COMPARISON BETWEEN FREQUENCY TUNING AND FREQUENCY – AMPLITUDE RATIO OF CERVICAL VESTIBULAR EVOKED MYOGENIC POTENTIAL FOR IDENTIFICATION OF MENIERE'S DISEASE

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May, 2014

CERTIFICATE

This is to certify that the dissertation entitled "**Comparison between Frequency Tuning and Frequency –Amplitude Ratio of Cervical Vestibular Evoked Myogenic Potential for identification of Meniere's disease"** is a bonafide work submitted in part fulfillment for the Degree of Master of Science (Audiology) of the student (Registration no. 12AUD021). This has been carried out under the guidance of a faculty of this institute and has not been submitted earlier to any Universities for the award of any Degree or Diploma.

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CERTIFICATE

This is to certify that the dissertation entitled "**Comparison between Frequency Tuning and Frequency –Amplitude Ratio of Cervical Vestibular Evoked Myogenic Potential for identification of Meniere's disease"** has been prepared under my supervision and guidance. It is also certified that this has not been submitted earlier in other University for the award of any Degree or Diploma.

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DECLARATION

This is to certify that this dissertation entitled "**Comparison between Frequency Tuning and Frequency –Amplitude Ratio of Cervical Vestibular Evoked Myogenic Potential for identification of Meniere's disease"** is the result of my own study under the guidance of Mr. Niraj Kumar Singh, Lecturer, Department of Audiology, All India Institute of Speech and Hearing, Mysore, and has not been submitted earlier in other University for the award of any Degree or Diploma.

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TABLE OF CONTENTS

List of figures

List of Tables

CHAPTER 1

Introduction

The vestibular evoked myogenic potentials (VEMP) are short latency myogenic potentials generated in response to stimulation of the otoloith organs either by acoustic stimulation (Colebatch & Halmagyi, 1992; Colebatch, Halmagyi, & Skuse, 1994; Welgampola & Colebatch, 2004), mechanical stimulation (Halmagyi , Yavor, & Colebatch, 1995) or electrical (galvanic) stimulation (Watson & Colebatch, 1998; Murofushi, Takegoshi, Ohki, & Ozeki, 2002). VEMP assesses two of the major vestibular reflex pathways, the vestibulo-occular reflex pathway and the vestibulo-spinal reflex pathway. Vestibulo-occular reflex pathway is assessed by ocular vestibular evoked myogenic potential (oVEMP) and the vestibulo-spinal reflex pathway can be assessed by the cervical vestibular evoked myogenic potential (cVEMP).

cVEMP is an inhibitory reflex recorded from tonically contracted ipsilateral sternocleidomastoid (SCM) muscle in response to stimulation of the saccule and the inferior vestibular nerve (Colebatch & Halmagyi, 1992). Although this response can be recorded from many different muscles, Colebatch et al. (1994) described a reliable method for recording this response from the upper half of the sternocleidomastoid (SCM) muscle using high-intensity click stimuli. The response is believed to reflect a stimulus synchronized reduction in tonic muscle activity (McCaslin, Jackobson, Hatton, Fowler, & De Long, 2013). Colebatch et al (1994) described the characteristics of cVEMP as an initial large biphasic positive-negative response (P13-N23) seen in all individuals with an intact vestibular system. Even individuals with severe to profound hearing loss with normal caloric responses have been found to show normal VEMP responses

(Ozeki, Matsuzaki, & Murofushi, 1999). However, these responses are usually absent or affected in case of most of the vestibular disorders like acoustic neuroma (Murofushi, Matsuzaki, & Mizuno, 1998; Murofushi, Shimzu, Takegoshi, & Cheng, 2001; Streubel, Cremer, Carey, Weg, & Minor, 2001; Suzuki, Yamada, Inoeu, Kashio, Saito, & Nakanishi, 2008), superior canal dehiscence syndrome (Brantberg, Bergenius & Tribukait, 1999; Brantberg, Berginius & Tribukait., 2001), vestibular neuritis (Ochi, Ohasi & Watanabe, 2003), auditory/audiovestibular neuropathy (Kumar, Sinha, Singh, Bharati, & Barman, 2008; Sazgar, Yazdani, Yazdi, & Rezazadeh, 2010; Sinha, Shankar & Sharanya, 2013), benign paroxysmal positional vertigo (Akkuzu, Akkuzu, & Okluolzu, 2006; Yang, Kim, Lee & Lee, 2008), cerebellopontine angle tumor (Iwasaki, Takai, Ito, & Murofushi, 2009), multiple sclerosis (Murofushi, Shimzu, Takegoshi, & Cheng, 2001) and Meniere's disease (Murofushi, Shimzu, & Takegoshi, 2001; Iwasaki, Takai, Ito, & Murofushi, 2005).

Meniere's disease is defined as the idiopathic syndrome of endolymphatic hydrops (Fraysse, Alonso & House, 1980). It is characterized by the classic symptoms of recurrent rotary vertigo, hearing loss, aural fullness, and tinnitus [Committee on hearing and equilibrium guidelines for the diagnosis and evaluation of therapy in Meniere's disease, American Academy of Otolaryngology, Head and Neck Surgery (AAO-HNS, 1995)]. Its underlying pathophysiology is believed to be derived from an excess of inner ear fluid (endolymphatic hydrops), which over time can contribute to permanent loss of hearing and vestibular function. Histopathological findings of patients with Meniere's disease suggest that the most involved structure of the inner ear is cochlea, followed by saccule, utricle, and semicircular canals (Okuno & Sando, 1987).

The Committee of Hearing and Equilibrium (AAO-HNS, 1995) has classified Meniere's disease into four categories based on certain cardinal signs and symptoms and histopathological findings. These include possible, probable, definite and certain Meniere's disease. 'Possible Meniere's disease is characterized by the presence of episodic rotatory vertigo, without documented hearing loss or with sensorineural hearing loss which is fluctuating or fixed. This category also holds good if symptoms are associated with disequilibrium, but without definitive episodes. Further, other causes that could result in similar presentations have to be eliminated before a tag of 'possible Meniere's disease' is applied. 'Probable Meniere's disease' is defined by the complaint of one definitive episode of vertigo along with audiometrically documented hearing loss on at least one occasion, and tinnitus or aural fullness, with other causes of such precipitations excluded. 'Definite Meniere's disease' is identified in case of presence of two or more definitive spontaneous episodes of vertigo, each lasting 20 minutes or longer along with audiometrically documented hearing loss on at least one occasion, and tinnitus or aural fullness, while excluding other causes. 'Certain Meniere's disease' is 'definite Meniere's disease' confirmed with histopathological findings.

The utility of cVEMP in the diagnosis of Meniere's disease has been explored by several studies (Murofushi et al, 2001; Iwasaki et al, 2005; Rauch, Zhou, Kujawa, Guinan, & Herrmann, 2004; Lee, Joong, Kim, &Yoon, 2009). de Waele et al showed abnormal cVEMP responses in 54% individuals with Meniere's disease (de Waele, Huy, Diard, Freyss, & Vidal, 1999). Similarly, abnormal cVEMP responses were found in 30 out of 79 (38%) of the individuals with Meniere's disease on the side of the pathology (Wang, Tsai, Chien, & Ho, 2012). In the study by Iwasaki et al (2005), 12 out

of 40 participants with abnormal VEMP responses and normal caloric responses were diagnosed with Meniere's disease. However, all the above studies have utilized different parameters of cVEMP for the identification of pathology. The parameters of cVEMP studied for the identification of Meniere's disease include threshold (de Waele et al., 1999; Murofushi et al., 2002), peak-to-peak amplitude (Murofushi et al., 2001; Akkuzu et al., 2006), inter-aural amplitude ratio (Cheng, Huang, & Young, 2003), frequency tuning (Rauch, Zhou, Kujawa, Guinan, & Herrmann et al, 2004) and frequency-amplitude ratio (Kim-Lee et al, 2009).

Threshold abnormalities in individuals with Meniere's disease were reported in several studies, but the sensitivity of this measure in all the studies were quite low, ranging from 54% to 67% (de Waele et al., 1999; Ribeiro et al., 2005; Kuo et al., 2005). Osei-lah, Ceranic and Luxon (2008) reported that threshold differences between normal individuals and individuals with Meniere's disease lacked statistical significance.

Baier and Dieterich (2009) found reduced amplitudes of VEMP responses in 11 out of 16 participants (69%) with Meniere's disease; however similar results (68% prevalence) were obtained for individuals with vestibular migraine. Akkuzzu et al. (2006) also found no statistical difference in latencies and peak-to-peak amplitudes between cVEMP responses in BPPV and Meniere's disease.

Interaural amplitude asymmetry (IAD) is defined as the percentage ratio between the difference and sum of the peak-to-peak amplitude of the two ears. Using the criteria of 36% as normal IAD ratio, Wang et al (2012) reported abnormal IAD in 38% of the individuals with Meniere's disease. In addition to Meniere's disease, Serra, Dorigueto, De Almeida and Gananca (2012) found abnormal IAD in 20 out of 21 individuals with

unilateral vestibular hypofunction (which included Meniere's disease, BPPV, Labyrinthitis, vestibular neuritis, vestibular migraine and unknown etiologies). Therefore this is not a specific finding to Meniere's disease.

cVEMP recorded using various tone burst frequencies has been reported to result in variation in threshold and amplitude due to the phenomenon of frequency tuning. Todd, Cody and Banks (2000) estimated the best average frequency of cVEMP between 300 Hz to 350 Hz, while, while Welgempola and Colebatch (2001) obtained best frequency tuning at 700 Hz. In case of Meniere's disease, Rauch et al (2004) found a shift in the frequency tuning to 1000 Hz. Such alteration/broadening of frequency tuning of cVEMP has been observed only in one other pathology, superior semicircular canal dehiscence syndrome (Taylor, Bradshaw, Welgempola, & Halmagyi, 2012). Taking the test-retest reliability into account, the difference between the peak amplitude at tuned frequency and the surrounding frequencies does seem to be significant enough to consider. In the study by Rauch et al (2004) on individuals with MD, although the averaged data showed peaked tuning pattern, there was considerable scatter across individual data. In individuals with Meniere's disease, it becomes difficult to identify the exact frequency of tuning considering the test retest reliability of cVEMP amplitude, which has been reported to be around 2 μV to 9 μV (Isaradisaikul, Strong, Moushey, Gabbard, Ackley, & Jenkins, 2008). Although, there might be difference in the amplitude between frequencies, the likelihood of them falling within the test retest reliability is high. Therefore, the chance that an individual shows frequency tuning to 500 Hz on one occasion and to 750 Hz or 1000 Hz on another is possible.

Kim-Lee et al (2009) recorded cVEMP for tone bursts at 500 Hz and 1 kHz on healthy individuals and individuals with MD and obtained the Frequency amplitude ratio (FAR). FAR was defined as the ratio between the peak-to-peak amplitude of ipsilateral cVEMP responses at 1 kHz and 500 Hz (1/0.5 FAR). They reported a sensitivity of 93% and a false alarm rate of 5% for using FAR in identification of Meniere's disease. Taylor et al (2012) obtained interaural amplitude asymmetry ratios and amplitude frequency ratios for individuals with Meniere's disease and found that the FAR was most effective means of quantifying this tuning shift in Meniere's disease ears. However in all these studies, FAR was obtained only between two frequencies, i.e., 500 Hz and 1000 Hz, although studies have shown considerable scatter in the tuned frequency in individuals with Meniere's disease.

Need for the Study

Although several parameters of cVEMP has been utilized for identification of Meniere's disease, their efficiency has been found to be questionable. The threshold and amplitude measures of cVEMP have not only been found to be lacking sensitivity and specificity in identifying Meniere's disease, but also shown to have reduced value in the differential diagnosis among vestibulopathies. (de Waele et al., 1999; Ribeiro et al., 2005; Kuo et al., 2005; Osei-lah, et al., 2008; Baier et al., 2009 & Akkuzzu et al., 2006). Abnormal IAD is consistent finding in MD, but it is also a common finding in all other unilateral vestibular disorders like BPPV, labyrinthitis and vestibular neuritis (Wang et al., 2012; Serra et al., 2012). Thus IAD does not provide information specific to Meniere's disease.

Although shift in frequency tuning of cVEMP could provide a unique tool in the identification of Meniere's disease, lack of test retest reliability of amplitude measure of cVEMP (Isaradisaikul et al., 2008; Anoop & Singh, 2010-11) and broad tuning curve shapes makes it difficult to objectively identify shift in frequency tuning. Further no data regarding the sensitivity and specificity of frequency tuning measures of cVEMP in identification of Meniere's disease has been reported by the studies so far. In addition, recording across a number of octave and mid-octave frequencies is time consuming.

Frequency-amplitude ratio (FAR) appears to have potential to be a more objective and less time consuming measure to identify Meniere's disease. However, the main drawback in the studies done in this regard (Kim-Lee et al., 2009; Taylor et al., 2012) is the use of only two frequencies to obtain the frequency amplitude ratio. This was owing to a presumption that frequency tuning in individuals with Meniere's disease is shifted to 1000 Hz from 500 Hz in healthy population. The frequency tuning data in individuals with Meniere's disease, however, has shown shift in frequency tuning from 500 Hz (as seen in healthy individuals) to 750 Hz, 1000Hz and even 1500Hz in a number of cases. Restricting the frequency amplitude ratio (FAR) measure thus may restrict its efficiency in identifying Meniere's disease. Thus, there is a need to obtain FAR data across different frequency pairs and compare them for their efficiency in the identification of Meniere's disease.

Aim of the Study

The aim of the study is to compare frequency tuning and FAR for identification of Meniere's disease.

Objectives of the study

1. To obtain frequency tuning properties for cVEMP in healthy individuals and individuals with Meniere's disease.
2. To obtain FAR of cVEMP for different frequency pairs and compare the frequency pairs for identification of Meniere's disease.
3. To compare frequency tuning and FAR of cVEMP for identification of Meniere's disease.

Hypothesis:

1. There is no statistically significant difference in frequency tuning curves between healthy individuals and individuals with Meniere's disease.
2. There is no statistically significant difference in the FAR of cVEMP between different frequency pairs for identification of Meniere's disease.
3. There is no statistically significant difference frequency tuning and FAR of cVEMP for identification of Meniere's disease.

CHAPTER 2

Review of literature

Meniere's disease is an inner-ear disorder of unknown pathophysiology, although main histopathological correlate is believed to be endolymphatic hydrops (Osei-Lah, Ceranic & Luxon, 2008). The disease in its typical form is characterized by acute phases, with attacks of vertigo, tinnitus, aural fullness and hearing loss, separated by variable periods of remission (Osei-Lah et al., 2008). Paparella (1984, 1991) described an aura that precedes the attacks, which is usually characterized by the presence of tinnitus in about 91.9% of individuals and/or aural fullness reported in about 74.1% of the individuals.

