CERVICAL AND OCULAR VESTIBULAR EVOKED MYOGENIC POTENTIALS IN SMOKERS

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This Dissertation is submitted as a part of fulfilment

for the Degree of Master of Science in Audiology

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July 2020

CERTIFICATE

This is to certify that this dissertation entitled **'Cervical and Ocular vestibular evoked myogenic potentials in smokers'** is the bonafide work submitted in part fulfilment for the Degree of Master of Science (Audiology) of the student with Registration No: **18AUD031**. This has been carried out under the guidance of a faculty of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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CERTIFICATE

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DECLARATION

This is to certify that this Master's dissertation entitled **'Cervical and Ocular vestibular evoked myogenic potentials in smokers'** is the result of my own study under the guidance of **Ms Mamatha N.M**, Assistant Professor in Audiology, 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 Diploma or Degree.

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ABSTRACT

Purpose: The literature on effects of smoking on auditory-vestibular system has shown that smoking has an adverse effect on hair cells of both auditory and vestibular system. However, the previous studies have only shown that there is presence of vestibular symptoms in smokers and also there is limited literature on effects of smoking on vestibular structures. Hence, the current study was designed with the aim of determining the effect of smoking on otolith organs using cervical and ocular vestibular evoked myogenic potentials.

Methods: Two groups of participants aged 24-40 were taken for the study. Group I consisted of 18 smokers with a history of smoking for a duration of 1year or more and smoking minimum of 10 cigarettes per day and Group II consisted of 22 non-smokers who doesn't have any history of smoking. All the participants in both the groups underwent a detailed case history, pure tone Audiometry, immittance and cervical and ocular vestibular evoked myogenic potential testing.

Results: Absolute latency of the p1, n1 peak of the cervical evoked myogenic potentials and n1, p1 peak of ocular vestibular evoked myogenic potentials were significantly more ($p < 0.05$) in smokers than non-smokers. Also, smokers had significantly reduced peak to peak amplitude of cervical and ocular vestibular evoked myogenic potential than non-smokers.

Conclusions: The adverse effect of smoking on vestibular evoked myogenic potentials is seen in the study, which was evidenced through reduction of peak to peak amplitude and prolonged absolute latency in the smoker group. This suggests that there is an adverse effect of smoking on the utricle and saccule. This can be attributed to the endothelial dysfunction caused by the smoking and adverse effect of nicotine receptors on various synaptic levels in the central vestibular system.

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Chapter 1

Introduction

The inner ear consists of auditory and vestibular structures responsible for both hearing and balance functions respectively. The vestibular system consists of the otolith organs (utricle & saccule) and the semi-circular canals. The semi-circular canals and otolith organs sense angular acceleration and linear acceleration respectively. When there is a head motion, angular and linear accelerations are detected by the peripheral vestibular structures and are transduced to electrochemical signals for transmission to the central nervous system. The information collected as a result of linear and angular acceleration is integrated by the central nervous system and is used to stabilize gaze using the vestibule-ocular reflex (VOR), and to modulate muscle tone by means of vestibule-spinal (VSR) and vestibulo-collic (VCR) reflexes (Moore, Hirasaki, Raphan & Cohen 2001).

The cochlea and the vestibular organs shares the same membranous labyrinth of the inner ear and hence the abnormality or the dysfunction of one part may lead to dysfunction of the other part too. This is due to similarities seen in the vestibular hair cells and the cochlear hair cells and the blood supply to both the systems (Starr et al., 2003) The causative factors of sensory neural hearing loss can also accompany vestibular impairments because both the systems share the same membranous labyrinth. In the vestibular system it can lead to an impairment of either semi-circular canals or the otolith organs or both. Pajor, Gryczyński, Łukomski, & Józefowicz-Korczyńska (2002) reported that 50% of the individuals with sensorineural hearing loss complain of vertigo and 30% of them reported of dizziness. The prevalence of vestibular dysfunction in children with severe to profound hearing loss was found to be 18.75% (Wolter.et al. 2016).

There are various risk factors that have been noted for causes of hearing impairment, such as presence of diabetes, industrial and/or recreational noise exposure, lower socioeconomic status, exposure to noisy environmental conditions, a history of coronary heart disease, symptoms of peripheral neuropathy, alcohol abuse (Bainbridge, Hoffman& Cowie 2011). Along with the above risk factors, exposure to cigarette smoke was also found to be associated with a 4.9 times increase in the prevalence of hearing deficits (Lyons, 1992). Thus smoking is well known causative factor for inner ear disorder. Disorder of inner ear may lead to a different type of manifestation including, spatial disorientation, vertigo, blur vision, and hearing impairment.

