VEMP has been used for the assessment of cases with Meniere's disease and has shown positive results in terms of higher inter-aural asymmetry ratio and post-glycerol changes in amplitude. However, a high inter-aural asymmetry ratio is also consistent with other peripheral vestibular pathologies like Labyrinthitis, Vestibular neuritis and Vestibular schwannomas involving the inferior vestibular nerve (Murufoshi, Shimizu, Takegoshi, & Cheng, 2001; Honaker & Samy , 2007; Suzuki, Yamada, Inoue, Kashio, Saito, & Nakanishi, 2008; Baier, Stieber, & Dieterich, 2009; Nola, Guastini, Crippa, Deiana, Mora, & Ralli, 2011). Thus, this finding does not become clinically specific to Meniere's disease. Glycerol test is slightly invasive and there are reports of it evoking vegetative symptoms like vomiting, nausea, vertigo and headache. Also, glycerol is contraindicated in patients with diabetes. So, it may not be viable to administer it on all patients.

Despite the fact that various other tests are available for the assessment of Meniere's disease, frequency tuning of vestibular evoked myogenic potentials could provide more reliable information as the frequency dynamics of cVEMP depends on the resonance frequency of the Saccular membrane (Node, Seo, Miyamoto, Adachi, Hashimoto & Sakagami, 2005) which in turn depends upon the integrity of the Saccule.

Rauch et al. (2004) examined vestibular evoked myogenic potential testing using ipsilateral broad-band click and short tone-burst stimuli at 250, 500, 1,000, 2,000, and 4,000 Hz on 14 healthy individuals as well as 34 individuals with unilateral Meniere's disease. They reported a frequency-dependent cVEMP threshold, with best response ("frequency tuning") at 500 Hz. The affected ears of individuals with Meniere's disease showed significantly increased cVEMP thresholds with tuning apparent at 1000 Hz in majority of ears with Meniere's disease. Node et al. (2005), while exploring a similar aspect, found frequency tuning of cVEMP at 500 Hz among healthy individuals and a shift in frequency tuning to 1000 Hz or beyond in the endolymphatic hydrops group. Later in 2012, Sandhu, Low, Rea and Saunders further complemented the findings of Rauch et al. (2004) and Node et al. (2005) by reporting a shift in frequency tuning of cVEMP in ears of individuals with Meniere's disease. However, the above studies either used a small sample size or did not account for the effect of age which could cause a change in frequency tuning of cVEMP (Piker, 2012). Thus, there is the need to carry out a study with better control of age-related changes in frequency tuning of cVEMP and assess its efficacy as a diagnostic tool for identification of endolymphatic hydrops (Meniere's disease).

Aim

The present study aimed at characterizing the cVEMP response in participants with classical unilateral endolymphatic hydrops (mainly Definite Meniere's disease) and examining its efficacy in the diagnosis of Meniere's disease.

Objectives

a. To compare the frequency tuning of cVEMP of healthy individual to the vestibular pathologies.
b. To study the effect of frequency of tone-bursts on the response rate, latency, amplitude and threshold of cVEMP.

Method

Research design

The study used a 'Non-experimental static group comparison' research design.

Participants

The study incorporated two sets of participants who were assigned to two groups- a normal group and an endolymphatic hydrops group. The endolymphatic hydrops group consisted of 20 consecutive non-diabetic participants fulfilling the criteria of unilateral definite endolymphatic hydrops (Meniere's disease) based on the criteria defined by the American Academy of Otolaryngology- Head and Neck Surgery (AAO-HNS; 1995). Among these, 13 were females and the remaining 7 males. The age range of participants in the endolymphatic hydrops group was 27-55 years (mean age = 41.23 years; median age = 46 years). The normal group comprised of 20 adults with normal audio-vestibular function who were age and gender matched to the participants of endolymphatic hydrops group.

Inclusion criteria.

As per the AAO-HNS (1995) criteria for 'Definite Meniere's disease', the participants were required to present with the history of at least two episodes of spinning vertigo spanning over 20 minutes each. Further, they were also required to have at least one instance of documented hearing loss, episodes of tinnitus accompanied by aural fullness in absence of other causes of hearing loss and vertigo. In addition to the fulfilment of the AAO-HNS criteria for the diagnosis of Meniere's disease, the participants were required to have loudness discomfort levels beyond 100 dB HL, assessed using speech stimuli, in order to facilitate discomfort free testing through VEMP.

Exclusion criteria.

The presence of associated or other neurological deficits, as identified through a neurological screening by a Neurologist, served to exclude the participants from both the groups. Individuals with conductive components to their hearing loss were not included. The tympanogram types other than type 'A' and/or absent or elevated acoustic reflex thresholds (≥100 dB HL) on immittance evaluation served to exclude the participants from the study. Further, the presence of Diabetes and Hypertension also served as contra-indicators of subject selection in this group and its presence was ruled out by an experienced medical professional. Individuals with eye defects like squints, blindness or spontaneous nystagmus were not considered for this group.

Instrumentation

A calibrated Madsen Orbiter OB-922 version 2 diagnostic audiometer with Telephonics TDH-39 supra-aural headphones housed in MX-41 AR ear cushions and Radioear B-71 bone vibrator were used for pure-tone and speech audiometry. A calibrated Grason-Stadler Inc.

Tympstar was used to obtain tympanograms and acoustic reflex thresholds to rule out middle ear pathology in the participants. A calibrated Biologic Navigator Pro (version 7.0.0), with the default Etymotic ER-3A insert earphones, was used for recording auditory brainstem response (ABR) and cVEMP responses.

Procedure

All the participants of the study underwent detailed case history using the case history form used in the Audiology clinic at All India Institute of Speech and Hearing and the Neuro-Otological questionnaire (unpublished) developed by the Department of Ear, Nose and Throat, All India Institute of Speech and Hearing (Appendix I). This was done to rule out middle ear pathologies, neural pathologies and also to exclude the participants with metabolic disorders. The criterion for Definite Meniere's disease was established by using the questionnaire developed by the American Academy of Otolaryngology- Head and Neck Surgery (AAO-HNS, 1995). The results of pure-tone audiometry, immittance evaluation and auditory brainstem responses were considered for the fulfilment of the subject selection criteia.

Pure- tone and speech audiometry.

Pure-tone thresholds were established at octave frequencies from 250 Hz through 8000 Hz for air-conduction and 250 Hz through 4000 Hz for bone-conduction stimuli using modified Hughson and Westlake procedure for threshold estimation (Carhart & Jerger, 1959). The mid-octave frequency threshold was also obtained in case of a difference exceeding 20 dB HL between the adjacent octave frequencies. The Bracketing method was adopted for obtaining speech recognition threshold using the paired-words lists in the participants' native language. The speech identification scores were obtained using the phonemically balanced (PB) word list in the participants' native language using intensity levels recommended for the

respective word lists. The uncomfortable level was obtained for speech stimuli using the standard instruction given by British Society of Audiology (2011).

Immittance evaluation.