The AAO-HNS in 1995 defined the stages of Meniere's disease based on the average puretone thresholds at 0.5, 1, 2 and 3 kHz using the worst audiogram during a 6 months interval before starting of treatment. Individuals with PTA of less than 26 dB were classified under stage I, those between 26-40 dB were grouped under stage II, between 41-70 dB were put under stage III, and PTA greater than 70 dB were referred to as stage IV. In addition to pure tone audiometry, several other tests are being used to characterize Meniere's disease, which include the bithermal caloric test, electronystagmography, electrocochleography, CHAMP and VEMP

Electronystagmography

Electronystagmography (ENG) evaluates the functioning of the vestibulo-occular reflex system and determines impact of eye/body movement and thermal stimulation on the inner ear (Li & Lorenzo, 2013). It comprises of a host of subtests which evaluates the

central and peripheral vestibular function and can help localize the site of the lesion (Li & Lorenzo, 2013). Typically, the ENG test battery may include calibration/saccade test, gaze test, smooth pursuit test, optokinetic test, positional test and caloric test. The saccade test involves tracking a light moving towards either side from the centre by 10º, 20º or 30º. It checks for overshoot or undershoot of eye movements, also known as occular dysmetria (Silman & Silverman, 1991). Gaze test involves fixating the gaze for about 30 seconds on a static target either at the centre or 30º to the left or right. This test checks for spontaneous nystagmus, in the absence of vestibular stimulation (Silman & Silverman, 1991). The smooth pursuit or pendulum tracking test involves the patient tracking a sinusoidally moving target at speed of around 10º/sec. The test checks the ability to track a moving target smoothly within the visual field (Silman & Silverman, 1991). Optokinetic test involves tracking a visual target repetitively moving from left to right or right to left at a faster speed of 20º or 40º/sec. It checks for the ability to quickly trace targets moving across our visual field (Silman & Silverman, 1991). Positional tests involve moving the head or the body from the neutral position and recording any eye movement/nystagmus induced by it. It helps in detecting various central and peripheral vestibular lesions (Coats, 1975; Stockwell, 1983). Caloric test involves thermal irrigation of the ear canals with warm and cold water or air. Warm water is maintained at 44º C while cold water at 30ºC.

Meniere's disease typically causes a reduced vestibular response in the affected ear, although response may be increased secondary to an acute lesion (Li & Lorenzo, 2013). Generally, the direction of nystagmus is away from the affected ear typically indicating weakened vestibular response (paretic phase) in that ear due to Meniere's

disease (Li & Lorenzo, 2013). However, during an irritative phase, which may precede the paretic phase, the nystagmus is directed toward the involved ear (Li & Lorenzo, 2013). Since its difficult to determine exactly what phase the patient is in, direction of the spontaneous nystagmus during or after an attack of Meniere's disease is not always a reliable indicator of the site of the lesion.

Proctor (2000) obtained ENG responses from 122 individuals with MD. He found caloric weakness in 58% of patients on the involved side, while 19% showed weakness on the better side. Complete paralysis was found in 7%. During the course of the disease, responses became weaker in 26% of patients and stronger in 11%. Spontaneous nystagmus, seen within 24 hours of an attack in 54 cases, was directed away from the diseased ear in only about one half of the cases. The large scatter shown above in the results of the bithermal caloric tests, thus questions its reliability in establishing a diagnosis of Meniere's disease, especially in accurately localizing the pathology to one side.

Electrocochleography

Electrocochleography (ECochG), involves measurement of stimulus-related cochlear potentials as well as the whole nerve action potential or compound action potential (AP). The ratio of amplitudes of the SP and AP has been commonly used as an indicator of endolymphatic hydrops. Enlarged SP/AP ratio has been reported to indicate endolymphatic hydrops (Eggermont, 1976). However, the sensitivity of enlarged SP/AP ratio in MD in both transtympanic and extratympanic EcochG was reported to be 62% (Shelami, Pyykko, Ishizaki, & Ashammakhi; 2002) and 71% (Chung, Cho, Choi, &

Hong; 2004), respectively. In fact, SP has been shown to be present only in about 60% of the healthy individuals (Sinha & Vanaja, 2006). Hence, it is unlikely to be sensitive in identifying the presence of Meniere's disease in an individual in whom it is inherently absent. Ferraro and Tibbils (1999) combined both magnitude and duration features by measuring areas of the SP and AP curve. They found that all individuals with enlarged SP/AP ratio also had an enlarged SP/AP area ratio. However, patients with suspected Meniere's disease whose SP/AP magnitudes were within normal limits also showed an enlarged SP/AP area ratio. Thus, using SP/AP area ratio significantly increases the diagnostic sensitivity of EcochG in identifuing Meniere's disease compared to SP/AP magnitude ratio (Devaiah, Dawson, Ferraro & Ator, 2003). Another important feature of Meniere's disease is an abnormally large latency difference of the AP when recorded with condensation and rarefraction polarities (Margolis & Lilly, 1989; Orchik, Shea & Ge, 1998; Sass, Densert, Magnusson & Whitaker, 1998; Visu & Singh, 2012). The physiological basis for this is thought to be related to the difference in the velocity of the travelling wave along the basilar membrane when stimulated in different directions. Orchik et al. (1998) carried out transtympanic EcochG in 89 ears with Meniere's disease and 24 ears without Meniere's disease, in condensation and rarefraction polarity. They found that an AP latency shift between the two recording conditions of >0.2 msec was found in 62.2% of the ears with Meniere's disease and 8.3% of the non-Meniere's ears. This shows a sensitivity of 62.2% and a specificity of 91.7%. However, results of EcochG are highly dependent on the stage of the Meniere's disease (Yamamoto et al., 2010) and most successful diagnosis is obtained when recorded during symptomatic period, the practicality of this situation would be however limited.

Osmotic tests

These tests involve the application of osmotic diuretics, like glycerol or urea, which can absorb endolymph from the ear, thereby reducing/alleviating its affects. Thus their application is expected to result into improved vestibular and auditory functions. Several studies have found positive findings on glycerol test in 50-60% of the individuals (Synder, 1974; Akioka, Fujita, Kitaoku & Matsunaga, 1990). However, the results of the glycerol test have been found to vary with the progression of Meniere's disease, with more positive findings seen in the early fluctuating phase than the stable phase (Klockhoff & Lindblom, 1986). In addition to this, administration of glycerol carries the risk of temporary side effects like loose motions, headache, nausea and increase in tinnitus (Futaki, Kitahara & Morimoto, 1977). Further it is contraindicated in patients with diabetes and cardiac and renal diseases. Thus, utility of osmotic test is limited by its invasiveness as well as dependence on disease progression.

Cochlear Hydrops Analysis Masking Procedure

Cochlear hydrops analysis masking procedure is a tool to identify changes in the basilar membrane mechanics based on the principles of travelling wave movement. The procedure involves recording of auditory evoked brainstem potentials (wave V) elicited with click alone as well as click along with high pass masking noises (pink noise) with different cut-off frequencies from 8 kHz down to 0.5 kHz. The latency of wave V was reported to increase as the cut-off frequency of masking noise decrease (Don, Kwong & Tanaka, 2005). Increase in the mass and tension on the BM due to excess fluid (endolymph) is believed to alter its mechanics and therefore prevents effective masking.

This was believed to result in reduced difference between the wave V latencies in click alone and click with noise conditions (Don et. al., 2005).

Don et al, in 2005, carried out CHAMP in 23 individuals with Meniere's disease and 32 normal/healthy individuals. They found both sensitivity and specificity of the test in identification of Meniere's disease to be 100%. However, in the more recent studies, both the sensitivity and specificity of CHAMP in identification of MD has been found to be much lower. De valck, Clacks, Wuyts and Heying (2007) reported a sensitivity of 31% and specificity of 28%. Lee et al (2011) carried out CHAMP in 108 subjects divided into three groups of Definite Meniere's disease (N=47), non-Meniere's disease with other vestibular disorder (N=41) and control group with no vestibular problem (N=20). They reported sensitivity of 64% and specificity of 98% along with a diagnostic accuracy of 80%. Hence, although it can help in ruling out the presence of Meniere's disease and its differential diagnosis from other vestibulopathies, CHAMP in itself may not be an adequate tool for identification of Meniere's disease. Moreover, it is often contaminated by post auricular muscle activities and also effected by the degree of hearing loss (De valck et. al., 2007), which limits its use when the hearing loss supersedes moderate degree.

Cervical Vestibular Evoked Myogenic Potential

Cervical Vestibular Evoked Myogenic Potential (cVEMP) is an inhibitory potential recorded from the tonically contracted ipsilateral sternocleidomastoid (SCM) muscle in response to loud sound (Colebatch & Halmagyi, 1992). The response is characterized by a biphasic wave with a positive peak at a latency of around 13 ms name

P13 or P1 and a negative peak at a latency of around 23 ms termed as N23 or N1 (Colbatch, Halmagyi & Skuse, 1994). The origin of the potential in cVEMP is reported to originate from the saccule and travels along the inferior vestibular nerve, the vestibular nuclei and then via the vestibule-spinal tract to the sternocleidomastoid (SCM) mucle (Robertson & Ireland, 1995).

The histopathological findings suggest that saccule is the second most involved structure in Meniere's disease (Okuno & Sando, 1987). This makes it suitable test for identification of Meniere's disease. There have been a number of reports on cVEMPs in MD, with variable results. de Waele, Huy, Diard, Freyss and Vidal (1999) carried out cVEMP on 59 patients with MD and found absent responses in 54% of them. Iwasaki et al (2005) measured caloric responses as well as VEMPs in 811 participants with balance problems and found 40 individuals having abnormal VEMP responses but normal caloric responses. 12 out of the 40 participants were diagnosed with MD. Kuo, Yang and Young (2005) reported abnormal VEMPs in 8 out of the 12 individuals with MD measured within 24 hours of the attack. However, in 4 of these individuals VEMP responses returned normal to after 48 hours. The proportion of individuals showing abnormal results, however, varies with the parameter of VEMP that was being studied. Some of the parameters studied in individuals with MD includes latency, peak-peak amplitude, threshold, inter-aural amplitude ratio frequency tuning and, more recently frequency-amplitude ratio.

Latency of cVEMP.

Latency of a response reflects the transmission of the acoustic or electrical signal. In Meniere's disease, as the transmission is not affected (Rauch et. al., 2004), studies have suggested no change in the latency of the peaks of cVEMP. Osei-Lah et al (2008) measured cVEMP in 20 individuals with Meniere's disease and 11 healthy individuals, using a 500 Hz tone burst and found no difference in the mean latency of P13 and N23 between the two groups. This supported the findings of previous study by de Waeles et al (1999). On the contrary, Akkuzu et al (2006) found significant prolongation in P13 latency in only 10% of the individuals with Meniere's disease. Hassan (2011) also reported prolonged latency of both P13 and N23 in the affected ears of individuals with Meniere's disease. However, the difference between the peak latencies in Meniere's disease group and the control group was not statistically significant. Thus, a latency measure of cVEMP has not been found to be a good indicator of Meniere's disease.

Peak-to-peak Amplitude.

Peak-to-peak amplitude of cVEMP is calculated as the absolute sum of the amplitudes of P13/P1 and the N23/N1 (Nguyen, Welgampola & Carey, 2010). Baier and Dieterich (2009) measured amplitude and latency of cVEMPs in 16 patients with Meniere's disease and 63 patients with vestibular migraine and compared with those of 63 age and gender matched healthy controls. They found reduced amplitudes of cVEMP responses in 11 out of 16 participants (69%) with MD; however similar results (68% prevalence) were obtained for individuals with vestibular migraine, thus reducing the specificity of the measure in terms of ability for differential diagnosis. Akkuzzu et al

(2006) also compared cVEMP responses in BPPV and MD and found no statistically significant difference peak-to-peak amplitudes between the two populations. The above discussion highlights that amplitude measures of cVEMP not only lack sensitivity and specificity in identifying MD but also has diminished value in the differential diagnosis among vestibulopathies.

Threshold of cVEMP.

Threshold of cVEMP is defined as the lowest intensity of stimulus at which response is judged to be present (Rauch et al, 2004). de Waele et al (1999) assessed 59 patients diagnosed with unilateral MD using cVEMP along with audiometric and caloric test. They found absent cVEMP responses on the affected side in 54% of the individuals. The absence was correlated with the degree of low frequency hearing loss, but not with the canal weakness. Kuo et. al. (2005) reported absent cVEMP or elevated cVEMP thresholds in 8 (67%) out of their 12 participants with Meniere's disease. Timmer et al (2006) estimated cVEMP thresholds of 100 individuals with MD out of which 12 were without drop attacks and 82 with drop attacks. Abnormal cVEMP thresholds were seen in 18 % of the affected ears of individuals without drop attacks and 41 % of the individuals with drop attacks. Osei-Lah et a. (2008) measured threshold of cVEMP in 20 individuals with MD and 18 healthy individuals using 500 Hz tone bursts cVEMPs were absent in 35% of the individuals with MD. The mean threshold in healthy controls was around 116 dB SPL, which did not differ significantly from the individuals with MD. Thus, sensitivity of threshold measure of cVEMP in identification of MD appears to be low and hence does not merit as a diagnostic measure for MD

Inter-aural amplitude asymmetry/differenace (IAD).

Interaural amplitude difference (IAD), also known as asymmetry ratio, is the comparison between the peak-to-peak amplitude of the cVEMP responses of the two ears, obtained using Jongkee's formula (Li, Houlden, Tomlinson, 1999), which is shown in Equation 1.

$$IAD = \frac{\text{amp P13}-\text{N23 (rt)} - \text{amp P13}-\text{N23 (lt)}}{\text{amp P13}-\text{N23 (rt)} + \text{amp P13}-\text{N23 (lt)}} \quad \text{Equation 1}$$

where, IAD refers to interaural amplitude difference, amp P13-N23 refers to the interpeak amplitude of P13-N23

Using the criteria of 36% as normal IAD ratio, Wang et al (2012) reported abnormal IAD in 38% of the individuals with MD. Cheng et al (2003) demonstrated that IAD ratios obtained from VEMP correlated with the progression stage of patients with definite Meniere's disease. Among his 40 participants with MD, 6 were classified in stage I with a mean IAD ratio of 0.02 (equivalent to 2%), 12 were classified in stage II with mean IAD ratio of 0.12 (equivalent to 12%), 17 classified in stage III with mean IAD ratio of 0.30 (equivalent to 30%) and 5 classified in stage IV had mean IAD ratio of 0.54 (equivalent to 54%). However such correlation was not seen in the study by Wang et al (2012).

In addition to MD, altered IAD has also been reported in several other unilateral vestibular pathologies like benign paroxysmal positional vertigo (BPPV), Vestibular neuritis, Labyrinthitis and vestibular migraine (Serra, Dorigueto, Almeida, & Gananca, 2012). Serra et al (2012) compared 33 individuals with unilateral vestibular hypofunction (which included MD, BPPV, labyrinthitis, vestibular neuritis, vestibular migraine, &

unknown etiologies) and found that abnormal VEMP was recorded in 21 individuals out of which 20 individuals showed abnormal IAD. Thus, although abnormal IAD is a significant finding in individuals with MD, it does not provide information specific to MD alone.

Frequency tuning.

cVEMP can be recorded using tone bursts from 250 Hz to 8000 Hz. Threshold and amplitude of cVEMP has been reported to vary across different recording frequencies. This phenomenon has led to the concept of frequency tuning. Frequency tuning of cVEMP corresponds to the frequency with largest amplitude and/or smallest/best threshold of response. Todd, Cody and Banks (2000) studied the frequency tuning properties of cVEMP in normal listeners, using tonebursts across several frequencies. They found that the greatest sensitivity of cVEMP ranges from 0.2 to 1 kHz, with the average best frequency estimated between 300 Hz to 350 Hz. Welgampola and Colebatch (2001) obtained best frequency tuning at 700 Hz. Patients with classical MD have cochleosaccular hydrops, which results in altered motion mechanics of the distended saccule (Don et al., 2005). As saccular afferents give rise to cVEMPs, it is logical to expect the alterations of saccular physiology to give rise to altered cVEMP characteristics these patients. Using this hypothesis, Rauch et al (2004) acquired cVEMP for clicks and tone burst stimuli at octave frequencies between 250 Hz to 2 kHz in 34 individuals with unilateral MD and 14 healthy controls. They found that in contrast to the control ears as well as the unaffected ears of the participants with MD, which showed frequency tuning at 500 Hz, the affected ears showed a shift in frequency tuning to higher frequencies (mostly 1 kHz). Similar results were obtained by Node, Seo, Miyamoto, Adachi,

Hashimoto and Sakagami (2005) who observed a general shift in peak amplitude of cVEMP from 500Hz in control group, to 1000 Hz in individuals with Meniere's disease. The concept was further reinforced by Sandhu, Low, Rea and Saunders (2012) and Taylor, Bradshaw, Welgampola and Halmagyi (2012), who also demonstrated similar shift in frequency tuning in the individuals with Meniere's disease. Such alteration/broadening of frequency tuning of cVEMP has been observed only in one other pathology, SCD (Taylor, Bradshaw, Welgampola, & Halmagyi, 2012). Thus, obtaining frequency tuning of cVEMP for the identification of Meniere's disease associated changes in the saccule appears to be a promising tool. However, there are several flipsides to it.