Although cigarette smoking causes many health related problems like cancer, heart disease, stroke, coronary spastic angina, acute coronary syndrome etc., but still it has become a common trend all over the world. it has been reported that approximately 1.3 billion people consume tobacco worldwide (Shafey, Dolwick & Guindon, 2003). Hazardous health effects of smoking is found to have depended on various factors such as , the age at which the smoking began and the number of cigarettes smoked per day, the type of inhalation and nicotine content in cigarette (Fletcher & Peto, 1977).

Tobacco smoking is found to affect the inner ear through several mechanisms that can be categorized as direct or indirect. Reactive oxygen species(ROS) and free radicals are generated due to the toxic substances in the cigarette. ROS induce direct oxidative damage and free radicals damages many cellular components such as DNA, protein, and lipids. All this damage leads to neurosensory hearing loss that affects especially the higher frequencies. Indirect damage is mediated by the vasospastic effect caused by nicotine on blood vessels.

Nicotine increases the acceleration of atherosclerosis process in the vascular loops of the auditory system. Moreover, it also increases levels of carboxy-hemoglobin in the blood. All these factors are observed to reduce oxygen perfusion in the inner ear (Yamaguchi, Haginaka & Morimoto, 2005; Therriault, Proulx, Castonguay, & Bissonnette, 2003).

Studies in the literature have concluded that smoking is found to harm the auditory system which is noted as an elevation in the pure tone hearing threshold, abnormal oto-acoustic emissions (reduced amplitude of evoked oto-acoustic emission) as well as auditory evoked potentials (Jedrzejczak, Koziel, Kochanek, & Skarzynski, 2015). Also, the toxic ingredients such as mercury and arsenic which can be seen in tobacco smoking can cause the degeneration of cochlear hair cells and also demyelination of nerves of auditory pathway (Cruickshank's et al., 1998) and also reduction in amplitude of distortion product oto-acoustic emissions(DPOAE) and wave V of auditory brainstem response (Gopal et al., 2009).

A hospital based study was done by Kumar et al. (2013) that consisted of total of 148 subjects, aged from 20 to 60 years among which 108 were smokers and 40 were age matched non-smokers. Based on smoking history audiological evaluations, it was found that, mild form (26-40 dB loss) was the most common (56.5%), while the severe type was the least common (2.8%) in the smokers and with 65.7% of the smokers and 15% of the non-smokers having hearing impairment. Also, as the age increased, the percentage of the affected individuals also increased, with greater percentages of the smokers being affected in comparison to the non-smokers. The most common type of hearing loss in the smokers was the sensorineural type (77.5%), followed by the mixed hearing loss (18.3%), while the mixed type was found in the non-smokers. Hence, it was concluded that severity of

the hearing loss in the smokers increased with an increase in the number of bidis/cigarettes.

Moreover, smoking can act as a risk factor for noise-induced hearing loss since smokers simultaneously exposed to noise in the workplace were affected by a higher degree of hearing loss compared to non-smokers (Zeilinger et al. 2013). Recent studies also have suggested atherosclerosis as a major cause for peripheral vestibular disorders and hearing loss (Oron, et al. in 2017, Wada et. al. in 2016). Early identification of these peripheral vestibular disorders can be done by using test like vestibular evoked myogenic potentials, video head impulse test caloric tests and rotatory chair test.

The vestibular evoked myogenic potentials (VEMP) is a short latency myogenic potential evoked by air conduction sound (Colebatch, Halmagyi, & Skuse,1994), bone conduction, vibration (Todd, Rosenberg, &Colebatch,2009) and the electric stimulation (Watson & Colebatch,1998). VEMP is recorded from surface electrodes placed over the muscles. These muscles can be triceps muscles, soleus muscle, gastrocnemius muscle, masseter muscle, extensor muscles of neck, sternocleidomastoid muscle and inferior oblique muscle (Cherchi et al in 2009, Rosenberg, Todd, & Colebatch in 2005). When the potential is recorded from the sternocleido mastoid muscle it is called as cervical VEMP(c-VEMP) and when it is recorded from the inferior oblique muscle it is called as ocular VEMP(o-VEMP). c-VEMP is useful tool to check the function of sacculo-collic reflex pathway and o-VEMP is mainly used to assess the function of otolith ocular pathway (Halmagyi, Curthoys & Ann, 1999).