The immittance evaluation incorporated tympanometry as well as reflexometry. The participants were seated comfortably and were instructed to avoid swallowing or any other head movements during the entire length of the test. The tympanometry was done using a probe tone frequency of 226 Hz at 85 dB SPL and by varying the pressure from +200 to -400 daPa. The pump speed for variation in pressure was set to 50 daPa/s. The reflexometry involved the use of same probe tone frequency as above. In addition, it also involved the use of reflex eliciting tones at octave frequencies from 500 through 4000 Hz. Both ipsilateral as well as contralateral acoustic reflex thresholds were obtained.

Auditory brainstem response.

ABR was acquired using double-channel recording. This was done to rule out retrocochlear pathologies. The participants were made to sit comfortably in a reclining chair and were instructed to relax while the test was being performed. The skin area, used for electrode placement, was scrubbed using an abrasive skin preparation gel (NuPrep) to reduce electrode impedance. The disc-type gold-plated electrodes with wire length of 1.5 meters were placed at Cz (non-inverting electrode), M1 and M2 (inverting electrode) and F_{PZ} (ground electrode) as per International 10/20 system for electrode placement (Jasper, 1958)). The electrodes were secured in place using surgical plaster. The absolute and inter-electrode impedance was maintained below 5 kΩ and 2 kΩ respectively. The stimulus and acquisition parameters to be used for ABR recording are shown in Table 1.

Table 1

The stimulus and acquisition parameters for recording Auditory brainstem responses.

Stimulus parameters		Acquisition Parameters	
Stimulus	Click	Amplifier Gain	100000 times
Duration	100 µs	Filter setting	100-3000 Hz
Onset phase	Rarefaction	Time window	10 ms
Gating	Blackman	Number of sweeps	1500
Intensity	90 dB nHL	Notch filter	On
Rate	11.1/s & 90.1/s		
Transducer	Etymotic ER-3A insert earphones		
Mode	Monaural with ipsilateral and contralateral recording		

Note: ‘s’- seconds; ‘µs’- microseconds; ‘ms’- milliseconds; ‘Hz’- Hertz; ‘dB nHL’- decibel normalised hearing level.

Cervical vestibular evoked myogenic potential.

Following the fulfilment of the criteria using the above mentioned audio-vestibular evaluations, the participants underwent VEMP recording. The participants were seated in an upright position on a straight back chair. The electrode montage involved placement of non-inverting electrode at two-thirds the way up the SCM muscle, inverting at the sterno-clavicular junction and ground on the forehead. Cervical VEMPs were acquired using unrectified procedure as it has been shown to yield better test-retest reliability than its rectified counterpart (Anoop & Singh, 2011, Isaradisaikul et al., 2008). In order to maintain

muscle activity at a constant level, the participants were instructed to turn their head in the direction opposite to the ear of stimulation to reach a specific point on the shoulder (Acromion, a bony projection on the shoulder) with the lateral side of their chin. This point (angle of rotation of the head) was kept constant across the recordings, ears and individuals. This method for muscle tension maintenance has been shown to be equally effective and produces similar inter-aural asymmetries to the visual feedback and EMG normalization procedures (McCaslin Jacobson, Hotton, Fowler, & DeLong, 2013). This method has also been shown to produce better inter-test reliability than the visual feedback procedure for feedback on EMG (Anoop & Singh, 2011). The series of alternating polarity tone-bursts at different octave and mid-octave frequencies was used for the recording of cVEMP. The frequencies used were 250 Hz, 500 Hz, 750 Hz, 1000 Hz, 1500 Hz, 2000 Hz, 3000 Hz and 4000 Hz. Using the Blackman gating function (rise time – plateau time – fall time = 2 ms – 0 ms – 2 ms), tone-burst were presented at a rate of 5.1 Hz and at an intensity of 95 dB nHL. The response was band-pass filtered between 10 and 1500 Hz and amplified by a factor 5000. The response window was set to 70 ms, which was inclusive of 15 ms pre-stimulus recording. A total of 200 averages were obtained per recording and the recordings were repeated once in case of unclear peaks or poor morphology. The presentation order of the stimuli was counter-balanced between the recordings to avoid order effect. The artifact rejection mode was switched off throughout the recording. An inter-recording rest period of 2 minutes was given to avoid the responses getting contaminated by fatigue. The resultant waveforms were analyzed in terms of response rate, amplitude, threshold and frequency tuning properties. Response rate was considered as the percentage of ears which showed response at a particular frequency. Frequency tuning of cVEMP was considered as a particular frequency that produced largest amplitude at maximum intensity and best threshold.

Results

The study aimed at investigating the frequency tuning properties of cVEMP in individuals with Meniere's disease as well as healthy individuals and compares the frequency tuning between these groups. Further, the study also sought to explore the effect of frequency of tone-burst on the response rate, latency, amplitude and threshold of cVEMP. The waveforms recorded from one individual from each of the groups across the frequencies have been shown in Figure 1.

Descriptive statistical analysis along with the subsequent statistical procedures was done using a commercially available statistical tool- Statistical Package for Social Sciences (SPSS) version 17.0. The within group comparison involved comparison between the two ears of the same individuals and also the frequencies of tone-bursts within the same individual and ear. The disease condition (Meniere's disease versus healthy) served to compare between the groups. Unrectified cVEMPs were recorded for all the four categories of ears at octave and mid-octave frequencies from 250 Hz to 4000 Hz. For the final data analysis, only the frequencies from 500 Hz to 1500 Hz were considered owing to absence of responses at 250 Hz and above 1500 Hz in a high percentage of ears. This was done in order to avoid the loss of data during comparative analysis between groups, ears and frequencies.