Sandhu, Robert, Peter and Nick (2011) obtained cVEMPs at octave and mid-octave frequencies between 250 Hz and 4 kHz from 12 individuals with Meniere's disease (8 definite and 4 probable Meniere's Disease) and 8 healthy participants. In the healthy volunteers, the acoustic stimulus frequency at which the response amplitudes were largest was 500 Hz. This shifted to higher frequencies in patients with definite Meniere's disease for cVEMP. The overall amplitude in the Meniere's ears was reduced compared to the normal ears. In definite Meniere's disease the maximum amplitude was found at 750 Hz, however average data of both the subgroups showed maximum amplitude at 1000 Hz. Singh, Sinha, Rajeshwari and Barman (2013) obtained cVEMPs in 22 individuals with unilateral Meniere's disease and 22 age and gender matched healthy controls, at octave and mid-octave frequencies between 250 Hz to 4 kHz. They also found a shift in the frequency tuning above 750 Hz in individuals with Meniere's disease.

Using a 750 Hz criteria, they found a sensitivity of 100% and specificity of 63.4% for the diagnosis of Meniere's disease using shift in frequency tuning.

Although shift in frequency tuning of cVEMP could provide a unique tool in the identification of MD, the major drawback of these data in both normal individuals as well as in MD is a large inter-subject variation. In the data obtained by Todd et al (2000) in healthy individuals, the peak-to-peak amplitude at 200 Hz, 400 Hz and 800 Hz were 127 μV, 137 μV and 103 μV respectively. However, the test-retest reliability of cVEMP amplitudes has been shown to be in the order of 2μV to 9μV (Isaradisaikul et al., 2008). Taking this into account, the difference between the peak amplitude between 200 Hz and 400 Hz is not significant enough to consider 400 Hz as the peak of the tuning curve. Even in the study by Rauch et al. (2004) on individuals with MD, although the averaged data showed peaked tuning pattern, there was considerable scatter across individual data. Node et al (2005) obtained frequency tuning data in 36 normal individuals and 28 individuals with endolymphatic hydrops. cVEMP showed tuning at 500 Hz, 750 Hz, 1000 Hz, and 1500 Hz in 14, 11, 10 and 1 ear, respectively, out of 36 healthy ears. In case of endolymphatic hydrops, out of 28 ears, 3 ears were tuned to 500 Hz whereas 4 ears showed shift in frequency tuning to 700 Hz, 13 ears to 1000 Hz, 4 ears to 1500 Hz, 3 ears to 2000 Hz and 1 ear to 4000 Hz. From this data a sensitivity of around 89% and a specificity of 39% could be derived for frequency tuning measures. However, sensitivity and specificity values have not been mentioned in the studies. A poor specificity value increases the rate of false alarms to a great extent, thereby reducing the utility of the test itself. In addition, recording across a number of octave and mid-octave frequencies is

time consuming and could have a possible effect on cochlear function due to excessively loud noise (≥ 125 dBSPL) used in most studies.

Frequency-amplitude ratio (FAR).

Frequency amplitude ratio can be defined as the ratio of amplitude of cVEMP at the test frequency to its amplitude at 500 Hz. It has also been mentioned as amplitude-frequency ratio by some authors (Kim-lee, Ahn, Kim, & Yoon, 2009). FAR might provide a more objective measure to identify the shift in frequency tuning of cVEMP in individuals with MD. Kim-Lee et al (2009) recorded cVEMP for tone bursts at 500 Hz and 1 kHz and obtained the FAR for 20 healthy individuals and 50 individuals with MD. They found that 1/0.5 FAR ratios in the MD group were significantly elevated compared to the normal group and put forward a cut-off value of 0.7 for the diagnosis of MD (1/0.5 FAR>0.7 indicating positive result for MD). Using this value they reported a sensitivity of 93% and a false alarm rate of 5% in the identification of MD. Taylor et al. (2012) obtained IAD ratios and FARs for cVEMP in 60 individuals with MD (30 probable and 30 definite) and 30 healthy controls. They found that the FAR was most effective means of quantifying the tuning shift in MD ears.

Though the above studies pointed towards an alternate means of identifying MD using cVEMP, the main drawback in both these studies is the use of only two frequencies to obtain the FAR. This was owing to a presumption that frequency tuning in individuals with MD is shifted to 1000 Hz from 500 Hz in healthy population. The frequency tuning data in individuals with MD, however, has shown shift in frequency tuning from 500 Hz (as seen in healthy individuals) to 750 Hz, 1000Hz and even beyond 1500Hz in a number of cases. Hence, the assumption of shift of frequency tuning to 1000 Hz, and therefore

obtaining FAR only between 1000 Hz and 500 Hz, may restrict its efficiency in identifying MD

CHAPTER 3

Method

Research Design

A static group comparison research design was adopted to compare the group of individuals with Meniere's disease against the group of healthy individuals.

Participants

Participants were divided into two groups. Group I consisted of 21 individuals diagnosed with unilateral 'definite Meniere's disease' based on the criteria put forward by AAO-HNS (1995) which identifies a definite Meniere's disease based on the presence of two or more definitive episodes of vertiginous attacks, each lasting 20 minutes or longer, audiometrically documented hearing loss on at least one occasion and tinnitus or aural fullness in the effected ear, in the absence of any other causes of such precipitations. In order to rule out other conditions with similar presentations, evaluation was done by an Otolaryngologist, who carried out the necessary tests. Group II consisted of 20 healthy individuals with no auditory and vestibular complaints. These participants were age and gender matched with the participants in group I. Individuals with a documented hearing loss above 15 dB HL in any of the ears at any frequency between 250 Hz to 8 kHz were excluded from this group. Further exclusions were based on the presence of any middle ear dysfunction, present complaint or past history of vestibular symptoms, persistent headache (amounting to similarity with vestibular migraine), high blood pressure, spondylitis or any other neuromuscular problem or a tolerance problem to loud sounds. Irrespective of the

group, informed written consent was obtained from all the participants and their participation in the study was on a non-payment basis.

Instrumentation

A calibrated two-channel diagnostic audiometer, Madsen Orbiter OB-922 with default Telephonics TDH-39 supra-aural headphones housed in MX41/AR ear cushions, was used for air-conduction audiometry, speech audiometry and uncomfortable level (UCL) measurement. Radioear B-71 bone vibrator, connected to the same audiometer, was used for bone-conduction audiometry. Calibrated Grason-Stadler Incorporated (GSI) Tympstar middle ear analyzer with default probe assembly and contralateral insert earphone was used to assess middle ear functioning. Biologic Navigator Pro auditory evoked potential system (version 7.0.0), with default Etymotic ER-3A insert earphones, was used for recording cVEMP and also auditory brainstem response (ABR) whenever necessary. Recorders and Medicare system (RMS) ENG instrument was used for electronystagmography.

Test Environment

All the tests were carried out in a well-illuminated, electrically shielded and sound treated room with ambient noise levels well within the permissible limits (ANSI S3.1, 1999), except ENG, which was carried out in a quiet, dark room. Pure-tone audiometry and speech audiometry were carried out in a double room set-up, whereas immittance evaluation, ABR, ENG and cVEMP were carried out in single room set-ups.

Procedure

The entire procedure consisted of two phases. Phase I included the tests used to fulfill the participant selection criteria. Phase II involved cVEMP recording and analysis.

Phase I (Tests for subject selection).

All the participants went through a series of evaluations beginning with a detailed case history for documenting the presence of any hearing and/or vestibular problem as well the precise nature and duration of the problem. For this Maryland Dizziness and Balance questionnaire (Metropolitan Neuroear Group, Balance Centre of Maryland, 2004) was administered, which probes into the nature and onset of the symptoms along with associated factors in the first section, the presence and nature of any associated auditory symptoms in the second section and information about general lifestyle and habits in the last section. Following this, an otoscopic evaluation was performed to ensure a clear ear canal and healthy tympanic membrane. Air-conduction audiometry was carried out at octave frequencies from 250 Hz to 8000 Hz and bone-conduction audiometry at octave frequencies from 250 Hz to 4000 Hz using modified Hughson-Westlake procedure (Carhart & Jerger, 1959). Speech recognition thresholds were obtained for bisyllabic wordlists in participants' native language using bracketing procedure. Speech identification scores were obtained for standardized phonetically balanced wordlists presented at prescribed levels in participants' native language. UCL for all the participants were measured with live speech stimulus using

ascending trial. They were given standard instructions put forward by the British Society of Audiology (2011) which is as follows:

"*I will gradually make the sound louder in your ear, and you must press the button (or raise your hand) as soon as the sound becomes uncomfortable (uncomfortably loud). This is not a test to find the loudest sound you can tolerate; it is a test to find what level of sound you find uncomfortable. You should press the button (or raise your hand) only when the sound becomes uncomfortable; but make sure you press (raise) it as soon as the sound reaches that level.*"

Immittance evaluation was done in both the ears to rule out middle ear abnormalities. A 226 Hz probe-tone, presented at 85 dB SPL with pressure sweep rate set at 50 daPa/s, was used for tympanometry. Acoustic reflexes were obtained at 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz, both ipsilaterally and contralaterally, using the same probe-tone frequency as mentioned above.

Vestibular test battery included subjective vestibular tests and Electronystagmography (ENG). Subjective vestibular tests included were Romberg test, Fukuda stepping test (FST), Tandem gait test and Past pointing test. For administration of Romberg test, subjects were instructed to stand erect at the centre of two concentric circles, with their hands stretched out and feet close together. This was performed with their eyes open at first and then with eyes closed. They were observed for any swaying movement to one side for a duration of 1 minute. The presence of swaying was considered as an indication vestibular pathology. The Fukuda stepping subjects were instructed to maintain the similar position to the Romberg test and to

march at the same place for 50 steps. A deviation towards any side by greater than 45^{o} was considered as abnormal (as per clinical norm developed at the Department of Otolaryngology, All India Institute of Speech and Hearing, Mysore). For Tandem gait testing subjects were instructed to walk heel to toe along an imaginary straight line across the room and observed for swaying or falling tendency, which was considered as a sign of vestibular pathology. During the past pointing test, the subjects were asked to extend their index finger and alternately touch the tip of their nose and the clinician's index finger whose position constantly kept changing in terms of distance as well as direction. Overshooting or undershooting movements were considered as abnormal and so was the presence of tremors during the task.

ENG test battery included gaze test, optokinetic test and saccade test for evaluation of central vestibular system and positional test, Dix-Hallpike maneuver (positioning test) and bithermal caloric evaluation for peripheral vestibular evaluation. For ENG evaluation, electrode sites were prepared with commercially available skin preparation gel and electrodes were placed using the conduction paste. Corneo-retinal potentials generated by horizontal eye movements were recorded with non-inverting electrode at the outer canthi of the right eye, the inverting electrode at the outer canthi of the left eye and the ground electrode at the forehead. To calibrate the instrument, the participants were seated facing a light bar at a distance of 1meter and instructed to follow the LED target that randomly appeared 10^{o} from the centre on either side. Instrument's sensitivity was adjusted such that every 10^{o} eye movement corresponded to 10 mm movement on the recording paper and a corneo-retinal potential of 200 μV.

The central tests started with saccade test. This test was similar to the calibration test where LED targets appeared at 10^{o} on either side of the centre and the subject's task was to track the target. The presence of catch up saccade on five consecutive or more than five out of ten recordings was considered positive for central pathology (Smith & Cogan, 1960). Gaze test was used to check for spontaneous nystagmus, by asking subjects to fixate their gaze for about 30 seconds on a static target either at the centre or 30º on either side. Presence of spontaneous nystagmus at any position or direction changing nystagmus was considered abnormal result for central pathology (Coats, 1975; Osterveld, 1982). Optokinetic test required the subjects to track the LED target moving repetitively from left to right or right to left at a speed of 20º/sec. the presence of asymmetry between left to right and right to left, or absence of triangular pattern was considered for abnormality and was taken positive result for central pathology (Barber & Stockwell, 1980; Smith & Cogan, 1960).

During the positional test subjects were placed in seven different positions (sitting, supine, supine with head hanging, supine with head turned right, lateral right positioning of whole body and lateral left positioning of whole body). Each position was maintained for 30 seconds and presence of nystagmus was noted. Direction fixed nystagmus exceeding 6º/sec in atleast three positions was considered as an indicator of peripheral vestibular pathology, while direction changing, non-fatigable nystagmus indicated central lesion (Barber & Stockwell, 1980; Coats, 1975; Stockwell, 1983). Dix-Hallpike (positioning) test was carried out to rule out Benign Paroxysmal Positional Vertigo. Caloric testing involved bithermal irrigation of the ear canals with warm water at 44º C and cold water at 30ºC with a sterile syringe. The irrigation was always in right

first and warm first sequence. Weak caloric response on the affected side with nystagmus beating in the opposite direction confirmed presence of Meniere's disease (Li & Lorenzo, 2013).

Phase II (cVEMP recording).

For recording cVEMP, participants were seated in a comfortable chair in an upright position. The electrode sites were prepared using a commercially available skin preparation gel. The gold-plated disc-type electrodes were placed with conduction paste at the respective electrode sites and secured in place with the help of surgical plaster. Absolute electrode impedance and inter-electrode impedance were maintained below 5 kΩ and 2 kΩ respectively. Single-channel recording with electrodes placed on the side ipsilateral to the stimulus side was carried out. The inverting (negative) electrode was placed at the sternoclavicular junction, the non-inverting (positive) electrode at upper one third of the sternocleidomastoid muscle and the ground electrode at forehead. This electrode placement was similar to those used in previous studies (Colebatch & Halmagyi, 1992; Murofushi et al., 1999; Kim Lee et al., 2009; Taylor et al., 2012; Singh, Supreetha, Kashyap, & Sahana, 2013). Stimulus used was alternating polarity tone-bursts of 1 ms of rise/fall time and 2 ms of plateau time, which has been found to be appropriate (Cheng & Murofushi, 2001a, b; Singh & Apeksha, 2014). Participants were instructed to turn their head away from the side of stimulation in order to tense the SCM muscle. To monitor the amount muscle tension in SCM muscle throughout the recordings, a fixed pointer was placed at the shoulder level of the participants at an angle of about 60^{o}-70^{o} and the participants were required to touch the pointer with the lateral aspect of their chin. This method has been shown

to be equally effective in maintaining muscle tension during cVEMP recording as the visual feedback and EMG normalization systems (McCaslin, Jackobson, Hatton, Fowler, & DeLong, 2013). This method has also been shown to have similar or better test-retest reliability compared to the visual feedback system (Isaradisaikul et al., 2008; Anoop & Singh, 2011). The tone bursts were presented at 125 dB SPL with a repetition rate of 5.1 per second at octave and mid-octave frequencies from 250 Hz to 2000 Hz. Analysis window was set to 74 ms, which included 10 ms pre-stimulus recording. The data was averaged across 200 sweeps after being band-pass filtered between 10 Hz and 1500 Hz and multiplied by a factor of 5000.

Analysis

Two independent experienced audiologists marked the peaks on the waveforms for each recording from each subject. Upon obtaining high inter-judge reliability for these markings using Chronbach's alpha test ($\alpha \geq 0.8$) and high correlation between the judges using Pearson's correlation analysis ($r \geq 0.8$), the markings of only one judge was used for further analysis. Frequency tuning was obtained by measuring the P1-N1 wave amplitude across different frequencies and identifying the waveform with the largest amplitude. For calculating the frequency-amplitude ratio, the amplitude of P1-N1 at 750 Hz, 1000 Hz, 1500 Hz and tuned frequency (frequency with largest P1-N1 amplitude) were divided by its amplitude at 500 Hz. This yielded FAR across frequency pairs like 750 /500, 1000 /500, 1500 /500 and tuned frequency (TF)/500. Only these frequency pairs were considered for analysis, due to lack of responses above 1500 Hz and below 500Hz for many individuals.