1.1 Need for the study

1.1.1 Association between cochlear disorders and vestibular disorders

There is a report of existence of great association between vestibular or balance disorders in sensorineural hearing loss as vestibule and cochlea is found to share a continuous membranous labyrinth and also has a similar receptors cell (Zhou, Wu, & Wang, 2016). Thus the damage to the cochlea can lead to damage to the vestibular organ.

There are many reports of vestibular and balance dysfunction in hearing-impaired children, as it was reported that 70% of children persisting with sensorineural hearing loss (SNHL) have vestibular system disorders (Santos, Venosa, & Sampaio, 2015). The prevalence of vestibular related problems in SNHL individuals differs across studies and age groups. Pajor, Gryczyński, Łukomski, & Józefowicz-Korczyńska (2002) reported that 50% of the individuals with sensorineural hearing loss complain of vertigo and 30% of them reported of dizziness. The prevalence of vestibular dysfunction in children with severe to profound hearing loss was found to be 18.75% (Wolter.et al. 2016). The vestibular symptoms such as difficulty in balancing among the individuals with unilateral sensorineural hearing loss (Schunknecht 1993, Volker & Chole 2010) has also been reported.

1.1.2 Smoking as a risk factor for inner ear diseases

The epidemiology of hearing loss based studies have reported that current smoking was associated with an increased risk of hearing impairment (Cruickshank’s et al. 2015). According to a study done on Bangladeshi population, it has been

shown that smokers had significantly higher hearing thresholds at 4, 8, and 12 kHz frequencies than non-smokers (Sumit, et al. 2015). Smokers are found to be more susceptible to get hearing loss compared to non-smokers (Cruickshank's, et al. 2015).

Vinay et.al. (2009) studied the effect of smoking on the amplitudes of transient evoked otoacoustic emissions (TEOAEs) in two groups aged from 20 years to 69 years having normal hearing sensitivity. Group I consisted of fifty smokers and group II consisted of fifty non-smokers. Their results showed that TEOAEs amplitude (SNR Values) was significantly reduced in smokers compared to non-smokers across all the age range. Authors concluded that smoking have adverse effect on the outer hair cell functioning, which lead to poor SNR in smoking group.

Munjal et al. (2017), concluded that smoking leads to hair cell loss in smokers. He found that the extended high frequency pure tone thresholds were worsen in the smokers than non-smokers. Moreover, the mean amplitude of the both distortion product otoacoustic emission and transient evoked myogenic potentials were reduced in smokers. Similar findings has been reported by the Rogha et al. (2015), where they found that the mean hearing threshold at 8000 Hz in smoker group deteriorated significantly compared with non-smoker group and concluded that smoking causes high frequency hearing loss.

Thus from the above mentioned studies it can be concluded that smoking can lead to adverse effect on auditory system as it mainly affects the functioning of outer hair cells which is shown as reduced amplitude of both transient and distortion product otoacoustic emission in various studies.

1.1.3 Effects of smoking on vestibular system

Smoking is reported to result in endothelial dysfunction and vasospasm which leads to impaired vasodilatation. This impaired vasodilation may be found to cause peripheral vestibular disorders (Nezamoddin et al. 2016). Smoke contains the nicotine which acts on the nicotinic receptor which includes a non-selective cation channel and causes depolarization and excitation of the cells. These receptors are found in different parts of vestibular systems (both peripheral & central), spinal system, sensorimotor systems. Thus, nicotine affects the overall vestibular physiology that is needed to maintain the body balance which leads to increase in the body sway in the smokers (Cristiana Borges Pereira, Michael Strupp, Thomas Holzleitner & Thomas Brand, 2001).

Smoking has irreversible hazardous effects on saccular hair cells. Mustafa et al (2013), reported that absolute latency of the p1 and n1 peaks of cervical vestibular evoked myogenic potentials in smokers were significantly more than non-smokers. These effects could be imputed to the impact of nicotine on the microvascular dynamics. All these previous studies (Mustafa et al.2013, Cristiana et al.2001) indicate that there isadverse effect of smoking on the hearing and vestibular system (saccule & utricle).