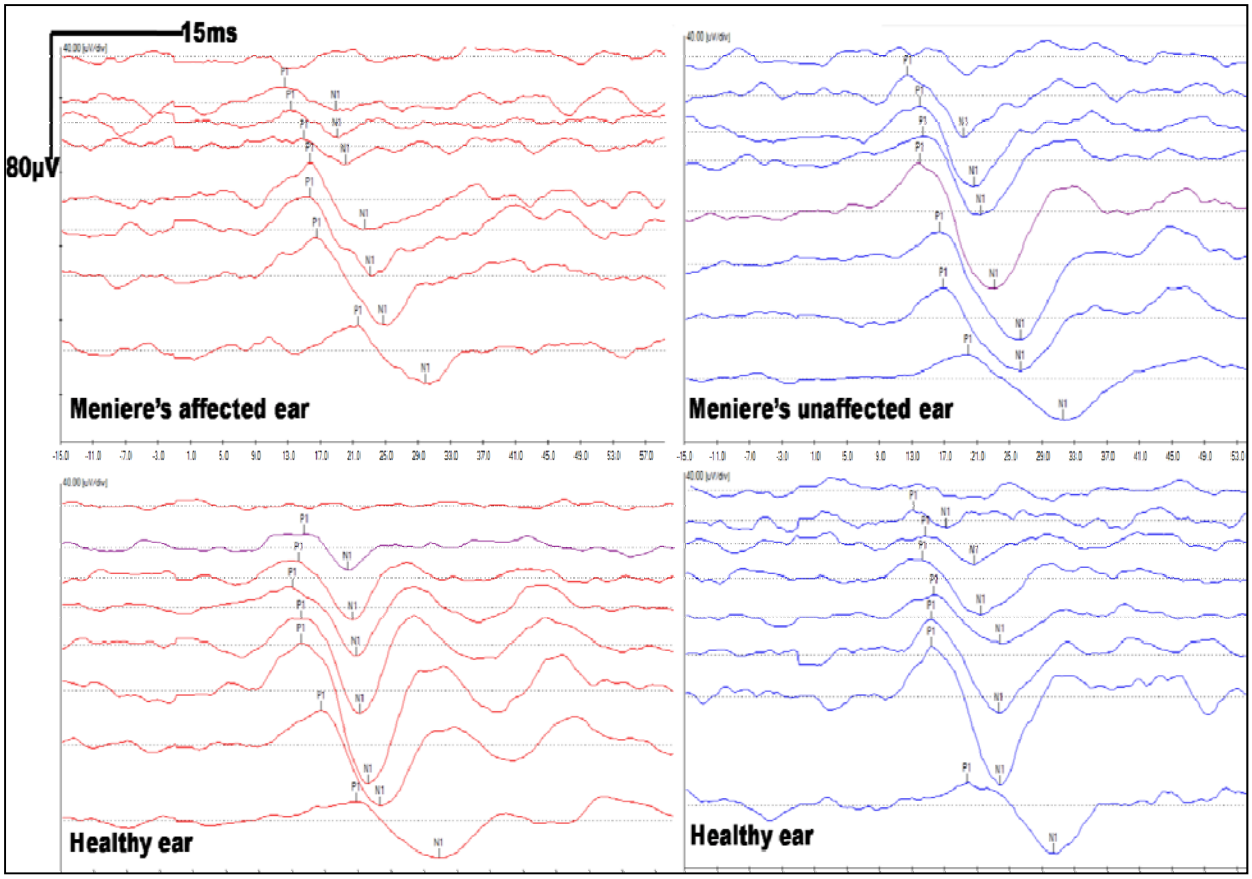

Figure 1: Representative waveforms acquired from healthy individuals and individuals with Meniere's disease arranged in increasing order of frequency from 250 to 4000 Hz from bottom to top in each panel

Response rate

The response rate, defined by percentage of ears in which the responses were present at a particular frequency and at maximum intensity, was 100% at all the frequencies up to 1000 Hz in healthy individuals and reduced dramatically for 3000 Hz and 4000 Hz. In case of the unaffected ears of individuals with MD, the response rate was lower overall compared to the healthy individuals except at 500 Hz and 750 Hz, where the response rate was 100%. The response rates in the unaffected ears of individuals with MD were largest at 500 Hz and 750 Hz and thereafter subsequently declined in either direction. In contrast, the ears affected with Meniere's disease demonstrated highest response prevalence at 750 Hz and 1000 Hz followed

by 500 Hz and 1500 Hz. The responses for other frequencies revealed a sharp decline, both for within and between group comparisons. The response rates for ears affected with Meniere's disease, unaffected ears of individuals with Meniere's disease and ears of healthy individuals across frequencies have been shown in Table 2.

Table 2

Response rates for different categories of ears across frequencies

Ears	Response rate (in %)							
	0.25 kHz	0.5 kHz	0.75 kHz	1 kHz	1.5 kHz	2 kHz	3 kHz	4 kHz
MD	62.63	77.27	90.90	95.45	77.27	62.63	31.80	13.63
Non- MD	90.90	100	100	95.45	77.27	77.27	31.80	4.54
Healthy	100	100	100	100	90.90	81.81	62.63	13.63

Note: 'kHz'- Kilo Hertz; 'MD'- affected ears of individuals with Meniere's disease; Non-MD'- unaffected ears of individuals with Meniere's disease; 'Healthy'- ears of healthy individuals.

Latency

The VEMP responses were analyzed for two prominent and consistently occurring peaks namely P13 and N23. The mean along with 95% confidence intervals for the P13 as well as N23 latencies is shown in Figure 2. The latencies for both the peaks were nearly overlapping across the categories. However within each of the groups, there was a trend towards reduction in latency with increase in frequency for both the peaks. On visual inspection, the slope of N23 appeared to be sharper compared to P13 latency.

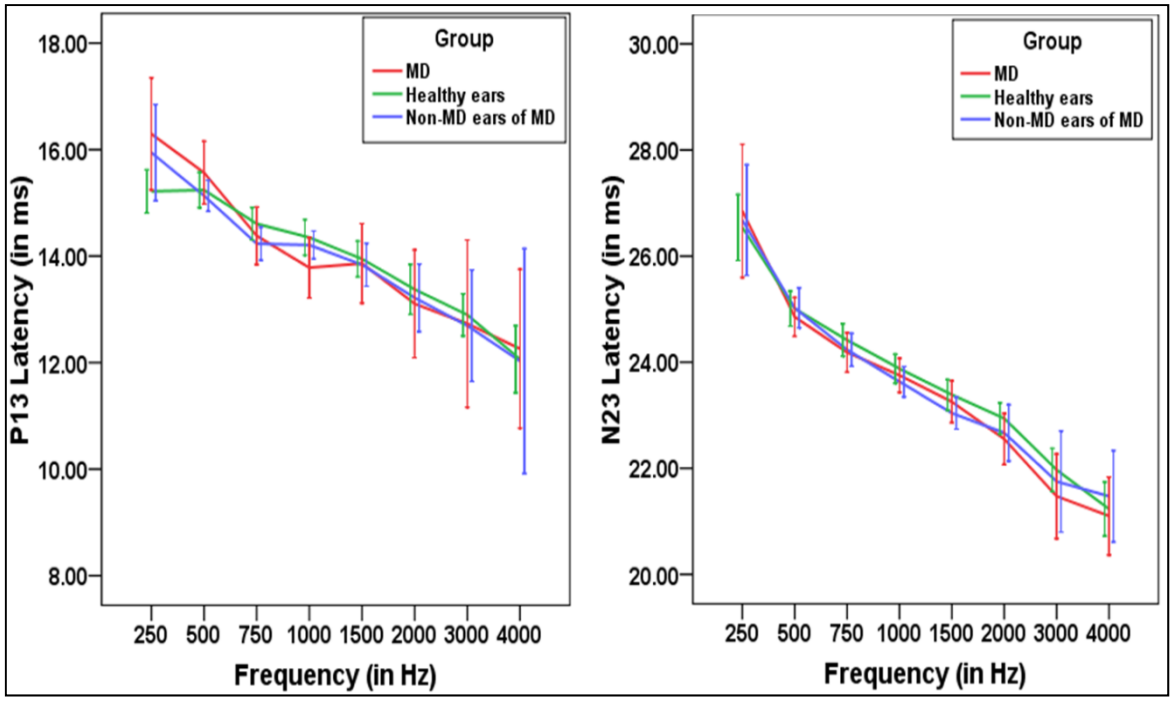

Figure 2: P13 (left panel) and N23 (right panel) latencies (mean and 95% confidence intervals) of cVEMP for all the four categories across frequencies