Statistical analysis

All necessary statistical procedures were carried out using a commercially available SPSS (Statistical Package for Social Sciences) software version 17.0. A static group comparison research design was adopted in which the within group comparisons involved comparison of each frequency pair between the two ears and comparison across different frequency pairs in each ear. Comparison of FAR for different frequency pairs between the individuals with Meniere's disease and the healthy individuals served as the between group factor. Two way repeated measures ANOVA was carried out with ears and FARs as the within subject factors and group as the between subject factor. One way repeated measures ANOVA for ears and frequency pairs were obtained separately and MANOVA was used for between group comparison for each ear type and each FAR due to the presence of significant interactions between the variables. Bonferroni adjusted multiple comparisons were used for pairwise comparisons, whenever necessary. Receiver Operating Criterion (ROC) curves were drawn to obtain the optimum criterion point for diagnosis of Meniere's disease using FAR across all the frequency pairs and to measure the sensitivity and specificity of each of the frequency pairs.

CHAPTER 4

Results

Cervical VEMPs were recorded from 21 individuals with Meniere's disease and 21 age and gender matched healthy individuals across six octave and mid-octave frequencies from 250 Hz to 2000 Hz. Individual waveforms and grand averaged waveforms across the six frequencies for the ears of healthy individuals and the unaffected and affected ears of individuals with Meniere's disease are shown in Figure 1, 2 and 3 respectively.

Frequency tuning of cVEMP in healthy individuals and individuals with Meniere's disease

The response rate, which was defined as the proportion of ears in which the cVEMP response was present, was 100% at all the frequencies for the healthy group. For the Meniere's group, the response rate was found to be 100% at all six frequencies in the unaffected ear, except for one individual in whom there was presence of middle ear pathology (active ear discharge) and hence absence of responses across the range of frequencies. In their affected ears, the response rate was observed to be 100% at all the frequencies but 250 Hz and 2000 Hz, which demonstrated response rates of 85.7 % and 90.4% respectively.

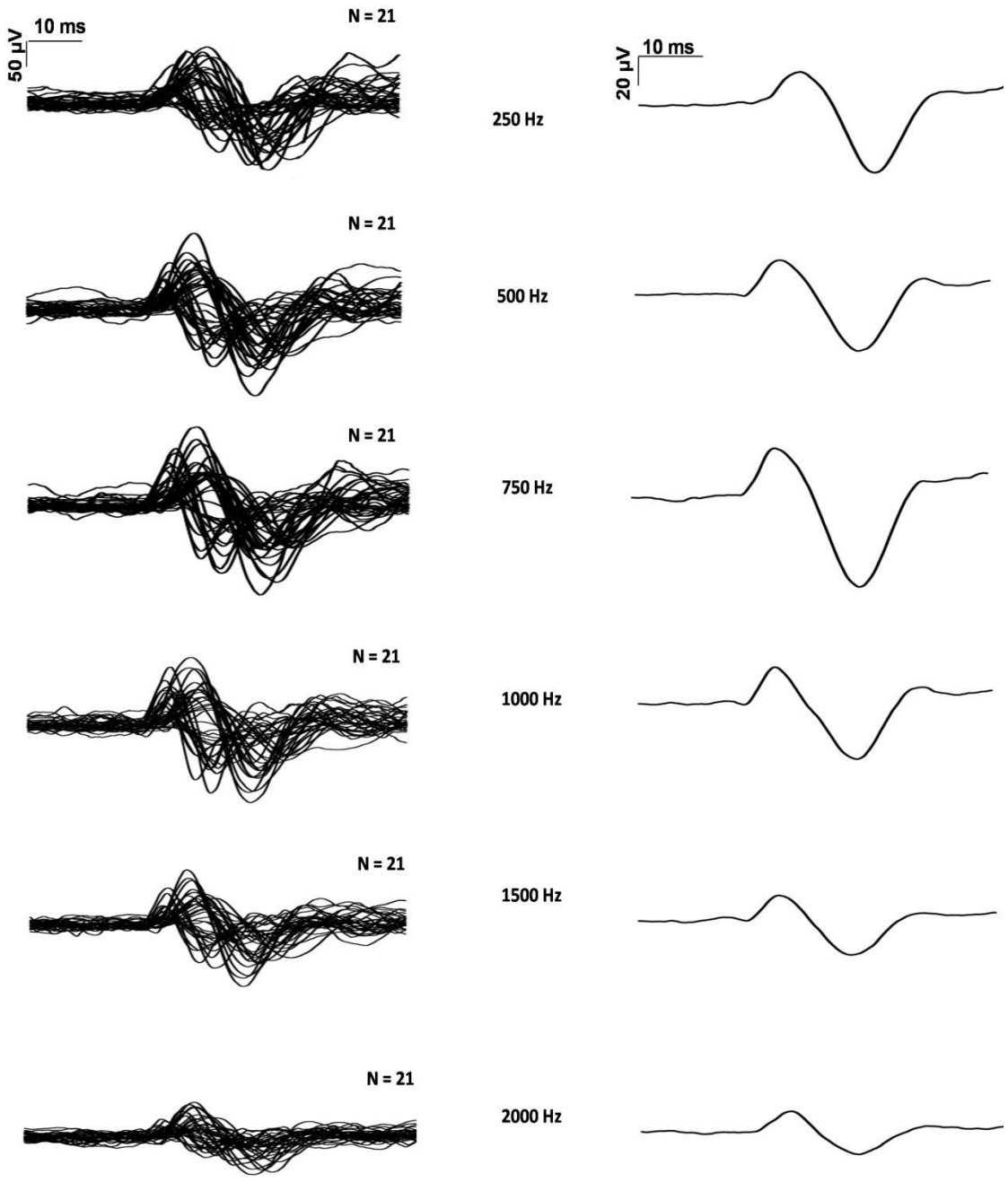

Figure 1: Individual waveforms (left panels) and grand averaged waveforms (right panels) of healthy individuals across frequencies from 250 Hz to 2000 Hz. 'N' represents the number of individuals in whom responses were present at the corresponding frequency.

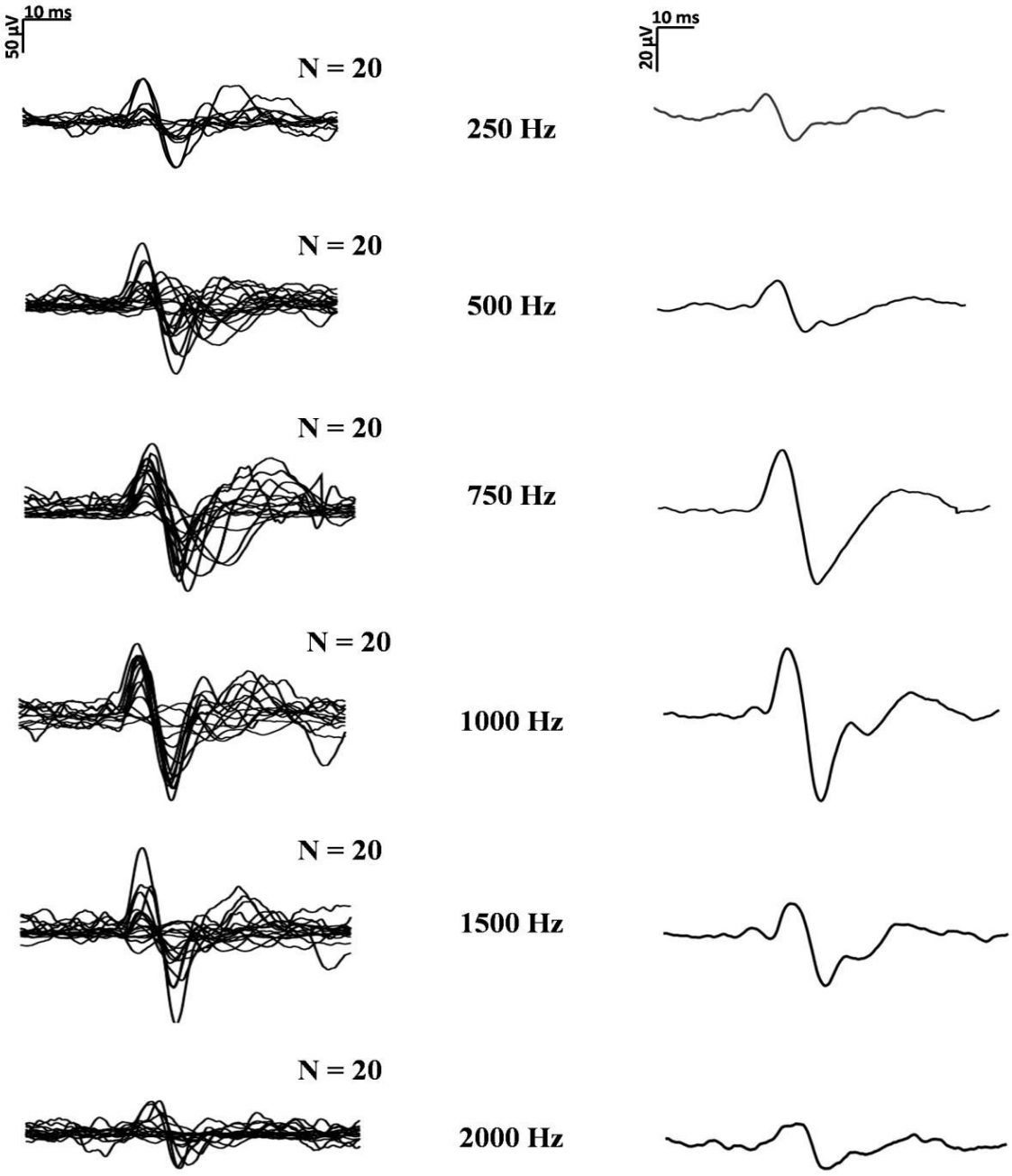

Figure 2: Individual waveforms (left panels) and grand averaged waveforms (right panels) of unaffected ears of individuals with Meniere's disease across frequencies from 250 Hz to 2000 Hz. 'N' represents the number of ears in which responses were present at the corresponding frequency.

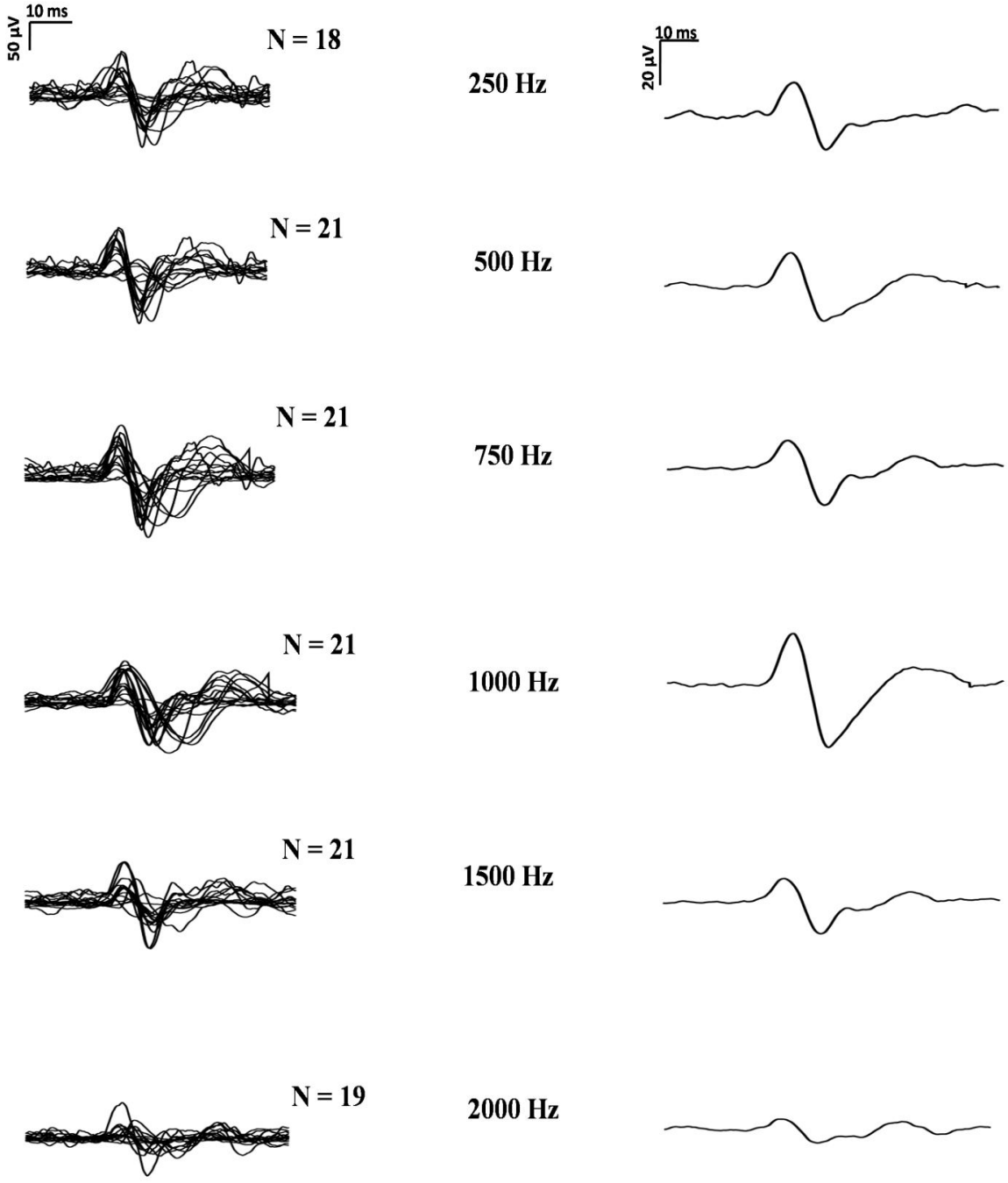

Figure 3: Individual waveforms (left panels) and grand averaged waveforms (right panels) of affected ears of individuals with Meniere's disease across frequencies from 250 Hz to 2000 Hz. 'N' represents the number of ears in which responses were present at the corresponding frequency.

Frequency tuning of cVEMP, which refers to the frequency producing largest amplitude, was obtained for all the participants. Most of the healthy ears were tuned to the frequency of 750 Hz and only a few showed tuning at 500 Hz. In the ears affected with Meniere's disease, the frequency tuning was seen primarily at 1000 Hz. The results were more scattered in the unaffected ears of the Meniere's disease group. Table 1 shows the spread of frequency tuning in both the groups. Frequency tuning curves were obtained plotting the amplitude against frequency. Figure 4 represents the amplitude of cVEMP across frequencies in the affected and unaffected ears of Meniere's disease and the healthy individuals' ears.

Table 1.

Spread of frequency tuning (percentage of ears tuned at various frequencies).