Since there are very few studies conducted on the effect of smoking on the vestibular system. Moreover, all these studies are done in western population, and racial difference in may be present between Indian and western population. Hence, the present study is taken to study the effect of smoking on peripheral vestibular system with respect to Indian context.

1.2 Aim of the Study

Present study aims to know the effect of smoking on cervical and ocular vestibular evoked myogenic potentials.

1.3 Objectives

- To compare the peak to peak amplitude of both cervical vestibular evoked myogenic potentials and ocular vestibular evoked myogenic potentials between the smokers and non-smokers.
- To compare the absolute latency of both cervical vestibular evoked myogenic potentials and ocular vestibular evoked myogenic potentials between the smokers and non-smokers.

Chapter 2

Review of literature

2.1 Smoking and its effect on health

Epidemiological and clinical studies indicate that Smoking is an explicit and definite risk factor in a group of diseases. Cigarette smoke contains many harmful chemicals such as hydrogen cyanide, formaldehyde, lead, arsenic, ammonia, radioactive elements, benzene, carbon monoxide, nitrosamines, polycyclic aromatic hydrocarbons (PAHs) nicotine and carbon dioxide. All these chemicals are found to cause many diseases such as cancer, heart disease, stroke, coronary spastic angina, acute coronary syndrome, atherosclerosis, chronic bronchitis, emphysema, asthma, lupus, alzheimer's disease, cataracts, rheumatoid arthritis, crohn's disease, psoriasis, chronic obstructive pulmonary disease, hypertension and hearing loss. Smokers when compared to non-smokers, are noted to be at a greater risk of having bacterial respiratory infections, chronic viral diseases, cancers (oral, laryngeal, oesophageal, pancreatic, renal, and bladder cancer), circulatory diseases such as arteriosclerosis, aortic aneurism, stroke, and multiple organ disorders. Even passive smoking (second hand smoke) with a smoke exposure of about 10% that of active smoking is associated with approximately a 30% increase of coronary artery disease (CAD), compared with an 80% increase in active smokers (Black cl al. 1995; Pearl et al.in 1938; Willett el al., 1987, Barnoya and Glantz. 2005).

Moreover, some studies have suggested that maternal smoking during pregnancy can lead to intellectual delays, most likely caused by central nervous system impairment, or can adversely affect language ability through underlying physiologic mechanisms (e.g., the outer hair cells in the ear), thus leading to poorer

performance on auditory processing tasks, temporal auditory processing, auditory brainstem responses and attention deficit hyperactivity disorder (Cruickshanks et. al.1998 & McCartney et.al. 1994).

2.2 Biological effects of smoking on health

Cigarette smoke contains nicotine, which is a naturally occurring alkaloid found in the tobacco. Nicotine is primary addictive and bioactive agent in cigarette smoke (Doolittle et al. 1995; Goldberg et al, 1981, 1982, 1983; Corrigall et al.1999; Rabinoff et al., 2007). Inhalation of the cigarette smoke is found to result in rapid absorption of nicotine through the lungs into the blood stream. Once it crosses the blood-brain barrier and reaches the central nervous system (CNS), it stimulates nicotinic acetylcholine receptors (Pomerleau et al. 1984; Doolittle et al. 1995; Hukkanen et al. 2005; Mendelson et al. 2005). The stimulation of receptors in the CNS and the hypothalamic-pituitary-adrenal axis promotes release of many chemical messengers including acetylcholine adrenocorticotropic hormone, norepinephrine, epinephrine, arginine vasopressin and dopamine (Pomerleau et al. 1989; Koob and Le Moal. in 2001; Sinha. 2001; Contorcggi et al. 2003). The resultant increase of these compounds in the CNS has been implicated in the addictive nature of nicotine activation of the sympathetic nervous system (SNS) and results in elevation of these compounds outside the CNS, in blood plasma and are likely to be associated with the systemic cardiovascular effects of cigarette smoking (Ambrose et al., 2004; Mendelson et al., 2006).

Tobacco toxicity is directly proportional to the number of cigarettes smoked and inversely proportional to the age at which the habit was initiated (Slotking et al. 2007). Hearing sensitivity of smokers is found to be 1.5 times more likely to be

reduced compared to non- smokers. Cigarette smoking is also considered to be highly associated with development of hearing loss (Fransen et al., 2008).