Two-way repeated measures ANOVA was done for ears and frequencies with group as between subject factor in order to investigate the statistical significance of the above observed trend. The results revealed no significant main effect of ears [$F(1,42) = 0.001$, $p>0.05$] as well as groups [$F(1,42) = 0.50$, $p>0.05$] on the latency of P13. However, there was a significant main effect of frequencies on the P13 latency [$F(3, 126) = 58.83$, $p<0.001$]. Also, there was no interaction between any of the within and between subject variables ($p>0.05$). The Bonferroni adjusted multiple comparisons revealed significant difference between all the possible pairs ($p<0.001$). The only exception to this was the comparison of 1000 Hz with 750 Hz and 1500 Hz, both of which were not significantly different ($p>0.05$). In case of N23, the Two-way repeated measures ANOVA revealed similar results to those obtained for P13 latency. While there was no significant main effect of ears [$F(1,42) = 0.74$, $p>0.05$] as well as groups [$F(1,42) = 0.71$, $p>0.05$], there was a significant main effect of frequency [$F(3,126) = 58.83$, $p<0.001$] on N23 latency. Additionally, there was also a lack of

interaction between the variables of the study ($p>0.05$). The results of Bonferroni adjusted multiple comparisons demonstrated the existence of significant difference between all the possible pairs of frequencies ($p<0.001$).

Amplitude

The P13 and N23 peak-to-peak amplitude was analyzed. The peak-to-peak amplitude was observed to be largest for ears healthy individuals followed by unaffected ears of individuals with Meniere's disease. The affected ears of individuals with Meniere's disease produced smallest amplitudes. Table 3 shows the mean and standard deviation of cVEMP amplitudes for ears of both the groups across frequencies.

Table 3

Mean and standard deviation of peak-to-peak amplitude of cVEMP for ears of both ears across frequencies

Ears	Amplitude (in μV)							
	0.25 kHz	0.5 kHz	0.75 kHz	1 kHz	1.5 kHz	2 kHz	3 kHz	4 kHz
MD	26.84 ± 15.75	30.47 ± 20.76	40.95 ± 25.11	37.86 ± 25.41	26.04 ± 19.16	24.64 ± 24.64	16.99 ± 11.34	14.97 ± 4.08
Non- MD	31.84 ± 29.59	44.91 ± 36.53	45.63 ± 34.51	39.90 ± 26.88	30.20 ± 23.56	25.68 ± 26.25	27.04 ± 19.49	11.81 ± 3.35
Healthy	40.79 ± 22.14	55.53 ± 21.78	49.12 ± 23.11	43.27 ± 19.82	38.67 ± 21.67	23.18 ± 14.78	15.95 ± 6.07	13.67 ± 1.98

Note: 'MD'- affected ears of individuals with Meniere's disease; 'Non-MD'- unaffected ears of individuals with Meniere's disease; 'Healthy'- ears of healthy individuals.

The above observed difference in amplitudes between the categories was evaluated for their statistical significance using Two-way repeated measures ANOVA. This was done for ears and frequencies with group as between subject factor and the results revealed significant main effect of frequencies [$F(3, 126) = 40.03$, $p<0.001$] but not of ears [$F(1, 42) = 2.71$, $p>0.05$] and groups [$F(1,42) = 2.13$, $p>0.05$] on peak-to-peak amplitude. Further, there was no interaction between any of the variables ($p>0.05$). The Bonferroni adjusted multiple comparisons revealed the amplitude at 1500 Hz to be significantly smaller than the other three frequencies ($p<0.001$). The amplitude for 1000 Hz tone-burst was also observed to significantly smaller than that at 750 Hz and 500 Hz ($p<0.05$). However, there was no significant difference in amplitude between 750 Hz and 500 Hz ($p>0.05$).

Threshold

The threshold was defined as the lowest intensity at which reliably identifiable VEMP peaks could be observed. Table 4 shows the mean and standard deviation of thresholds at different frequencies among ears of both the groups.

Table 4

Mean and standard deviation of threshold of cVEMP for ears of both ears across frequencies

Ears	Threshold (in dB SPL)							
	0.25 kHz	0.5 kHz	0.75 kHz	1 kHz	1.5 kHz	2 kHz	3 kHz	4 kHz
MD	127.14 ± 4.68	122.72 ± 7.67	120.45 ± 8.98	120.00 ± 9.25	123.92 ± 7.89	129.28 ± 2.67	130.00 ± 0.00	130.00 ± 0.00
Non- MD	126.42 ± 6.33	120.90 ± 8.67	121.36 ± 8.88	121.36 ± 7.74	125.45 ± 5.95	126.46 ± 3.75	130.00 ± 0.00	130.00 ± 0.00
Healthy ears	117.72 ± 8.58	116.59 ± 6.07	117.50 ± 7.19	120.68 ± 5.86	123.63 ± 6.13	127.69 ± 4.26	128.92 ± 3.14	130.00 ± 0.00

Note: 'MD'- affected ears of individuals with Meniere's disease; 'Non-MD'- unaffected ears of individuals with Meniere's disease; 'Healthy'- ears of healthy individuals.

Two-way repeated measures ANOVA was done for ears and frequencies with group as between subject factor. The results revealed a significant main effect of frequencies [$F(3, 126) = 15.43$, $p<0.001$] and groups [$F(1,42) = 4.32$, $p<0.05$] but not of ears [$F(1,42) = 0.98$, $p>0.05$] on threshold. The significant interactional effect was observed only between frequencies and group [$F(3, 126) = 4.32$, $p<0.01$]. In order to resolve this interactional effect

between groups and frequencies, the groups were compared at each frequency separately. For this, MANOVA was done. The results revealed significant difference between the affected ears of individuals with MD and the healthy individuals [$F(1, 34) = 17.72$, $p<0.001$] at 500 Hz. It also showed significant difference between unaffected ears of individuals with MD and healthy individuals' ears at [$F(1, 34) = 11.49$, $p<0.01$] at 500 Hz. The MD group had significantly poorer threshold than the healthy individuals. In individuals with MD, the affected ears showed poorer threshold than the unaffected ears. Further, frequencies were also compared within each of the groups. In order to compare between frequencies within groups, repeated measures ANOVA was done. In individuals with MD, affected ears showed no significant main effect [$F (3, 63) = 1.17$, $p>0.05$] whereas unaffected ears showed marginally significant main effect of frequencies [$F(3, 63) = 2.62$, $p = 0.058$] on threshold. The Bonferroni adjusted multiple comparisons between the thresholds of different frequencies in the unaffected ears of MD revealed no difference between any of the pairs of frequencies ($p>0.05$). While evaluating the effect of frequency on the thresholds of healthy ears, a significant main effect was observed for both MD matched healthy ears [$F(3, 63) = 9.24$, $p<0.001$] as well as in unaffected ears of MD matched healthy ears [$F(3, 63) = 13.79$, $p<0.001$]. Bonferroni adjusted multiple comparisons revealed the threshold at 1500 Hz to be significantly different from all others ($p<0.05$) in both the categories of ears of healthy individuals. For all other pairs of frequencies, there was no significant difference in threshold ($p>0.05$).**Frequency tuning properties**

In a particular individual, the cVEMP was considered to be tuned to a particular frequency that produced largest amplitude at maximum intensity and smallest (best) threshold of cVEMP. Table 5 shows the ears of individuals of both the groups which produced largest (best) amplitudes and lowest (best) thresholds.