	500 Hz	750 Hz	1000 Hz	1500 Hz	2000 Hz
Ears affected with Meniere's disease	-	19%	71.4%	14.3%	-
Ears unaffected with Meniere's disease	25%	35%	35%	-	-
Healthy Ears	22.7%	70.5%	6.8%	-	-

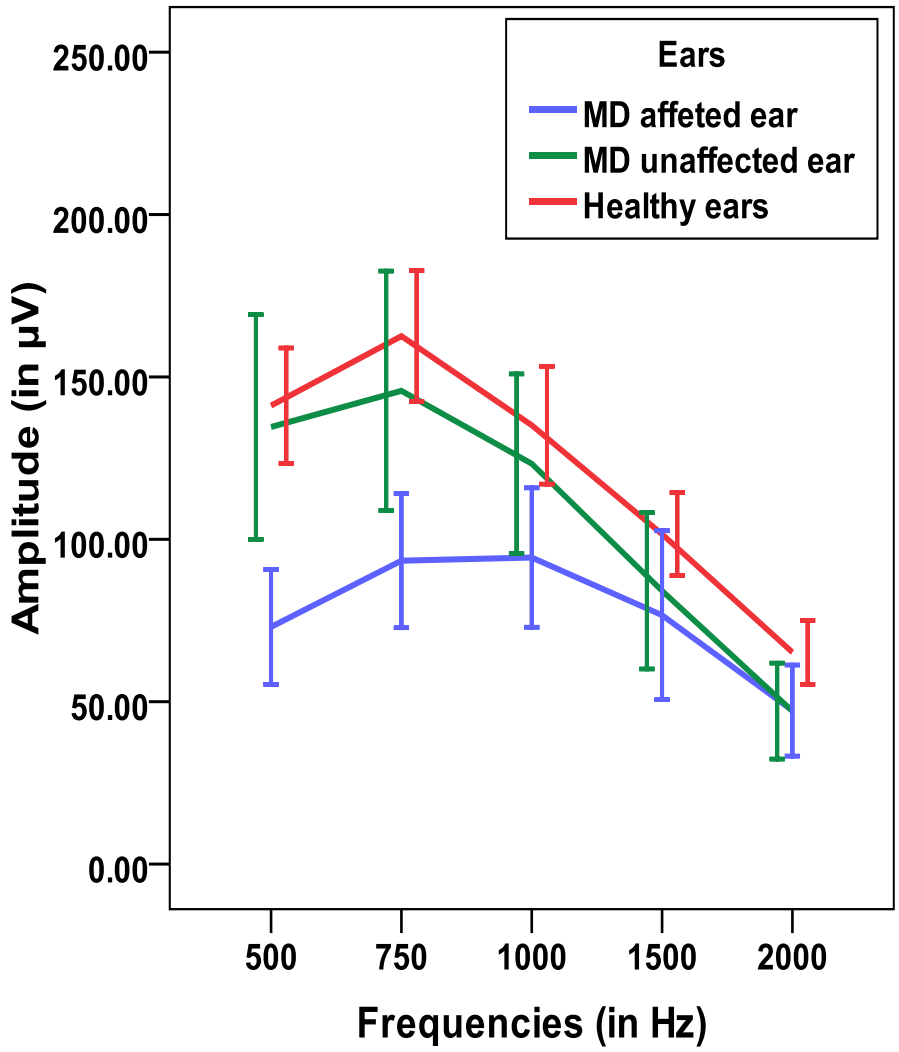

Figure 4: Frequency tuning of cVEMP in ears of healthy individuals and affected and unaffected ears of individuals with Meniere's disease.

The frequency tuning data presented above is based on the observation regarding amplitude of cVEMP across frequencies. To explore the statistical significance of these frequency tuning characteristics, two-way repeated measures ANOVA was administered. The results revealed a significant main effect of ear [$F(1,37) = 5.65$, $p < 0.05$] and frequency [$F(4,148) = 78.25$, $p < 0.05$] but no significant main effect of groups [$F(2.85) = 2.85$, $p < 0.05$]. It also showed significant interactions between frequencies and groups

[F(1,37) = 23.27, $p < 0.05$] as well as ears, frequency and group [F(1,37) = 59.93, $p < 0.05$]. In order to resolve the interactional effect found for the above test, the data was further analyzed using MANOVA for between group comparison under each ear and frequency. It also necessitated the use of separate one-way repeated measure ANOVAs for the comparison between the ear types for each frequency and for the comparison of amplitude across frequencies in each ear type.

For comparison between the affected ears of individuals with Meniere's disease and the matched ears (same sided ears of healthy individuals as the side of ears with pathology in Meniere's disease), the results of MANOVA showed a significant main affect of group on cVEMP amplitude only at the frequencies of 500 Hz [F(1,37) = 19.37, $p < 0.05$] and 750 Hz [F(1,37) = 13.15, $p < 0.05$] but not at any other frequency {1000 Hz [F(1,37) = 2.379, $p > 0.05$], 1500 Hz [F(1,37) = 1.65, $p > 0.05$ and 2000 Hz [F(1,37) = 3.95, $p > 0.05$]}. When comparing the unaffected ears of individuals with Meniere's disease against the matched ears of healthy individuals, MANOVA demonstrated no significant main effect of group on amplitude at any of the frequencies {500 Hz [F(1,37) = 0.05, $p > 0.05$], 750 Hz [F(1,37) = 0.1, $p > 0.05$], 1000 Hz [F(1,37) = 0.05, $p > 0.05$], 1500 Hz [F(1,37) = 0.47, $p > 0.05$] and 2000 Hz [F(1,37) = 1.18, $p > 0.05$]}.

The results of one-way repeated measures ANOVA for between ear comparison within the group revealed a significant main effect of ears on the amplitudes of cVEMP at the frequencies of 500 Hz [F(1,19) = 25.77, $p < 0.05$], 750 Hz [F(1,19) = 13.83, $p < 0.05$] and 1000 Hz [F(1,19) = 4.70, $p < 0.05$], but no significant main effect at 1500 Hz [F(1,19) = 0.38, $p > 0.05$] and 2000 Hz [F(1,19) = 0.7, $p > 0.05$] in the Meniere's disease group. In the group of healthy individuals however, there was no significant main effect

of ears at any of the frequencies {500Hz [F(1,20) = 0.72, $p > 0.05$], 750 Hz [F(1,20) = 0.20, $p > 0.05$], 1000 Hz [F(1,20) = 0.12, $p < 0.05$], 1500 Hz [F(1,37) = 0.48, $p < 0.05$ and 2000 Hz [F(1,37) = 1.80, $p < 0.05$]}.

Separate one-way repeated measures ANOVAs were carried out for frequencies within each ear type and the results demonstrated a significant main of frequencies on the amplitude within the affected [F(4,80) = 14.4, $p < 0.05$] and unaffected ears [F(4,76) = 32.06, $p < 0.05$]} of individuals in the Meniere's disease group. There was also a significant main effect of frequencies in the ears of individuals that were matched to the affected ears [F(4,80) = 38.84, $p<0.05$] and unaffected ears [F(4,80) = 47.74, $p<0.05$]} of individuals with Meniere's disease. The Bonferroni adjusted multiple comparisons was done for pair wise comparison between frequencies in each of the ear types. The results in the affected ears of the Meniere's disease group showed significantly larger amplitude for 1000 Hz compared to 500 Hz and 2000 Hz. There was no significant difference in the amplitudes between any of the other frequencies. In the unaffected ears, the largest amplitude was present at 750 Hz, which was significantly different from the amplitudes at 1500 Hz and 2000 Hz, but not significantly different from amplitude at 500 Hz and 1000 Hz. Similar comparison was carried out in the healthy ears which revealed similar results as obtained in the unaffected ears of Meniere's disease group. The only exception to this was the amplitude at 750 Hz which was significantly larger than that of 1000 Hz, in addition to the amplitude at 1500 Hz and 2000 Hz. Figure 5 and 6 represent the clustered graph comparing the amplitude at different frequencies between the two ears in healthy individuals and in individuals with Meniere's disease respectively.

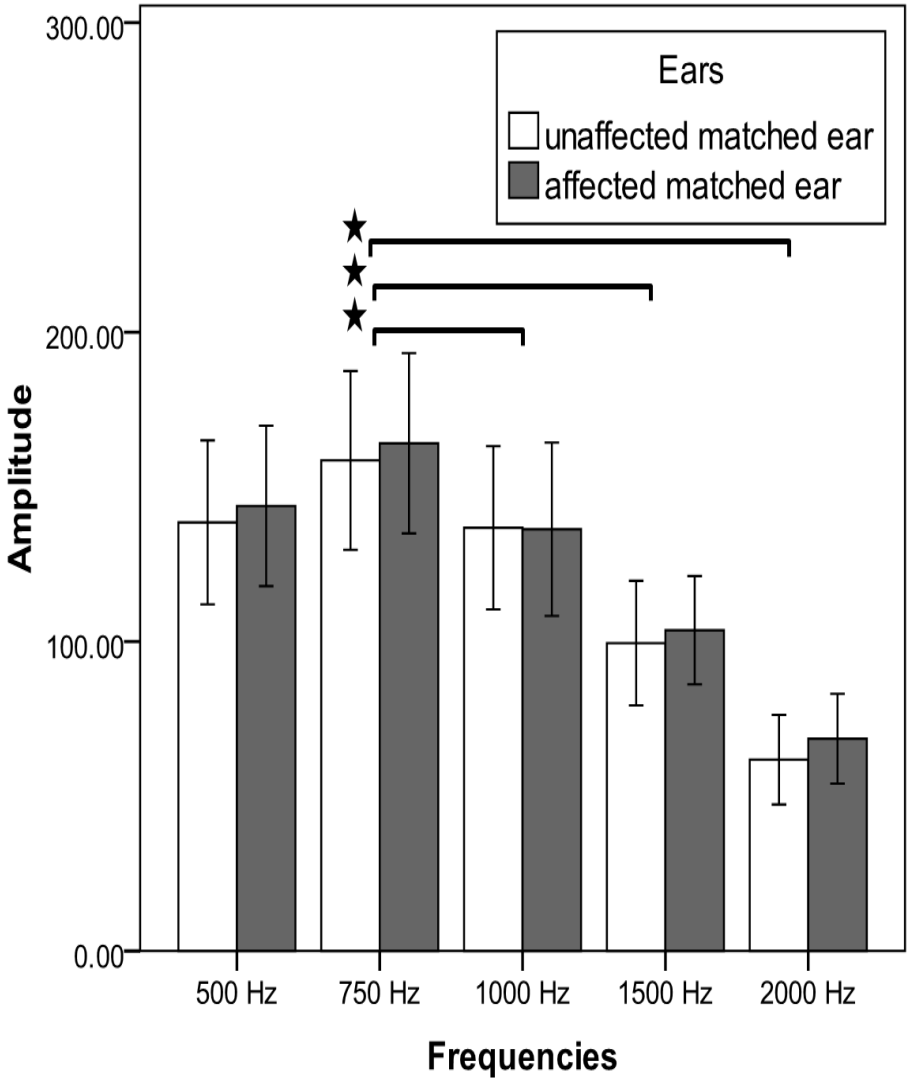

Figure 5: Mean amplitudes across frequencies in the ears of healthy individuals that were matched to unaffected ears and affected ears of Meniere's disease. Lines with asterisk represent significant difference between the amplitudes.

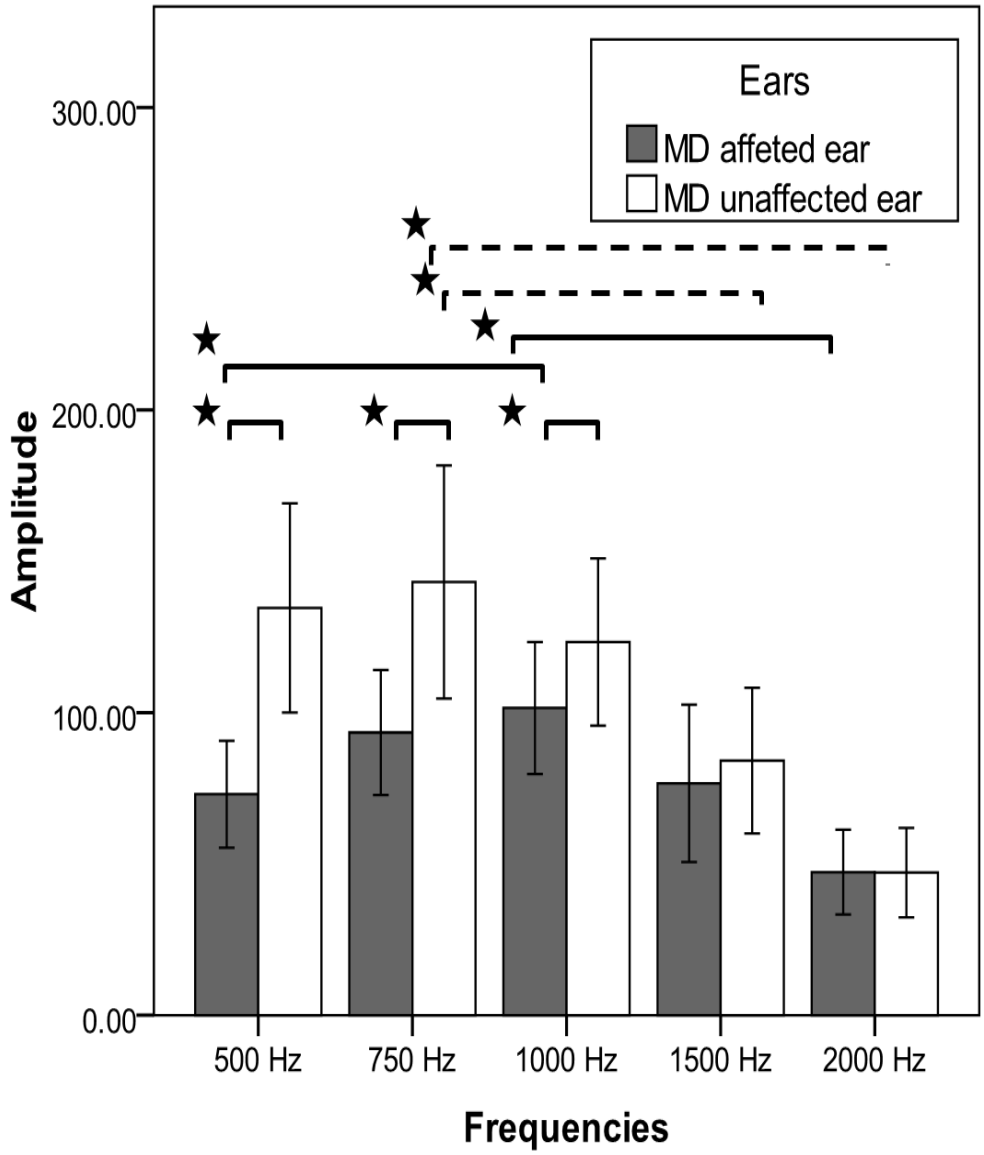

Figure 6: Mean amplitudes across frequencies in the affected ears and unaffected ears of individuals with Meniere's disease. Lines with asterisk represent significant difference between the amplitudes.

The first hypothesis stated that there is no statistically significant difference between significant difference between frequency tuning of cVEMP for healthy

individuals and individuals with Meniere's disease. However, the results demonstrated that the healthy ears and the unaffected ears of individuals with Meniere's disease had significantly larger amplitude at 750 Hz, while this was seen at the frequency of 1000 Hz in the ears with Meniere's disease. The frequency tuning curves clearly demonstrated sharp frequency tuning in healthy ears at 750 Hz. In the Meniere's ears though, there was a broader tuning curve with its peak shifted towards 1000 Hz. Hence, there was a significant difference in the frequency tuning of cVEMP between the healthy ears and the ears with Meniere's disease, and thus H_o (first hypothesis) is rejected.

Frequency amplitude ratio in healthy individuals and individuals with Meniere's disease

In order to obtain frequency amplitude ratio, the peak-to-peak amplitude at 750 Hz, 1000 Hz, 1500 Hz and the tuned frequency (TF) were divided by the amplitude of peak-to-peak amplitude at 500 Hz. Only these four frequencies were compared with 500 Hz to obtain FAR as the response rate was 100% at these frequencies in all the participants. Descriptive statistics was carried out to obtain the mean FARs across the four frequency pairs described above. The mean FAR across all the frequency pairs was greater in the affected ears of the individuals with Meniere's disease compared to their unaffected ears as well as both the ears of the healthy group. The largest FAR was observed for the frequency pair TF/500 irrespective of the group or the ear. The frequency pair of 750/500 produced the largest FAR in both ears of the healthy individuals and the unaffected ears of the individuals with Meniere's disease. In the affected ears with Meniere's disease, this was the case for the frequency pair of

1000/500. Table 2 shows the FARs for the four frequency pairs in both ears of both the groups.