2.3 Smoking and its effect on auditory system

Smoking is a noted as a serious public health problem and several studies have shown its many harmful effects on individuals. Smoking damages the endothelium layer of the labyrinthine arteries and allows a collection of substances, known as plaque, to build up in the artery wall. Accumulation of these plaque leads to the narrowing of labyrinthine artery and eventually block the artery which consequently decrease the net cardiac output to the inner ear resulting in symptoms like vertigo, hearing loss and tinnitus. Thus, tobacco is found to cause vascular changes that is found to affect cochlea and also results in reduced blood supply to the cochlea (Lowe, Drummond, Forbes, & Barbenel, 1980). Smoking consequently lowers blood oxygen levels, vascular obstruction, alters blood viscosity, and possibly ototoxicity. Toxic ingredients such as mercury and arsenic which are seen in smoking can cause the degeneration of cochlear hair cells and also demyelination of nerves of auditory pathway (Cruickshanks et al. 1998). Exposure to cigarette smoke was found to be associated with a 4.9 times increase in the prevalence of hearing deficits (Lyons, 1992).

According to a study done on Bangladeshi population, it has been shown that smokers had significantly higher hearing thresholds at 4, 8, and 12 kHz frequencies than non-smokers (Sumit, et al. 2015). Smokers are found to be more susceptible to get hearing loss compared to non-smokers (Cruickshank's, et al. 2015). Even the prenatal and neonatal exposure to nicotine from smoking has shown to alter or diminish the functioning of the cortical nicotinic acetylcholine receptors leading to

long-term negative effects on auditory-cognitive functions in adult rats (Liang et al., 2006).

Vinay et. al. (2009) studied the effect of smoking on the amplitudes of transient evoked otoacoustic emissions (TEOAEs). He conducted study in two groups containing fifty participants in each group. All the participants were having normal hearing sensitivity with age ranged from 20 years to 69 years. Their results showed that TEOAEs amplitude (SNR Values) was significantly reduced in smokers compared to non-smokers across all the age range. Authors concluded that smoking have adverse effect on the outer hair cell functioning, which lead to poor SNR in smoking group.

Munjal et al. (2017) revealed a statistically significant difference between chronic male smokers (age 24-40 years) and the non-smoker group (age 20-31 years) for behavioural thresholds (right ear: 250 Hz, 500 Hz, 2000 Hz and 4000 Hz; left ear: 250 Hz-8000 Hz). Also smokers were found to have different forms of degree of hearing loss that was shown in extended high-frequency audiometry. DPOAE and TEOAE results also revealed a statistically significant difference between the two groups at 2 kHz and 4 kHz and no significant difference was observed at 0.5 kHz, 1.4 kHz, 2 kHz, 3 kHz, 4 kHz, and 8 kHz.The study also revealed that chronic smokers were found to have reduced amplitude for OAEs, especially in high frequencies compared to non-smokers. Hence, the results here suggest the need for OAEs & HFA as essential tools for early identification of hearing loss related to smoking.

Similarly, Rogha et al. (2015) evaluated a hearing threshold of the smoker group versus non-smoker by measuring pure tone thresholds, TEOAE's, and DPOAE's. Some of the factors like smoking intensity (number of cigarettes smoked

per day) which categorized in three-level: mild (<10 cigarettes smoked per day), moderate (10-20 cigarette smoked per day) and severe (>20 cigarettes smoked per day) and use of other tobacco products and drugs are being used were recorded. Results indicated significant differences between smoking intensity (mild & severe level) and hearing threshold at 2000, 4000 and 8000 Hz, but statistically no significant difference was found between smoking intensity and DPOAE/ TEOAE test results.

Mehrparvar et al. (2015) assessed the simultaneous effect of noise exposure and smoking on standard pure tone audiometry (PTA) and distortion product otoacoustic emissions (DPOAEs). A total of 224 workers who were exposed to noise and were divided into two groups: Smokers (n = 105) that served as case group and non-smokers (n = 119) served as control group and measured DPOAE's response amplitudes. All the subjects were exposed to 91.08 ± 2.29 dB A [time-weighted average (TWA) for an 8 h work shift]. The results indicated that mean DPOAE response amplitude at frequencies higher than 1,000 Hz was significantly lower in the smokers compared to non-smokers. From this study, it can be observed that, the smoking can aggravate the effect of noise on hearing.