Table 5

Frequency corresponding to best amplitude in ears of healthy individuals and individuals with Meniere's disease

Age (in years) /Gender	Individuals with MD		Healthy individuals	
	Affected	Unaffected	Affected matched	Unaffected matched
50/F	1500 Hz	750 Hz	750 Hz	750 Hz
35/M	750 Hz	750 Hz	750 Hz	750 Hz
47/M	1000 Hz	750 Hz	500 Hz	500 Hz
47/F	1000 Hz	750 Hz	750 Hz	750 Hz
35/F	1000 Hz	1000 Hz	500 Hz	500 Hz
28/M	750 Hz	500 Hz	750 Hz	500 Hz
32/F	750 Hz	500 Hz	500 Hz	500 Hz
40/M	750 Hz	750 Hz	500 Hz	500 Hz
45/F	1500 Hz	1000 Hz	500 Hz	750 Hz
50/M	1500 Hz	1500 Hz	750 Hz	750 Hz
46/F	1000 Hz	750 Hz	500 Hz	500 Hz
38/M	1000 Hz	750 Hz	750 Hz	750 Hz
31/M	1000 Hz	750 Hz	500 Hz	750 Hz
24/M	750 Hz	500 Hz	750 Hz	750 Hz

32/M	750 Hz	750 Hz	500 Hz	500 Hz
39/F	750 Hz	750 Hz	500 Hz	500 Hz
41/F	750 Hz	500 Hz	500 Hz	500 Hz
48/F	750 Hz	500 Hz	500 Hz	500 Hz
44/M	750 Hz	500 Hz	500 Hz	500 Hz
49/F	1000 Hz	750 Hz	500 Hz	500 Hz
41/F	750 Hz	750 Hz	500 Hz	500 Hz
44/F	1000 Hz	1000 Hz	750 Hz	500 Hz

Note: ‘M’- Male; ‘F’- female; ‘Hz’- Hertz; ‘MD’- Meniere’s disease.

The comparison between the frequencies demonstrated largest amplitudes at 500 Hz in 63.64% and at 750 Hz in the remaining 36.36% of healthy ears. There were no healthy ears with highest amplitudes at any other frequency. Thus the mode frequency was 500 Hz among the ears of healthy individuals. In the ears with Meniere’s disease, largest amplitudes were observed in 11 (50%) ears at 750 Hz. This was followed by 1000 Hz, where 8 (36.36%) ears demonstrated largest amplitudes. Apart from these two frequencies, largest amplitudes were also noticed at 1500 Hz in 3 (13.63%) ears. The category with Meniere’s ears failed to show largest amplitude at 500 Hz in even a single ear. Hence the mode cVEMP frequency was 750 Hz among the affected ears of the individuals with Meniere’s disease. The unaffected ears of individuals with Meniere’s disease produced results which were overlapping with both the above discussed categories and portrayed the most widespread peak frequency distribution ranging from 500 Hz to 1500 Hz. The largest amplitudes were observable, though in different proportions, at all the frequencies where largest amplitudes were observed in the above

groups. While lion's share was exhibited by 750 Hz for largest amplitude (54.54% ears showing largest amplitude), 1500 Hz demonstrated highest amplitude in only one (4.5%) ear. Out of the remaining ears, 6 (27.27%) ears showed largest amplitude at 500 Hz and 3 (13.63%) ears were found to have largest amplitude at 1000 Hz thereby resulting in mode frequency at 750 Hz. Figure 3 shows the graphical representation of category comparison across frequencies.

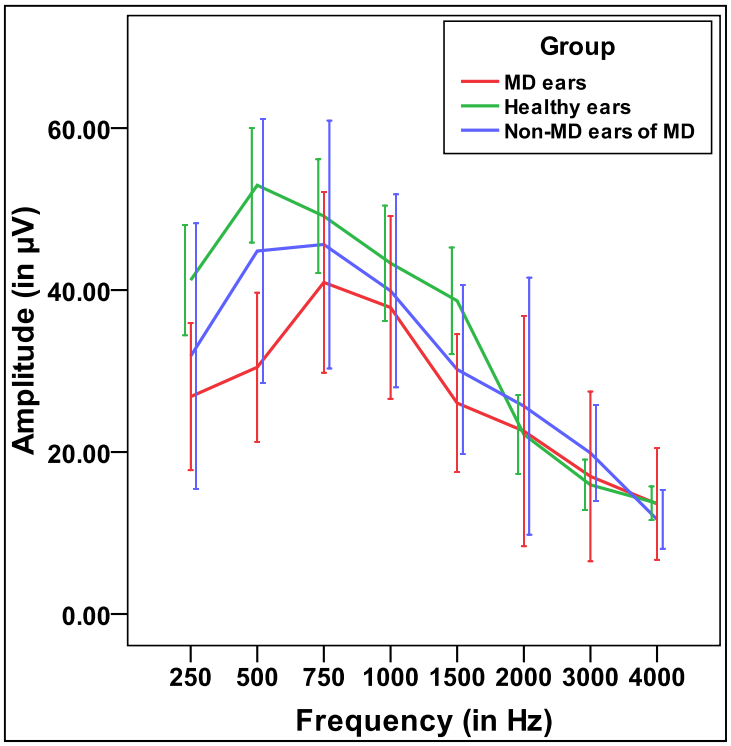

Figure 3: Amplitude (mean and 95% confidence intervals) of cVEMP across frequencies for ears with Meniere's disease, unaffected ears of individuals with Meniere's disease and ears of healthy individuals

In terms of threshold, there were several individuals in both the groups who demonstrated lowest (best) thresholds at more than one frequency. Nonetheless, the best mean and mode thresholds were observed at 500 Hz for both the ears of healthy individuals. The affected ears with Meniere's disease revealed best mean and mode thresholds at 1000 Hz, which was closely followed by 750 Hz. In stark contrast to these, the unaffected ears of

individuals with Meniere's disease failed to show any clear leader in terms of best mean threshold at any particular frequency. The best mean thresholds were shared between 500 Hz, 750 Hz and 1000 Hz, with a large chunk of the ears showing equal thresholds across these frequencies. Figure 4 shows the plot of threshold against frequency for ears of healthy individuals and affected as well as unaffected ears of individual with Meniere's disease.