Table 2.

Mean and standard deviation of frequency-amplitude ratios for the group of individuals with Meniere's disease and the group of healthy individuals

		750/500 Hz	1000/500 Hz	1500/500 Hz	TF/500 Hz
Meniere's Disease group	MD ear	1.33 (0.37)	1.50 (0.50)	1.11(0.54)	1.63(0.48)
	NMD ear	1.06 (0.24)	1.00 (0.36)	0.69 (0.40)	1.19 (0.28)
Healthy Group	MD matched ear	1.19 (0.33)	1.18 (0.33)	0.76 (0.19)	1.25 (0.26)
	NMD matched ear	1.21 (0.28)	1.2 (0.28)	0.76 (0.31)	1.23 (0.24)

Note: 'MD ear'- affected ears of individuals with Meniere's disease; 'NMD ear'- unaffected ears of individuals with Meniere's disease; 'MD matched ear'- same sided ears of healthy individuals as the affected ears of individuals with Meniere's disease; 'NMD matched ear'- same sided ears of healthy individuals as the unaffected ears of individuals with Meniere's disease.

In order to evaluate the statistical significance of these observations, two-way repeated measures ANOVA was carried out for ears and frequency pairs with group serving as the between subject factor. The results showed significant main effect of ear [$F(1,39) = 11.82$, $p < 0.05$], frequency pair [$F(3,117) = 59.93$, $p < 0.05$] and group [$F(1,39) = 5.60$, $p < 0.05$]. Also, there was a significant interaction between ear and group [$F(1,39) = 11.32$, $p<0.05$] as well as frequency pair and group [$F(3,117) = 4.03$, $p < 0.05$].

However, there was no significant interaction between ear and frequency pair [$F(3,117) = 1.86$, $p > 0.05$] as well as ear, frequency pair and group [$F(3,117) = 2.07$, $p > 0.05$].

The interactions were resolved using MANOVA for between group comparison under each ear and frequency pair, and separate one-way repeated measures ANOVAs for the comparison of ear types for each frequency pair and for the comparison of FARs for each frequency pair in each ear type. Results of MANOVA revealed no significant main effect of group on any of the frequency pairs {750/500 [$F(1, 39) = 2.57$, $p > 0.05$], 1000/500 [$F(1, 39) = 0.00$, $p > 0.05$], 1500/500 [$F(1,39) = 0.20$, $p > 0.05$] and TF/500 [$F(1, 39) = 0.08$, $p > 0.05$]} when unaffected ears of Meniere's disease group and the matched ears of healthy groups were compared. Nonetheless, there was a significant main effect of group for all the frequency pairs {1000/500 [$F(1, 39) = 17.25$, $p < 0.05$], 1500/500 [$F(1, 39) = 9.68$, $p < 0.05$], and TF/500 [$F(1, 39) = 11.75$, $p < 0.05$]), except 750/500 Hz [$F(1, 39) = 2.05$, $p > 0.05$], when the affected ears of the Meniere's disease group were compared against the matched ears of the healthy group.

A one-way repeated measures ANOVA with ears as the within subject variable revealed significant main effect of ear on the FARs for each of the frequency pairs {750/500 [$F(1,19) = 11.13$, $p < 0.05$], 1000/500 [$F(1,19) = 13.02$, $p < 0.05$], 1500/500 [$F(1,19) = 14.52$, $p < 0.05$] and TF/500 [$F(1,19) = 16.36$, $p < 0.05$]} in the Meniere's disease group. However, in the healthy group, there was no significant main effect of ear on FARs of any of the frequency pairs {750/500 [$F(1,20) = 0.00$, $p > 0.05$], 1000/500 [$F(1,20) = 0.79$, $p > 0.05$], 1500/500 [$F(1,20) = 0.04$, $p > 0.05$] and TF/500 [$F(1,20) = 0.23$, $p > 0.05$]}.

Separate one-way repeated measures ANOVA was carried out with frequency pairs as the within subject variable for each ear type. The results revealed a significant main effect of frequency pairs on FARs in each ear {affected [$F(3,60) = 12.07$, $p < 0.05$] and unaffected ears [$F(3,57) = 21.03$, $p < 0.05$]} in the Meniere's disease group and also in the group of healthy individuals {ears matched to the affected ears [$F(3,60) = 45.24$, $p<0.05$] and unaffected ears [$F(3,60) = 44.33$, $p<0.05$]}. The Bonferroni adjusted multiple comparisons between the FARs in each ear showed the FAR of the frequency pairs 750/500 and 1500/500 to be significantly lower than TF/500 ($p < 0.05$) in the affected ear of Meniere's disease group. However, there was no significant difference between the frequency pairs 1000/500 and TF/500 in the same group. The FAR of 1500/500 was also significantly lower than the FAR of 1000/500. In their unaffected ear, there was no significant difference between the FAR of the frequency pair 750/500 and TF/500 ($p >0.05$), but significant difference existed between all the other frequency pairs ($p < 0.05$). In the healthy group, both the ears revealed significantly larger FAR of the frequency pair TF/500 compared to all other frequency pairs ($p <0.05$), except 750/500. The clustered bar graphs for FAR of different frequency pairs in healthy individuals and both ears of individuals with Meniere's disease are shown in figure 7 and 8 respectively.

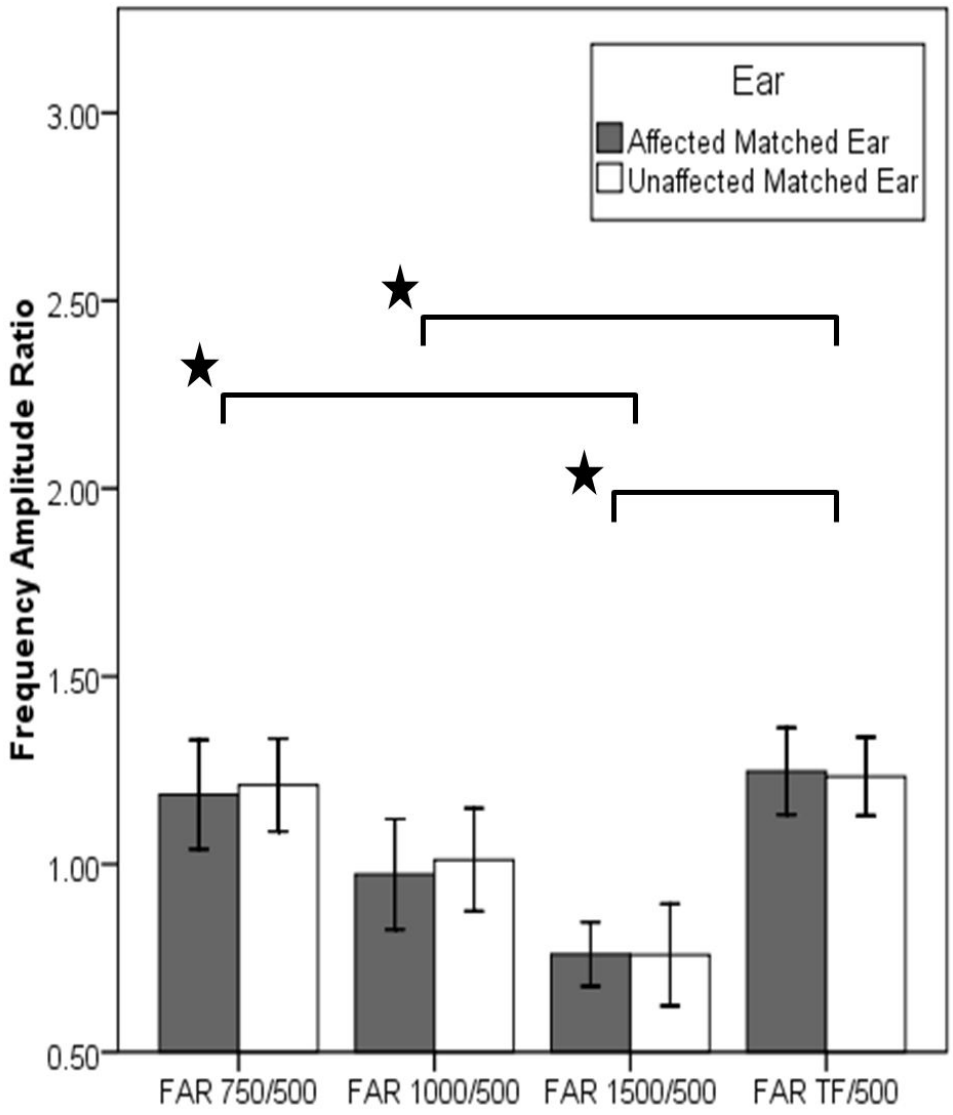

Figure 7: Mean FAR across frequency pairs in the ears matched to the Meniere's affected and unaffected ears of healthy group. The horizontal lines with asterisks depict significant difference in the FAR between the two frequency pairs.

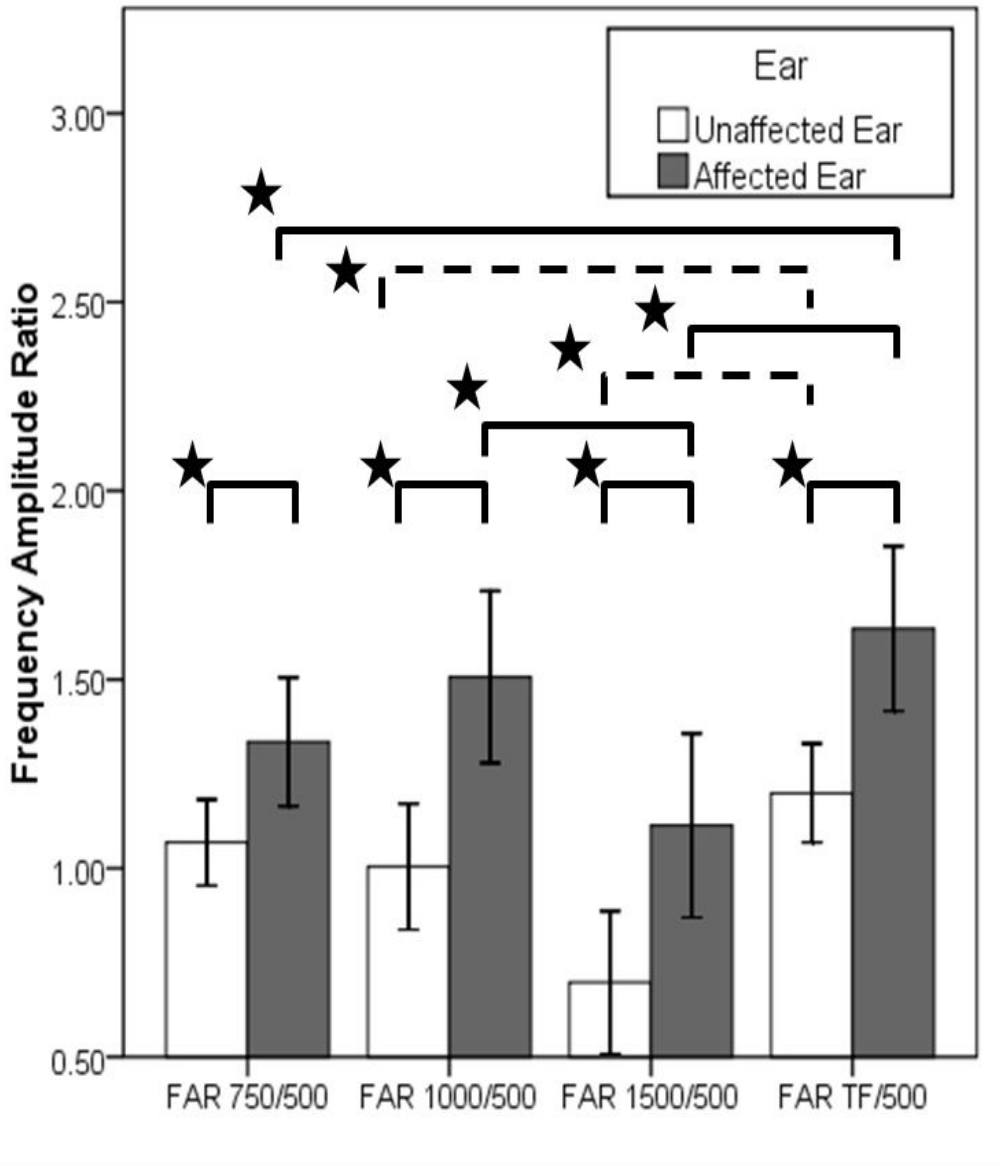

Figure 8: Mean FAR across frequency pairs in the affected and unaffected ears of Meniere's disease group. The horizontal lines with asterisks depict significant difference in the FAR. Dotted lines are used for unaffected ears and solid lines for the affected ears of Meniere's disease.

The second hypothesis stated that there is no statistically significant difference in FAR of cVEMP between different frequency pairs for identification of MD. However, the results demonstrated significantly larger FARs corresponding to the frequency pairs of TF/500 and 1000/500 in Meniere's disease compare to other frequency pairs. This was the case for the frequency pairs TF/500 and 750/500 in healthy individuals. Hence, there was a statistically significant difference between FAR of cVEMP of different frequency pairs for the identification of Meniere's disease and therefore, H_o (second hypothesis) is rejected.

Comparison of frequency tuning and frequency amplitude ratio for identification of Meniere's disease

In order to obtain an optimum cut off frequency that can be used for the identification of Meniere's disease and to obtain the sensitivity and specificity of the frequency tuning measure using the optimum criterion point, ROC curves were drawn for tuned frequencies in all the individuals. Figure 9 represents the ROC curve for frequency tuning. The area under the ROC curve obtained was 0.86 and using a criterion of frequency tuning above the frequency of 875 Hz for the identification of Meniere's disease, the sensitivity was found to be 76.2 % and specificity 85.3%.

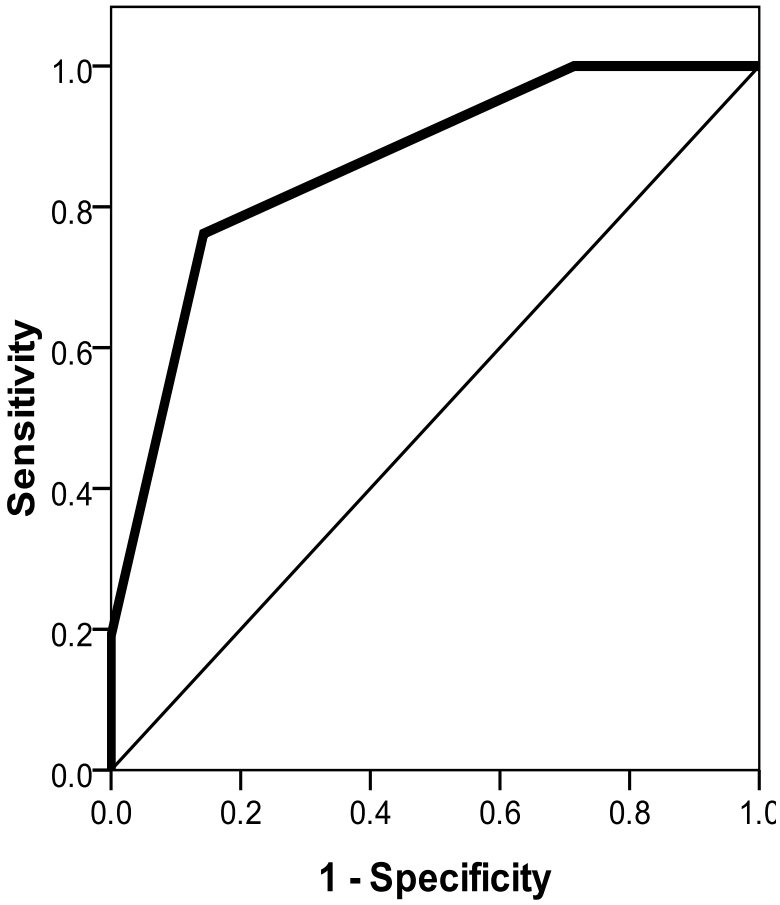

Figure 9: Receiver Operating Characteristics Curve for frequency tuning of cVEMP

ROC curves were also drawn in order to find out an optimum criterion points of FARs for each of the frequency pairs that can be used to differentiate between ears with and without Meniere's disease and to obtain the sensitivity and specificity of the FARs at the criterion point across different frequency pairs. The ROC curves for different frequency pairs are shown in Figure 10. The largest area under the curve of 0.82 was obtained for the frequency pair of 1000/500, which was followed by TF/500, 1500/500 and 750/500 with area under the curve of 0.75, 0.72 and 0.64, respectively. Using a criterion point >1.05 for identification of Meniere's disease, the frequency pair of 1000/500 yielded a sensitivity of 76.2% and specificity of 70%. The sensitivity and

specificity obtained for the frequency pair of TF/500, using a criterion point of >1.19, were 71.4% and 62% respectively. The FAR of 1500/500 resulted in a sensitivity of 66.7% and a specificity of 71.4% when using a criterion point of >.81. Lastly, the frequency pair of 750/500, while using >1.25 as the criterion point, resulted in 42.9% sensitivity and 61.9% specificity. The coordinates of the ROC curves for frequency tuning and for FARs across different frequency pairs along with corresponding sensitivity and specificity values is provided in appendix 1 and 2.