Prabhu, Varma, Dutta, Kumar and Goyal, (2017) attempted to determine the influence of smoking on contralateral suppression of distortion product otoacoustic emissions (DPOAE). The he differences in the amount of contralateral suppression of DPOAE between smokers and non-smokers was determined. In addition, correlation was also determined among duration of smoking, frequency of smoking and number of cigarettes per day on contralateral suppression of DPOAE's. The study was carried out on 25 smokers and non-smokers. Their results of the study showed that the amount of suppression was reduced in smokers at all the frequencies

suggesting an efferent auditory system dysfunction. The increase in the duration of smoking, the frequency of smoking and the number of cigarettes smoked correlated negatively with the amount of suppression. Authors concluded that chronic smoking habits increases the risk of efferent auditory damage.

Prabhu, Varma, Dutta, Kumar and Goyal, (2017) determined the effect of smoking on ultra-high-frequency auditory sensitivity and relationship between the nature of smoking and ultra-high-frequency otoacoustic emissions (OAEs) and high frequency pure tone thresholds. Their study sample included 25 smokers and 25 non-smokers. High-frequency audiometric thresholds and amplitudes of high-frequency DPOAE's were analysed for both ears from all participants. The results showed that the ultra-high-frequency thresholds were elevated and that there was reduction in the amplitudes of ultra-high-frequency OAEs in smokers. Authors concluded that chronic smoking leads to increased risk of damage of auditory system and high ultra-high-frequency OAEs and ultra-high-frequency audiometry can be used for the early detection of auditory impairment.

2.4 Effect of smoking on vestibular system

The smoke from a cigarette has over 4500 complex chemicals in them including carbon monoxide (CO), nicotine, and carbon dioxide. Nicotine is found to decrease blood supply through vasoconstriction which may increase in susceptibility to peripheral vascular disease. Cigarette smoking induces endothelial dysfunction and inflammatory responses. Nezamoddin et al. (2015) determined of the relationship between endothelial dysfunction and smoking. A Flow-mediated dilation (FMD) of brachial artery was measured to assess the endothelial function in 30 patients with SSNHL and 30 healthy individuals using a high-resolution B-mode sonogram. Their results showed that Flow-mediated dilation (FMD) was

significantly lower in patients than controls. Since, the blood supply to the cochlea is maintained by the labyrinthine artery, which has no other collateral vasculature. Vascular disorders may cause inner ear injury and dysfunction secondary to anoxia or hypoxia Furthermore, endothelial dysfunction, high levels of haemostatic factors, and a disturbed blood flow are the fundamentals of thromboembolic diseases and can impair the microcirculation of the cochlea (Pasquale, Francesco & Valeria et al. in 2007). This smoking-induced changes have been found to cause transient bloodstream disruption to the labyrinthine artery, a feeding artery to the inner ear, potentially leading to new peripheral vestibular disorder (PVD) events.

Smoking also leads to an elevation in white blood cell counts, cytokines, reactive oxygen species (ROS), cyclooxygenase-2 (COX-2), and increased lipid peroxidation levels. These changes are associated with amount of dose (number of cigarette), and potentially reversible form of arterial dysfunction, induced by cigarette smoking (Barbieri et al. 2011).

Smoke contains the nicotine which acts on the nicotinic receptor, it includes a non -selective cation channel and causes depolarization and excitation of the cells. These receptors are found in different parts of vestibular systems (both peripheral & central), spinal system, sensorimotor systems. Thus, nicotine is noted to affect the overall vestibular physiology that is needed to maintain the body balance which leads to increase in the body sway in the smokers. (Pereira, Strupp, Holzleitner & Brand, 2001).

Wada et al. (2017) also reported that the smoking is associated with the origin of new peripheral vestibular disorders. He conducted retrospective study in which 393 participants aged ≥20 years [mean age 65.3 years; males 133 (33.8%)]

treated for hypertension, dyslipidaemia, or diabetes mellitus at a primary care clinic between November 2011 and March 2013 were enrolled. Participants were categorized as ever-smokers (including current and past -smokers; divided per <30 and ≥30 pack-years), and never-smokers. New peripheral vestibular disorders (PVD) events were reported over a 1-year follow-up period. Hazard ratios (HR) for new onset PVD were estimated using the Cox proportional hazard regression model. He found that hazard ratios (HR) was more in smokers when compared with non-smokers.