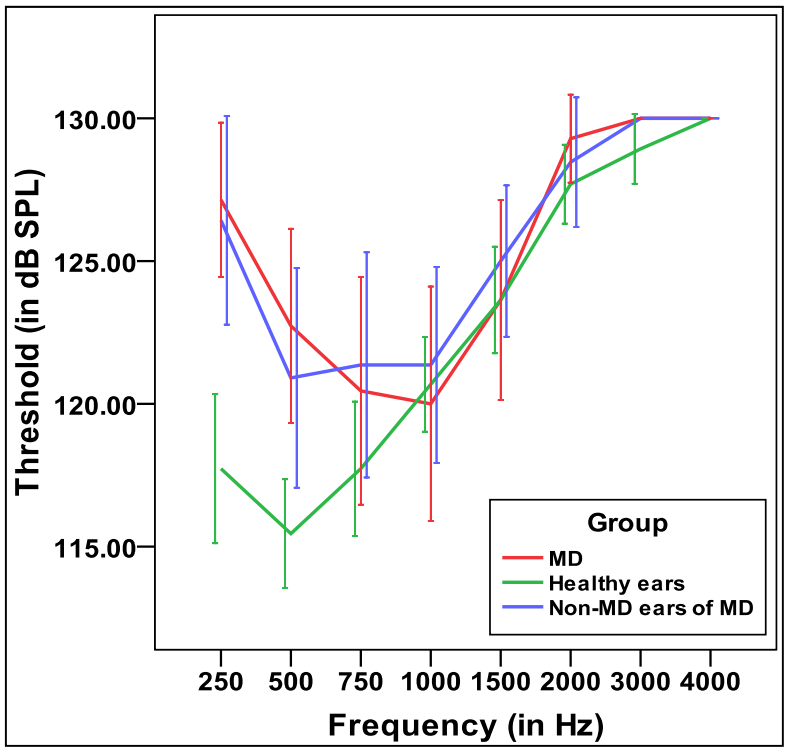

Figure 4: Threshold (mean and 95% confidence interval) of cVEMP for affected ears of individuals with Meniere's disease, unaffected ears of individuals with Meniere's disease and ears of healthy individuals

Thus, combining the results of amplitude and threshold, largest amplitude along with lowest thresholds were observed at 500 Hz among healthy individuals. The unaffected ears also demonstrated largest amplitude and lowest threshold in majority of individuals at 500 Hz and 750 Hz, though 3 ears showed this to occur at 1000 and 1 ear at 1500 Hz. In the affected ears with Meniere's disease, the largest amplitudes and lowest thresholds were observable at 750 Hz as well as 1000 Hz.

The sensitivity and specificity values for diagnosis of Meniere's disease using the frequency tuning properties were assessed using Receiver operating characteristic (ROC) curve. The ROC curves were obtained using commercially available MediCal software. The ROC curve for frequency tuning has been shown in Figure 5. The area under the curve was 0.90. The optimum frequency tuning was decided as criterion point that produced the best combination of sensitivity and specificity. This was found to be a frequency tuning of >500 Hz. Use of this criterion produced a sensitivity of 100% and specificity of 63.64%. Based on this criterion, 68.18% of unaffected ears of individuals with Meniere's disease tested positive for Meniere's disease. The sensitivity and specificity corresponding to other criterion points has been shown in Appendix-I

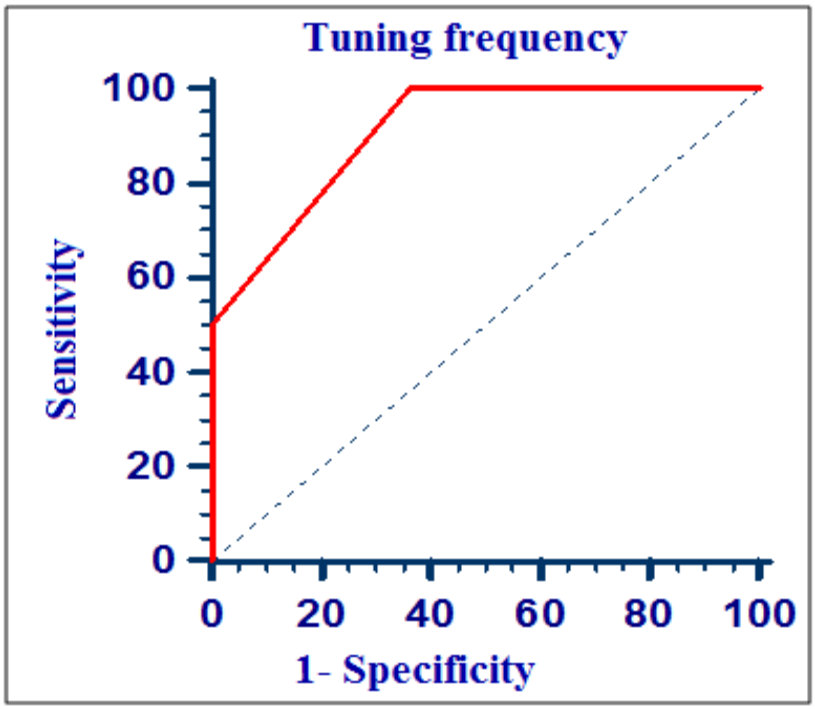

Figure 5: Receiver operating characteristic curve for diagnosis of Meniere's disease using frequency tuning properties. The dotted diagonal line represents the reference. The red curve shows the sensitivity and specificity value.

Discussion

The present study evaluated the frequency tuning properties of cVEMP among healthy individuals as well as individuals with Endolymphatic hydrops (Meniere's disease). It also evaluated the effect of varying frequency of stimulus on several cVEMP parameters like response rate, latency, amplitude and threshold.

Response rate

The response rates were found to be 100% for frequencies up to 1000 Hz and reduced thereafter towards high frequencies among healthy individuals. This is in agreement with those reported previously in this regard (Janky & Shepard, 2009; Piker, 2012;Sandhu et al., 2012). Janky and Shepard (2009), in their study on age-related changes in VEMP response parameters, reported nearly 100% response rates at different frequencies till 1000 Hz but only 50% for clicks. Though they did not explore the effect of tone-burst frequencies beyond 1000 Hz on response rates, the response rate for clicks can be similar to those of high frequency stimuli. This is owing to the fact that maximum energy content of click stimuli has been shown to occur in the region of 2000 Hz to 4000 Hz and the auditory brainstem responses, to click stimuli have been reported to be well correlated with behavioral responses in this frequency region (Coats & Martin, 1977; Gorga, Worthington, Reiland, Beauchaine, & Goldgar, 1985; Jerger & Mauldine, 1978). This viewpoint draws support from Piker (2012) who showed only 46% response rate at 2000 Hz as against 100% at frequencies before 2000 Hz. Sandhu et al. (2012) presented a very similar picture of the response rates in healthy individuals to the findings in the present study.