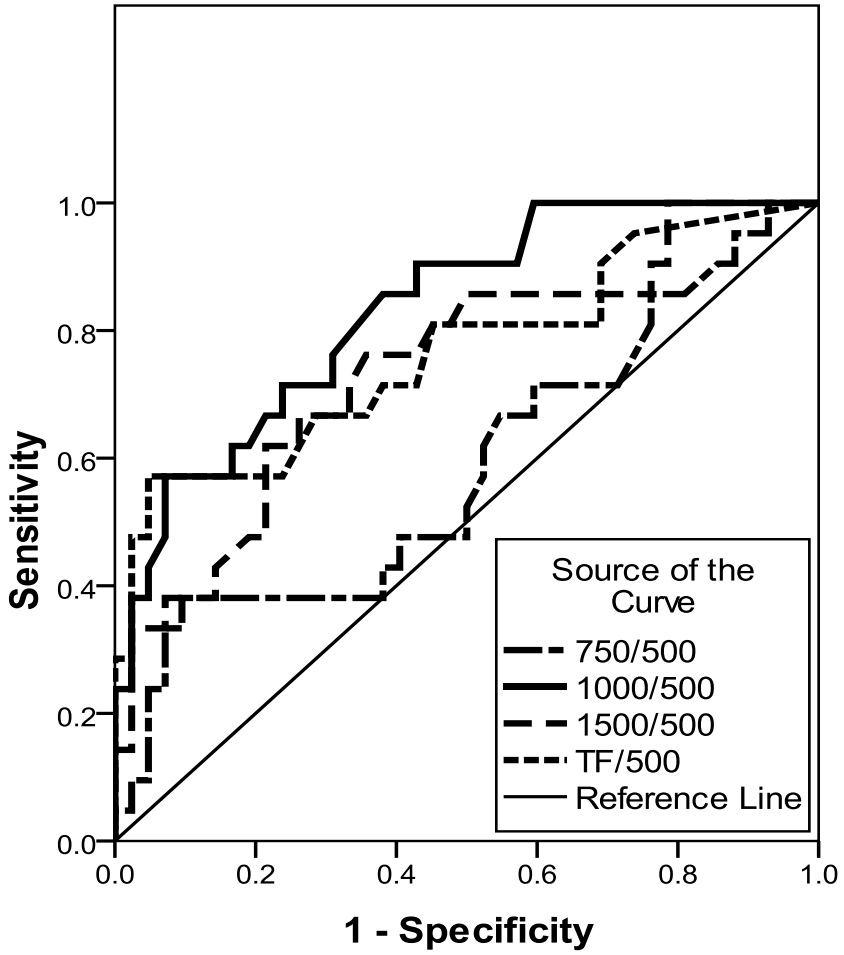

Figure 10: Receiver operating characteristics curves for frequency amplitude ratios of different frequency pairs.

The third hypothesis stated that there is no significant difference between frequency tuning and frequency amplitude ratio of cVEMP in identification of Meniere's disease. Based on the analysis using ROC curves the sensitivity and specificity obtained for frequency tuning curve using a cut off frequency of 1000 Hz is similar to that obtained for frequency amplitude ratio at the optimum criterion value for the FAR of frequency pair 1000/500. Hence, null hypothesis is accepted.

CHAPTER 5

Discussion

The study was conducted with an aim to compare the diagnostic utility of frequency tuning and frequency amplitude ratio of cVEMP for the identification of Meniere's disease. It was also aimed at identifying which of the frequency pairs would give the FAR that would be most suitable for identifying Meniere's disease associated changes in the saccule. Keeping these objectives in mind, cVEMPs were recorded across six octave and midocatve frequencies from 250 Hz to 2000 Hz from a group of individuals with Meniere's disease and another group of age and gender matched healthy individuals in order to the obtain tuned frequency in each individual. FARs were obtained for the frequency pairs 750/500, 1000/500, 1500/500 and the tuned frequency (TF)/500 Hz which were compared between the ears in a group and also between the groups in addition to comparison of frequency pairs within each ear.

There was a 100% response rate for the healthy individuals across all the frequencies, and even in individuals with Meniere's disease, 100% response rates were obtained for the frequencies between 500 Hz to 1500 Hz. The response rates reduced slightly at 250 Hz (85.7%) and 2000 Hz (90.4%). These results for response rates in healthy individuals are in agreement with those reported previously (Rauch et al., 2004; Sandhu et al., 2012; Piker et al., 2013). Sandhu et al (2012) obtained 100% response for frequency from 500 Hz to 1000 Hz whereas Piker et al (2013) reported 100% response rates for frequencies up to 1500 Hz. Rauch et al (2004) reported exactly similar response rate to the one found in the present study across the range of frequencies used. In healthy

individuals, 100% responses were reported up to 1000 Hz by several authors (Janky & Shepard, 2009; Sandhu et al., 2012). In the present study even at 1500 Hz and 2000 Hz, there was 100% response from the healthy ears although there was considerable deterioration in terms of amplitude and morphology of the waveforms at these frequencies. Piker et al (2013) obtained cVEMP responses across octave and mid-octave frequencies from 125 Hz to 2000 Hz, and reported 100% response rates between 500 Hz to 1500 Hz. Rauch et al (2001) recorded cVEMP for octave frequencies from 250 Hz to 4000 Hz and found 100% response rates for frequencies up to 2000 Hz in the healthy individuals, which was the finding in the present study as well.

In the ears affected with Meniere's disease, Sandhu et al (2012) reported 100% response prevalence at 500 Hz followed by 88% at both 750 Hz and 1500 Hz and a decline in the response rates at both higher and lower frequencies. The present study thus conforms to these findings, with 100% response prevalence between 500 Hz to 1500 Hz and a decline at both lower and higher frequencies. The response rate at 250 Hz is in agreement with the previous findings of Rauch et al (2004) who reported 82% response prevalence at 250 Hz in Meniere's ears. The response rate at 2000 Hz in the present study (90.4%) is much higher than those reported previously. Sandhu et al (2012) reported only 13% prevalence of response at 2000 Hz in individuals with Meniere's disease. However the stimulus used in their study was presented at 120 dB peak SPL in contrast to 125 dB SPL used in the present study. At higher frequencies, even a small difference in the stimulus intensity can alter the amount vibration being transmitted to the saccule which in turn will affect the cVEMP response. This difference in the presentation level of stimulus

could be the reason behind the discrepancies in the response prevalence between the two studies.

A large proportion of the healthy ears were tuned to the frequency of 750 Hz in the present study. Most of previous findings on frequency tuning of cVEMP in healthy ears reported frequency tuning at lower frequencies, than the finding of the present study (Todd et al., 2000; Welgempola & Colebatch, 2001), although Welgempola and Colebatch (2001) did report largest amplitude of cVEMP to be present at 1000 Hz for some of the individuals. However they did not explore the frequency of 750 Hz. Singh et al (2011) reported largest amplitude of cVEMP at 750 Hz in a large number of their healthy subjects. Further, the frequency tuning of cVEMP in Meniere's disease was seen at 1000 Hz in most of the individuals, although, there were a few individuals in this group who demonstrated frequency tuning to 1500 Hz. Thus, the comparison of healthy individual's data and data from ears with Meniere's disease shows a shift in the tuning from 750 Hz in healthy ears to 1000 Hz in ears with Meniere's disease. The shift in the frequency tuning in the ears with Meniere's disease has been reported by several authors previously (Rauch et al., 2004; Node et al., 2005; Sandhu et al., 2012). Although the shift in tuning was reported mainly to 1000 Hz, there are reports of shifts in tuning to other frequencies, like 1500 Hz as well (Sandhu et al., 2012).

Several hypotheses had been put forward to explain these alterations, which includes dampening of mass-spring property of saccule (Todd et al., 2001) and changes in the electrical resonances of the hair cells of the saccule (Welgempola & Colebatch, 2005). As per the electrical resonance hypothesis of Welgempola and Colebatch (2005), the tuning of cVEMP corresponds to the electrical resonance of the saccular hair cells.

Loss or damage to these hair cells due to prolonged increased endolymphatic pressure leads to changes in the resonance property. However, previous study by Node et al (2005) did not show any relation between the duration or severity of Meniere's disease to the alterations of cVEMP frequency tuning, which makes the validity of the electrical resonance hypothesis questionable. According to the mass-spring hypothesis, the increased stiffness of the saccular membrane due to the accompanying endolymphatic hydrops leads to the shift in the tuning of the system to a higher frequency range. In further support of the altered mechanical properties of the saccule leading to alterations of frequency tuning, Node et al (2005) explained the relation between the resonant frequency of the saccule to its density and tension, which is mentioned in Equation 2. Based on this relation the increase in the resonant frequency can be linked to the increase of the saccular membrane tension due to increased endolymphatic pressure as well as its reduced density, for which there is existing anatomical support (Yamakava, 1938; Shinozaki & Kimura, 1980).

$$\text{Resonant frequency (f)} = k\sqrt{[t/d]} \qquad \text{Equation 2}$$

where, k is a constant; 't' and 'd' represent the tension and density, respectively, of the saccular membrane.

FAR across all the frequency pairs were found to be significantly elevated in the affected ears of the Meniere's disease group compared to the healthy group as well as the unaffected ears of the Meniere's disease group. This is in accordance with the findings of the previous studies (Lee et al., 2009; Taylor et al., 2012), although these studies were done using only one frequency pair (1000/500). This elevation of FARs in Meniere's

affected ears is most likely associated with shift in the maximum amplitude of cVEMP from 500 Hz, in most of the healthy ears, to higher frequencies in ears with Meniere's disease. Although, the previous studies have used the amplitude ratio only between the frequencies of 500 Hz and 1000 Hz to study the altered frequency tuning properties in Meniere's disease, they have reported the shifts in maximum cVEMP amplitude to other frequencies such as 750Hz and 1500 Hz as well. Thus the same underlying hypotheses of change in the mechanical resonance due to altered relationship between tension and density in the saccule could apply at these frequencies also. This explains the increased FAR obtained for the other frequency pairs.

The overall data showed largest FAR values for the frequency pair TF/500, which was followed by the frequency pair 750/500 in the unaffected ears of the Meniere's disease group as well as both ears of the healthy group. However, in the affected ears of Meniere's disease group, the largest FAR following the frequency pair TF/500 was seen for the frequency pair 1000/500. An indirect inference from this could be that of larger mean amplitude at 750 Hz in healthy individuals as well as unaffected ears of individuals with Meniere's disease. However, the largest mean amplitude in Meniere's ears corresponded to 1000 Hz. This indirect inference from the data is similar to the direct observations in this regard on healthy individuals (Welgempola & Colebatch, 2001) and in individuals with Meniere's disease (Rauch et al., 2004; Node et al., 2005). This could again be explained by the proposed mass-spring model by Todd et al (2001), explained in the above section.

Comparison across frequency pairs in each ear of the participants showed no significant difference between the FAR of 750/500 and TF/500 in healthy ears and

unaffected ears of individuals with Meniere's disease. This means that in most of these ears 750 Hz was the tuned frequency. Similarly in the ears affected by Meniere's disease, FAR of 1000/500 showed no significant difference from the FAR of TF/500, implying frequency tuning of cVEMP at 1000 Hz in most of these ears. Also, significantly larger differences between the FAR at 750/500 and the other two frequency pairs, 1000/500 and 1500/500, pointed towards a sharper tuning in the healthy ears, which was lacking in both the affected as well as unaffected ears of the Meniere's disease group. The studies reporting frequency tuning of cVEMP in healthy individuals and individuals with Meniere's disease have reported similar difference in tuned frequency and sharpening of tuning frequencies between the groups (Rauch et al., 2004; Node et al., 2005, Sandhu et al., 2012). The mass spring hypothesis proposed by Todd et al (2001) can again be used to explain this observation.

Comparison of FARs between the ears showed significant difference in case of individuals with Meniere's disease, whereas no significant difference was found between the ears for healthy individuals. Changes in frequency tuning as well as overall amplitude and morphology of responses, compared to healthy ears, were also observed in the unaffected ears of the individuals with Meniere's disease, which is consistent with earlier findings (Rauch et al., 2004; Node et al., 2005). These changes have been attributed to non-superficial involvement of both the ears in the disease. There have been reports in the literature of tendency of unilateral Meniere's disease to spread to the unaffected ear within a span of 2-7 years in up to 50% of cases (Salvinelli, Trivelli, Grecko, Silverstrini, & Pallini, 1999; Jackson & Silverstein, 2002). However, in the present study, the

alterations were not as prominent, and hence a significant difference existed between the two ears of these individuals.

Between groups comparison of the frequency pairs revealed that the largest difference between the ears affected by Meniere's disease and the healthy ears existed for the frequency pair of 1000/500 followed by the frequency pair TF/500. This means that considering FAR of 1000/500 is more likely to result in correct identification of Meniere's disease compared to other frequency pairs, including TF/500. The ROC curves drawn to compare the sensitivity and specificity of FARs at the optimum criterion point across the four frequency pairs confirmed the above prediction. The largest area under the curve as well as the highest sensitivity and specificity was obtained for the FAR of 1000/500.

Previous studies on abnormal frequency amplitude ratios in Meniere's disease were done considering only the frequency pair of 1000/500 (Kim Lee et al., 2009; Taylor et al.,2012). This was mainly due to an assumption that frequency shift occurs from 500 Hz to 1000 Hz in Meniere's disease. Since there are reports of shift in frequency tuning in ears with Meniere's disease to frequencies other than 1000 Hz also, the present study was done to explore the best possible frequency pair among 750/500, 1000/500, 1500/500 and tuned frequency/500 for the identification of the same. The outcomes of the study confirm the use of 1000/500 as the best diagnostic indicator Meniere's disease. Lee et al (2009) reported a much higher sensitivity of 95% and a low false alarm rate of 5% using FAR of 1000/500 with a cut off value of >0.7, in the identification of Meniere's disease in their study. Using a criterion point of >0.7 gives a sensitivity of 95.2% in the present study too, however the specificity at this level reduces to almost 10%. The sensitivity of

76.2% and specificity of 70% observed in the present study is similar to that obtained by Taylor et al (2012), who reported 75% sensitivity and 80% specificity using FAR of 1000/500.