Mustafa et al. (2013) compared the amplitude of transient evoked otoacoustic emissions (TEOAEs) and latencies of vestibular evoked myogenic potentials (VEMPs) among non-smokers, cigarette smokers, water pipe smokers, mixed smokers and ex-smokers. A total of 50 non-smokers, 28 water pipe smokers, 34 pure cigarette smokers, 28 mixed cigarette-water pipe smokers, and 21 ex-smokers with age ranged from 20 to 40 years were evaluated in the study. All had normal hearing sensitivity and normal middle ear functions and amplitude of TEOAEs and cervical vestibular evoked myogenic potentials (c-VEMP) were measured for all these participants. Results of their study showed that smoking had deleterious effects on the hair cells in the labyrinth. Damage to the outer hair cells was evidenced by the reduced amplitude of the TEOAEs in smokers and ex-smokers when compared with control group. Similarly, harm to the saccular hair cells was detected by the increased latency of the c-VEMPs which was significantly prolonged in smokers than non-smokers. Results also suggested that cessation of smoking could not change the profile of TEOAEs or VEMPs. Authors suggested that smoking could have irreversible hazardous effects on the labyrinthine hair cell functions.

These effects could be attributed to the impact of nicotine on the micro vascular dynamics.

To conclude, Smoking is a known cause of inner ear dysfunction and it ranges from loss of hearing and balance sensitivity to the hyperactive disorders like tinnitus. Smoking mainly affects the hair cells of inner ear by a process known as atherosclerosis. Atherosclerosis leads to narrowing of the inner ear vessels thus reducing the overall input of blood supply and affecting the microcirculation of inner ear. Some studies have also shown the ill effects of smoking on central nervous system. It mainly includes improper activation of the various nicotine receptors which may leads to the poor perception of sound and problems in maintaining the balance.

Chapter 3

Methods

The aim of the current study was to assess and compare the sacculo-collic and otolith ocular pathway function using cervical vestibular evoked myogenic potentials (c-VEMP) and ocular vestibular myogenic potentials (o-VEMP) between smokers and non-smokers. In order to achieve the aim, a standard group comparison research design was used.

3.1 Participants

Two groups of subjects with age range of 24 to 40 years were included in the study. Group I included 18 individuals with the habit of cigarette smoking and Group II included 22normal hearing individuals without smoking habits. Both the groups underwent the same procedure of testing which included taking of case history, diagnosis for normal hearing, normal middle ear function and recording of vestibular evoked myogenic potentials including both cervical vestibular evoked myogenic potential and ocular vestibular evoked myogenic potentials.

3.2 Participants selection criteria

- Both Group I and Group II included participants in the age range between 24 to 40 years. For Group I, smoking at least a pack of 10 cigarettes per day and average time they had smoked is more than 1 year was considered. For the selection of participants for both group I and Group II, the following inclusion criteria were used.

3.3 Instrumentation

- A calibrated Inventis Piano Plus was used to perform threshold estimation (pure tone audiometry and speech audiometry in both the groups. Calibrated TDH 39headphones for AC threshold and calibrated B-71 bone vibrator for BC threshold was used.
- A calibrated GSI Tympstar Immittance meter was used to measure tympanometry with a probe tone frequency of 226 Hz. The same equipment was used for measuring ipsilateral as well as contralateral acoustic reflexes at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz.
- A vestibular evoked myogenic potentials were recorded using the bio-logic navigator pro with an Insert ER-3A earphone (Etymotic Research, Inc., Elk Grove Village, IL, USA).

3.4 Procedure

3.4.1 Case history

A detailed case history in the form of an interview was taken from all the participants before the commencement of the evaluation. During this, the individuals were asked about:

- General history was taken regarding the age and occupation of the participants.
- Detailed medical history was taken regarding the presence of medical conditions like hypertension, diabetes, neurological diseases, otological diseases, recent upper respiratory tract infection and significant past

diseases/illnesses as well as surgery. Also the history of auditory problems such as otitis externa, occlusion due to ear wax, and otitis media was taken.

- Participants were asked about the smoking habits i.e., total number of cigarettes they consume daily and the duration since they are smoking.
- Otoscopic examination was carried out for every participant to check for stenosis of canal, earwax and presence of any foreign body. Also, the tympanic membrane was viewed through otoscope to rule out any perforation, scar or infection.