The response rates in individuals with Meniere's disease were slightly different across the frequencies. While the response rates among healthy individuals were found to be 100% up to 1000 Hz, they were in excess of 90% only at 750 Hz and 1000 Hz and subsequently

declined on either side in individuals with Meniere's disease. This is in consonance with Rauch et al. (2004), who also reported response rates at 250 Hz, 500 Hz and 1000 Hz to be 82%, 85% and 94% respectively among the ears of individuals with Meniere's disease. While evaluating the patients with Meniere's disease, Timmer et al. (2006), reported response rate of 87% at 500 Hz. This response rate appears close to the response rate observed at 500 Hz (≈78%) in the present study. Though Timmer et al. (2006) evaluated the responses at 250 Hz as well as 1000 Hz, they did not present the data of response rates at these frequencies in their study. Sandhu et al. (2012) reported largest response prevalence at 500 Hz (100%) closely followed by 750 Hz and 1500 Hz (88% at each) and subsequent decline thereafter on either side. Except for the differences in frequency with maximum response rate, which was at 1000 Hz and 750 Hz (95.45% and 90.90%) in the present study as against 500 Hz in Sandhu et al. (2012), the findings of present study are in agreement with those of Sandhu et al. (2012). The differences in the response prevalence in the present study from Sandhu et al (2012) might be attributed to the differences in sample size and be compounded by use of four participants with 'Probable Meniere's disease' in addition to eight participants with 'Definite Meniere's disease as against the use of twenty participants with 'Definite Meniere's disease' in the present study. Thus, the highest response prevalence occurs at frequencies from 750 Hz to 1000 Hz in affected ears with Meniere's disease, at least in the 'Definite Meniere's disease' category.

Among the ears of individuals with Meniere's disease which were unaffected, the response rate was observed to be 100% at 500 Hz, 750 Hz and 1000 Hz in the present study and declined sharply thereafter on either side of these frequencies. This is in agreement with those that reported previously (Sandhu et al., 2012). They also observed largest response prevalence of cVEMP at 500 Hz, 750 Hz and 1000 Hz and decline in response rate before 500 Hz and after 1000 Hz.

Latency

Latencies were compared within and between groups and the results showed a lack of difference between the groups. The latencies across the frequencies were similar for ears of healthy individuals as well as the affected and unaffected ears of individuals with Meniere's disease. This is in agreement with those reported previously in this context (Rauch et al., 2004). Other previous studies have also shown a lack of diagnostic relevance of latencies for Meniere's disease (Ribeiro, de Almeida, Caovilla, & Gananca, 2005; Zerei, Ghahraman, Daneshi, Emamjomeh, Memari, Akbari, & Faghihzadeh, 2009). They reported the latencies of cVEMP to be similar between ears of healthy individuals and those of individuals with Meniere's disease and thus lend support to the findings of the present study.

In terms of within group comparisons, the results demonstrated a lack of ear effect thereby showing no difference in latencies between the ears irrespective of the groups. However, the latencies of P13 were observed to be significantly affected by changes in frequencies across both the groups. There was nearly a constant reduction in the latency of P13 with increasing frequencies from 250 Hz to 4000 Hz. This is in partial or complete consonance with a number of previously reported studies (Rauch et al., 2004; Janky & Shepard, 2009; Piker, 2012). Piker (2012) reported the latencies of P13 corresponding to 1500 Hz and 2000 Hz tone-bursts to be significantly shorter than those corresponding to 250 Hz, 500 Hz, 750 Hz and 1000 Hz and a lack of significant difference for comparison among the frequencies between 250 Hz and 1000 Hz. Janky and Shepard (2009), in their study evaluating the effect of age on cVEMP, obtained responses for different frequency tone-bursts (250 Hz, 500 Hz, 750 Hz, & 1000 Hz). However, they did not discuss the results with regards to effect of frequency on latency. In the supplementary table though, the decline in latencies with increasing frequency of stimuli could be observed. In yet another study, Rauch

et al. (2004) observed a slight effect of frequency on latency, similar to those observed in the present study, and opined that the effect could be more related to the changes in rise time of the stimulus than actual changes in frequency. In the present study, the rise/fall time was maintained at 2 ms irrespective of the stimulus frequency; still the trend of change in latency with increasing stimulus frequency was similar to those reported earlier by Rauch et al. (2004). Thus, it can be said that changes in P13 latency is a result of stimulus frequency change rather than the rise time.

In the present study, the effect of stimulus frequency on the latency of N23 was also assessed. The latencies of N23 portrayed a significant trend towards reduction with increasing stimulus frequency. However, the above discussed studies have not presented the data for the effect of stimulus frequency on the latency of N23. Being the part of same response complex as P13, a similar change in the response latency with changing stimulus frequency could be explained.

Amplitude

The VEMP amplitude for all the three categories showed a overlapping pattern. Majority of the ears of healthy individuals produced largest amplitude at 500 Hz followed by a smaller section with largest amplitude at 750 Hz. Apart from these two frequencies, not even one ear of healthy individuals produced largest amplitude at any other frequency. This is in consonance with the previous studies, majority of which demonstrated the incidence of largest amplitude at either 500 Hz (Rauch et al., 2004; Node et al., 2005; Piker, 2012; Sandhu et al., 2012). Among others who refuted 500 Hz as the frequency with largest corresponding amplitude, Welgampola and Colebatch (2001) reported the largest amplitude to occur at 500 Hz in some individuals and 1000 Hz in others. However, they did not explore 750 Hz specifically. Thus there may be a likelihood of largest amplitude at 750 Hz among those who

demonstrated largest amplitude at 500 Hz or 1000 Hz. The findings of the present study are in complete contrast with those reported earlier by Todd, Cody and Banks (2000). They reported largest amplitudes for frequencies in the region of 300 to 350 Hz. The differences might be attributed to the variable use of the stimulus and acquisition parameters between the two set of studies. For instance, Todd et al. (2000) used tone-pips compared to tone-bursts used in the present study. The tone-pips are believed to be less frequency specific with considerably larger amounts of energy availability in the frequency side-pockets to centre frequency when compared to tone-bursts (Roser, Valente & Dunn, 2007). In order to minimize such frequency splatter, the present study used tone-bursts. While Welgampola and Colebatch (2005) suggested that VEMP tuning (largest amplitude at a particular frequency) originated because of an electrical resonance of the hair cells, Todd et al. (2000) suggested that the frequency tuning properties of VEMP could be successfully modelled by a 2nd-order mechanical system containing elements of mass and stiffness. For the otolith organs, the inertial force might be expected to be supplied by the mass of the otoconia, which has a density equivalent to 2.71 g/cm ^{3} (Carlstrom, 1963). In addition to the coupling of the sensory hair cells with the overlying visco-elastic mesh-gel layer, the contribution to stiffness may be arising from the relatively inelastic nature of the membranous labyrinth (Rabbit, Damiano, & Grant 2004). These elements work against each other and differ in their property across frequencies; mass limits responses to high-frequency stimuli while stiffness acts as a limiting factor at low frequencies. This would result in resonance at a frequency where the relative effects of stiffness and mass are equivalent and thus end up cancelling each other. Thus, the incidence of largest amplitude at 500 Hz or 750 Hz among ears of healthy individuals might be attributed to the mechanical resonance of the Saccule around these frequencies in such individuals.