Sensitivity and specificity obtained for frequency tuning of cVEMP with optimum criterion point as 1000 Hz (>875 Hz) was 76.2% and 85% respectively, for the identification of Meniere's disease. FAR showed largest sensitivity and specificity of 76.2% and 70 % respectively, for the frequency pair of 1000/500, at an optimum criterion point of >1.05. Thus, both the measures seem to be equally sensitive in the identification of Meniere's disease, although specificity of frequency tuning of cVEMP was slightly greater than FAR. However, in order to obtain frequency tuning, cVEMP has to be recorded at atleast four frequencies, 500 Hz, 750 Hz, 1000 Hz and 1500 Hz, as there are overlapping reports regarding the frequency tuning of cVEMP in both healthy ears as well as ears with Meniere's disease. However for obtaining frequency amplitude ratio, considering 1000/500 as the most sensitive frequency pair, cVEMP recording needs to be obtained for only two frequencies (1000 Hz and 500 Hz). Thus, the test time required to obtain frequency amplitude ratio for cVEMP will be greatly reduced compared to obtaining frequency tuning of cVEMP. This will also expose an individual to lesser duration of loud acoustic stimulation, a concern that has slowly begun to set roots after a recent study on the effect of VEMP stimuli on cochlear function revealed depressed amplitude at 8000 Hz in distortion product oto-acoustic emissions (Krause et al., 2013). Thus, FAR can help in the identification of Meniere's disease in a more safe and time efficient manner without compromising on the sensitivity and specificity of the results and hence is a better test than frequency tuning for clinical use.

CHAPTER 7

Summary and Conclusion

Meniere's disease is defined as the idiopathic syndrome of endolymphatic hydrops (Fraysse, Alonso & House, 1980) which is characterized by the classic triad of symptoms including recurrent rotary vertigo, hearing loss, aural fullness, and tinnitus [Committee on hearing and equilibrium guidelines for the diagnosis and evaluation of therapy in Meniere's disease, American Academy of Otolaryngology, Head and Neck Surgery (AAO-HNS, 1995)]. Its underlying pathophysiology is believed to be due to an excess fluid in the inner ear (endolymphatic hydrops) causing temporary or permanent damage to the various inner ear structures, saccule being the second most involved structure (Okuno & Sando, 1987). While there are several tests for the assessment of cochlea, cervical vestibular evoked myogenic potential (cVEMP) is the only known test with an ability to assess saccular functioning (Colebatch & Halmagyi, 1992; Colebatch et al., 1994; Halmagyi et al., 1995; Watson & Colebatch, 1998).

The utility of cVEMP in the diagnosis of Meniere's disease has been explored by several investigators. The proportion of individuals showing abnormal results, however, varies with the parameter of VEMP that was being studied. Frequency tuning of cVEMP is one of the most explored parameters. It refers to the frequency at which maximum amplitude of cVEMP is obtained. In healthy individuals, cVEMP tuning has been reported to be around 500 Hz (Rauch et al., 2004; Node et al, 2005). However, in individuals with Meniere's disease the frequency tuning of cVEMP has been found to shift towards higher frequencies of, mostly reported around 1000 Hz (Rauch et al., 2004;

Node et al, 2005, Sandhu et al., 2012). Another measure, exploiting the phenomenon of altered frequency tuning of cVEMP in individuals with Meniere's disease is the frequency-amplitude ratio (FAR), which is defined as the ratio of amplitude of cVEMP at the test frequency to its amplitude at 500 Hz. Elevated FAR of the frequency pair 1000 Hz/500 Hz has been a positive diagnostic finding in individuals with Meniere's disease (Kim-Lee et al., 2009; Taylor et. al., 2011). However, frequency tuning can occur at any frequency and have been reported to be involving 750 and 1500 Hz in a large proportion of individuals with Meniere's disease. But the FARs for these frequencies have not been explored. Thus, the present study was conducted with an aim to evaluate and compare the diagnostic utility of frequency tuning and FAR of cVEMP for the identification of Meniere's disease. It also aimed at comparing FARs of different frequency pairs and finding the most optimum frequency pair for diagnosis of Meniere's disease.

To fulfill these aims, two groups of individuals were utilized for the study. First group included 21 individuals identified as having unilateral definite Meniere's disease, based on the guidelines given by American Academy of Otolaryngology, Head and Neck Surgery (AAO-HNS, 1995) and the report of an otolaryngologist. Second group comprised of 21 age and gender matched healthy individuals, without any audio-vestibular complaints. After fulfilling the subject selection criteria, unrectified cervical vestibular evoked myogenic potentials (cVEMP) were recorded for the participants of both groups across six octave and midocatve frequencies from 250 Hz to 2000 Hz. Single-channel recordings were carried out with inverting and non-inverting electrodes placed the ipsilateral sternoclavicular junction and upper one third of the sternocleidomastoid muscle, respectively and the ground electrode placed at the forehead.

Stimulus used was alternating polarity tone-bursts of 1 ms of rise/fall time and 2 ms of plateau time, presented at 125 dB SPL with a repetition rate of 5.1 per second at the above mentioned test frequencies. Participants were instructed to turn their head away from the side of stimulation in order to tense the SCM muscle. The response were acquired over a 74 msec window including 10 msec pre-stimulus recording and averaged across 200 sweeps after being band-pass filtered between 10 Hz and 1500 Hz and multiplied by a factor of 5000.

The responses were analyzed in terms of the peak-to-peak amplitude of P1-N1 complex across the test frequencies in order to find the frequency tuning of cVEMP in each individual. The peak-to-peak amplitude at the frequencies of 750 Hz, 1000 Hz, 1500 Hz and the tuned frequency (TF) were divided by the peak-to-peak amplitude at 500 Hz to obtain frequency amplitude ratio (FAR) for the four frequency pairs of 750/500, 1000/500, 1500/500 and TF/500.

The unaffected ears of the individuals with Meniere's disease showed frequency tuning at several frequencies, but primarily at 750 Hz as well as 1000 Hz. Cervical VEMP responses were tuned to 750 Hz in majority of ears of healthy individuals 1000 Hz in most individuals with Meniere's disease. However the peak of the tuning curve in these ears was also located around 750 Hz, like the healthy ears. These findings are consistent with the findings of other investigators (Rauch et al., 2004; Node et al., 2005; Sandhu et al., 2012). The altered frequency tuning in individuals with Meniere's disease has been explained by a mass spring hypothesis put forward by Todd et al (2001). The increased stiffness of the saccular membrane due to the accompanying endolymphatic hydrops leads to the shift in the tuning of the system to a higher frequency range.

FAR obtained for all the four frequency pairs were considerably elevated in the affected ears of the individuals with Meniere's disease compared to their unaffected ears as well as the healthy ears. This could be attributed to the shift in the maximum amplitude of cVEMP from 500 Hz towards higher frequencies. The largest FAR was obtained for the frequency pair of TF/500 in all the individuals irrespective of the groups. In the healthy individuals as well as the unaffected ears of the individuals with Meniere's disease, this was followed by the FAR for the frequency pairs of 750/500, however in the affected ears of the individuals with Meniere's disease, the highest FAR after TF/500 was obtained for 1000/500. There was no statistically significant difference between the FAR of 750/500 and TF/500 in the healthy ears and between the FAR of 1000/500 and TF/500 in ears affected with Meniere's disease. This could again be attributed to the shift in the frequency tuning of cVEMP from around 750 Hz in healthy ears towards 1000 Hz in the ears affected with Meniere's disease.

Receiver operating characteristics curve drawn for the frequency tuning of cVEMP revealed a sensitivity of 76.2% and a specificity of 85.3 % for this measure at an optimum criterion point of >875 Hz, for the identification of Meniere's disease. Similar analysis for FAR of different frequency pairs revealed greatest sensitivity and specificity of 76.2% and 70% respectively, for the FAR of the frequency par 1000/500 on considering optimum criterion point of >1.05. This confirmed the use of 1000/500 as the best frequency pair to obtain FAR for correct identification of Meniere's disease. The sensitivity of both frequency tuning and FAR for identification of Meniere's disease was found to be the same, although specificity was slightly greater for frequency tuning. However, obtaining frequency tuning requires recording cVEMP for several frequencies,

in contrast to only two frequencies required to obtain FAR. The limitation in terms of time efficiency for frequency tuning is a major factor to be considered for regular clinical use, which can be overcome by the use of FAR. Additionally, the subject will also be exposed to loud acoustic stimuli used for cVEMP recording for a much shorter duration when recording at only 1000 Hz and 500 Hz for FAR compared to minimum four frequencies (500, 750, 1000, & 1500 Hz) for frequency tuning. Thus, FAR is better suited to clinical used than frequency tuning.

Implications of the study

Frequency tuning and frequency-amplitude ratio of cVEMP both give information regarding the altered frequency tuning of otolith organs, which is a striking feature in individuals with Meniere's disease. The present study found almost equal sensitivity and specificity of the two measures for the identification of Meniere's disease. However, frequency-amplitude ratio measurement is more time efficient and less fatiguing for the patient, compared to frequency tuning. It also poses lesser risk for the subjected to be exposed to high levels of acoustic stimulation by virtue of its time efficiency and hence it would have more practical utility in routine clinical use. The present study thus provides with a sensitive and time efficient tool for the identification of Meniere's disease.

Future directions

Frequency tuning and frequency amplitude ratio of cVEMP both were found to be equally sensitive for the identification of Meniere's disease. However, the sample size chosen for the study was small, which might affect the sensitivity and specificity

results. Hence future research with a larger population is recommended. Additionally, comparing the frequency amplitude ratio across the clinical population with different vestibular pathologies is suggested to find out its utility in the differential diagnosis of Meniere's disease.

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APPENDIX

The appendix consists of the Coordinates of the ROC curves for Frequency amplitude ratio and Frequency tuning along with the corresponding Sensitivity and specificity values.

Coordinates of the ROC Curve for Frequency Amplitude Ratio

Test Result Variable(s)	Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
FAR1	-.3400	1.000	1.000
	.6650	1.000	.976
	.6900	1.000	.929
	.7350	.952	.929
	.7850	.952	.905
	.8300	.952	.881
	.8750	.952	.857
	.9100	.905	.857
	.9350	.905	.833
	.9600	.857	.810
	.9800	.857	.786
	1.0150	.857	.762
	1.0500	.810	.762
	1.0700	.762	.738
	1.0850	.714	.714
	1.0950	.714	.667
	1.1050	.714	.643
	1.1200	.714	.619
	1.1350	.714	.595
	1.1450	.667	.595
	1.1550	.667	.548

	1.1650	.619	.524
	1.1750	.571	.524
	1.1850	.524	.500
	1.1950	.476	.500
	1.2050	.476	.476
	1.2150	.476	.452
	1.2250	.476	.405
	1.2350	.476	.381
	1.2450	.429	.381
	1.2550	.429	.357
	1.2800	.381	.357
	1.3150	.381	.333
	1.3350	.381	.310
	1.3450	.381	.262
	1.3600	.381	.238
	1.3750	.381	.214
	1.3900	.381	.190
	1.4100	.381	.167
	1.4500	.381	.119
	1.5100	.381	.095
	1.5450	.333	.095
	1.5550	.333	.071
	1.5850	.286	.071
	1.6200	.238	.071
	1.6350	.238	.048
	1.6450	.190	.048
	1.7100	.143	.048
	1.8900	.095	.048
	2.0350	.095	.024
	2.0750	.048	.024

	2.1050	.048	.000
	3.1200	.000	.000
FAR2	-.4500	1.000	1.000
	.5550	1.000	.976
	.5650	1.000	.952
	.5750	1.000	.929
	.6050	1.000	.905
	.6350	1.000	.881
	.6450	1.000	.857
	.6550	1.000	.833
	.6850	1.000	.810
	.7250	1.000	.786
	.7450	1.000	.762
	.7550	1.000	.738
	.7650	1.000	.714
	.7850	1.000	.690
	.8150	1.000	.667
	.8350	1.000	.643
	.8700	1.000	.619
	.9050	.905	.595
	.9150	.905	.571
	.9250	.905	.548
	.9350	.905	.524
	.9450	.905	.500
	.9700	.905	.476
	.9950	.857	.476
	1.0100	.857	.452
	1.0250	.857	.429
	1.0350	.857	.405
	1.0500	.762	.357
	1.0650	.762	.333
	1.1000	.714	.333

	1.1350	.714	.310
	1.1450	.667	.310
	1.1550	.667	.286
	1.1700	.619	.262
	1.1900	.619	.238
	1.2100	.571	.238
	1.2350	.571	.214
	1.2550	.571	.143
	1.2800	.571	.119
	1.3100	.476	.119
	1.3250	.429	.119
	1.3350	.429	.095
	1.4000	.429	.071
	1.5600	.429	.048
	1.7100	.381	.048
	1.7900	.333	.048
	1.8500	.286	.048
	1.9250	.286	.024
	1.9750	.238	.024
	2.0500	.238	.000
	2.1550	.190	.000
	2.2050	.143	.000
	2.2250	.095	.000
	2.2700	.048	.000
	3.3100	.000	.000
FAR3	-.6800	1.000	1.000
	.3700	1.000	.976
	.4400	1.000	.952
	.4700	1.000	.929
	.4900	.952	.929
	.5050	.905	.929
	.5200	.905	.881

	.5350	.857	.857
	.5500	.857	.833
	.5650	.857	.786
	.5750	.857	.738
	.5950	.857	.714
	.6150	.857	.643
	.6300	.857	.595
	.6500	.810	.595
	.6650	.762	.571
	.6750	.762	.548
	.6850	.762	.524
	.6950	.762	.500
	.7150	.762	.476
	.7400	.762	.452
	.7550	.714	.429
	.7750	.667	.429
	.7950	.667	.405
	.8050	.667	.381
	.8150	.667	.333
	.8400	.619	.333
	.8650	.619	.286
	.8800	.619	.262
	.8950	.571	.262
	.9100	.524	.262
	.9300	.524	.214
	.9550	.476	.190
	.9800	.476	.167
	1.0000	.429	.143
	1.0150	.429	.119
	1.0250	.381	.095
	1.0500	.381	.071
	1.0900	.381	.048

	1.1150	.333	.048
	1.2600	.333	.024
	1.4100	.286	.024
	1.5250	.238	.024
	1.6900	.190	.024
	1.7800	.143	.024
	1.8350	.143	.000
	1.9150	.095	.000
	2.1650	.048	.000
	3.3600	.000	.000
FAR4	.0000	1.000	1.000
	1.0200	.952	.786
	1.0500	.905	.786
	1.0650	.857	.762
	1.0750	.810	.762
	1.0850	.810	.738
	1.0950	.810	.714
	1.1050	.810	.690
	1.1200	.810	.643
	1.1400	.810	.619
	1.1550	.810	.548
	1.1700	.714	.524
	1.1900	.714	.476
	1.2050	.667	.452
	1.2150	.667	.429
	1.2250	.667	.381
	1.2650	.667	.357
	1.3150	.571	.333
	1.3350	.571	.286
	1.3550	.571	.238
	1.3750	.571	.214
	1.3900	.571	.190

	1.4100	.571	.167
	1.4500	.571	.119
	1.5150	.571	.095
	1.5900	.571	.071
	1.6400	.571	.048
	1.6550	.524	.048
	1.7050	.476	.048
	1.7550	.429	.048
	1.8650	.381	.048
	1.9900	.286	.048
	2.0450	.286	.024
	2.0850	.238	.024
	2.1050	.238	.000
	2.1550	.190	.000
	2.2050	.143	.000
	2.2650	.095	.000
	2.3350	.048	.000
	3.3600	.000	.000

The test result variable(s): FAR1, FAR2, FAR3, FAR4 has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

Coordinates of the ROC Curve for Frequency tuning

Test Result Variable(s):TF

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
499.00	1.000	1.000
625.00	1.000	.714
875.00	.762	.143
1250.00	.190	.000
1750.00	.048	.000
2001.00	.000	.000

The test result variable(s): TF has at least one tie between the positive actual state group and the negative actual state group.

a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.