In the affected ears of individuals with Meniere's disease, the largest amplitude was shared between 750 Hz and 1000 Hz for a large majority of the individuals. While a small minority of subjects revealed largest amplitude at 1500 Hz, none of the ears with Meniere's disease produced largest amplitude at 500 Hz, which demarcates the ears of healthy individuals from the ears affected with Meniere's disease. Thus, there was a change in the dynamics of the response corresponding to different frequencies and a shift in frequency corresponding to largest amplitude. This is in agreement with those reported previously in this context (Rauch et al., 2004; Node et al., 2005; Sandhu et al., 2012). A distention of Saccular membrane has been assumed to result from the accumulation of fluid within the Saccule. In a simple mass-spring-damping system, increasing membrane elastance, as might be expected to occur with endolymphatic hydrops, would increase the resonant frequency. The observed responses in VEMP are consistent with increasing resonance (Todd et al., 2000). Thus, the larger amplitudes of cVEMP responses at ≥750 Hz among the affected ears of individuals with Meniere's disease when compared to ≤750 Hz in the ears of healthy individuals are very likely caused by the changes in the mechanical resonance of the Saccule.

As far as the unaffected ears of the individuals with Meniere's disease are concerned, the largest amplitude was observed to be spread across the frequencies from 500 Hz to 1500 Hz. This is in consonance with those reported in literature in this regard (Rauch et al. 2004; Node et al., 2005). From the findings of the present study and those reported earlier, it is clear that the unaffected ears of individuals with Meniere's disease also show abnormality in terms of amplitude, which could be the result of occult bilateral disease in individuals with unilateral Meniere's disease. The hint for such an incidence has been reported previously, though through the use of other testing methods, among individuals with unilateral Meniere's disease. The findings in literature have shown that Meniere's disease has a tendency to progress to the other ear within 2-7 years of its onset in one ear in up to 50% of cases

(Salvinelli, Trivelli, Greco, Silvestrini, Fernandez, & Pallini, 1999; Jackson & Silverstein, 2002). Additionally, there could also be a central binaural interaction, where vestibulocolic reflex plays a role in compensating for the loss by reducing the functioning of the contralateral side (Rauch et al., 2004).

Threshold

Like amplitude, the frequencies corresponding to the lowest (best) threshold differed between the groups. The ears of healthy individuals most often failed to centre the best threshold at any one frequency; most often showing two or more frequencies with best threshold in a particular ear. However, the healthy individuals produced best mean as well as mode threshold at 500 Hz for cVEMP. This is in agreement with that reported previously Rauch et al. (2004), who also observed best threshold at 500 Hz for healthy individuals. However, in another study, Node et al. (2005) reported a more widespread distribution of the frequency corresponding to best threshold among healthy individuals. They observed best thresholds at 500 Hz (in 14 individuals), 700 Hz (in 11 individuals) and 1000 Hz (in 10 individuals) for the healthy ears. In contrast to the above studies, Todd et al. (2000) reported best thresholds between 300 Hz and 350 Hz for healthy individuals. The differences between the findings, as for amplitude, might again be attributed to the variable use of stimulus and acquisition parameters by the studies showing differences in the outcomes.

The affected ears with MD showed best mean and mode threshold at 750 Hz followed by 1000 Hz. The findings of the present study are similar to those reported earlier (Rauch et al., 2004; Node et al., 2005). The shift found in the affected ears of individuals with Meniere's disease could be attributed to the changes in the biomechanical properties of Saccule as a result of increase endolymph volume within it, as explained earlier in the discussion segment on amplitude.

For the unaffected ears of individuals with Meniere's disease, best thresholds were almost equally shared between 500 Hz, 750 Hz and 1000 Hz. According to Rauch et al. (2004), the unaffected ears of individuals with Meniere's disease also showed best thresholds to shift towards higher frequencies. The threshold shift was less for the unaffected ears of individuals with Meniere's disease than their affected ears. The reason for shift in threshold for unaffected ears of individuals with Meniere's disease could be explained on the basis of two probable reasons. First, there could be prevalence of occult bilateral disease in unilateral MD. Many clinical studies have shown involvement of second ear within 2-7 years of its onset in the first ear in up to 50% of individuals with unilateral Meniere's disease (Thomas & Harrison, 1971; Green, Blum, & Harner, 1991, Salvinelli et al., 1999; Jackson & Silverstein, 2002). Second, there could also be a contribution from the central binaural interaction, where vestibulocolic reflex plays a role in compensating for the loss by reducing the functioning of the contralateral side. However, if the second reasoning was to hold good, other unilateral peripheral vestibular pathologies should also produce contralateral ear response patterns. Nonetheless, extensive research report is lacking in this regards and calls for further probing.

Frequency tuning properties

The results of the present study indicated towards the centring of frequency tuning between 500 Hz and 750 Hz among healthy individuals with a large majority exhibiting tuning at 500 Hz, both for amplitude as well as threshold. This, however, was observed to tilt more towards higher frequencies, specifically towards 750 Hz and 1000 Hz among the ears affected with Meniere's disease. There was also an observation of slight tilt in the frequency tuning of unaffected ears of individuals with Meniere's disease with 750 Hz emerging as the mode frequency for largest amplitude and best threshold in this category of ears. These findings are in consonance with those reported previously for frequency tuning among ears of

healthy individuals (Rauch et al., 2004; Node et al., 2005; Piker, 2012) and affected as well as unaffected ears of individuals with Meniere's disease (Rauch et al., 2004; Node et al., 2005). The reasons for such occurrences have been explained in the earlier sections on amplitude and threshold for healthy individuals as well as individuals with Meniere's disease.

The sensitivity and specificity of use of frequency tuning technique in the identification of Meniere's disease was found to be 100% and 63.64% respectively. Previous studies in this context have not assessed this aspect and hence the findings of the present study may be considered the first instance in this regard. Thus, an excellent sensitivity coupled with a high enough specificity appears to project the assessment through frequency tuning technique as a promising alternative for the identification of Meniere's disease.

Conclusion

The findings of the present study revealed that the amplitude is smaller and threshold higher (poorer) in individuals with Meniere's disease compared to their healthy counterparts. The latency, however, does not differ between the two groups. While amplitudes are largest and thresholds best at 500 Hz and 750 Hz among ears of healthy individuals thereby contributing for frequency tuning of cervical VEMP between 500 and 750 Hz, those with Meniere's disease demonstrate the shift in largest amplitude and best threshold towards higher frequencies, specifically ≥750 Hz. A considerable proportion of the subjects with Meniere's disease show shift in tuning property of VEMP, in both affected as well as unaffected ears with larger shift in the affected ears than the unaffected ears. This might be useful in predicting a future progression to bilateral condition from a monaural onset, however further longitudinal studies are required to ascertain this aspect. Thus, shift in frequency tuning of cVEMP could be useful tool in the diagnosis of Meniere's disease, if cVEMP responses are present. However, some of the above mentioned parameters did not

show statistically significant differences between the groups. The differences for these have been explained based on descriptive statistics.

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