Various behavioural vestibular tests such as Romberg, Fukuda and Tandem gait test and past-pointing test were carried out to rule out the vestibular pathology.

- Romberg test: Romberg test was carried out by instructing the participant to stand with his/her feet together and arm stretched forward so that they were parallel to the ground and also to each other. The test was carried out in both eyes open (vision enabled) and eyes closed (vision denied) conditions. Presence of sway/imbalance was considered as an abnormal result.
- Fukuda test: During the Fukuda stepping test, the participant was asked to march for 50 steps at the same place with his/her eyes closed and arms stretched forward (similar position as that used during the Romberg test). Finding of deviation greater than 45^{o} towards either side and/or distance of >1m from original standing point was considered abnormal.
- Tandem gait test: Tandem gait test was performed with the participant walking heel-to-toe with head held straight for about 5 meters on an imaginary straight line. Presence of sway or loss of balance was considered an abnormal finding.

Past-pointing test (finger to nose test): During the past-pointing test, the participant was asked to touch his/her nose tip and the clinician's fingertip with his/her fingertip alternately. The position of the clinician's finger was varied in the space in such a way that the distance and the direction were both unpredictable. Citing of undershoot/overshoot of the target and/or presence of evident tremors was considered abnormal.

3.4.2 Test Environment

All the tests will be performed within the noise permissible criteria of ANSI S3.1 (1991) in an acoustically treated room.

3.4.3 Pure tone Audiometry

Using the modified Hughson and Westlake procedure (Carhart & Jerger, 1959), air conduction threshold with the TDH 39 headphones and bone conduction thresholds with B-71 bone vibrator will be obtained for octave frequencies from 250 to 8000 Hz and 250 to 4000 Hz respectively to investigate the hearing sensitivity of each participant.

3.4.4 Immittance Evaluation

Tympanometry was done to rule out any middle ear pathology. Tympanometry was done at 226 Hz probe tone and both ipsilateral and contralateral stimulation acoustic reflex threshold will be elicited for 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz.

3.5 Vestibular Evoked Myogenic Potentials

3.5.1 Recording of cervical vestibular evoked myogenic potentials (c-VEMP)

The c-VEMP was recorded using ER-3A insert ear phones from the bio-logic navigator pro evoked potential system. The participant was seated on a comfortable chair in an upright position. The following steps were involved in recording of c-VEMP.

- Identification of sternocleidomastoid muscle: Sternocleidomastoid muscle was identified by palpating and finding the stiff part when the head was turned to the opposite side.
- Electrode placement: The electrode placement sites were scrubbed with a commercially available abrasive gel. The inverting (negative / reference) electrode was placed at the sterno-clavicular junction, the non-inverting (positive / active) electrode at the upper one-third of the sternocleidomastoid muscle and the ground (common) electrode on the forehead. These electrodes were secured with surgical tape. The absolute impedance and inter-electrode impedance were ensured within 5 kΩ and 2 kΩ, respectively.

The stimulus and acquisition parameters used for recording of cervical vestibular evoked myogenic potentials are given in Table 3.1

Table 3.1

Shows stimulus and acquisition parameters for recording of c-VEMP

Stimulus parameters		**Acquisition parameters**	
Stimulus type	Tone burst	Analysis time	0 - 50 ms
Frequency	500 & 1000 Hz	Filter setting	0.1 to 1000 Hz
Intensity	125 dB pe SPL	Averages	200
Gating	Blackman Window	Amplifier gain	30000
Stimulus duration	2-1-2 ms	No. of channels	1
Onset phase	Alternating	Electrode	Non inverting: half
Rate	5.1/s	montage	way or one third from
Mode	Monaural		mastoid.
Transducer	Insert earphones		Inverting: Sterno-
			Clavicular junction
			Ground : forehead

EMG monitoring and EMG normalization were used to control the effects of variable muscle tension on c-VEMP responses. The participants were given visual feedback by asking them to maintain the needle deflection within the green zone which was equated to an EMG range of 30-70 µV. Further, the raw amplitude was divided by the root mean square of the pre-stimulus EMG in order to achieve EMG normalized c-VEMP amplitude. c-VEMP was obtained only from one ear of each participant, with half of the participants in each group undergoing recording from his/her right ear and the other half from left ear. This was done in order to avoid ear order effect, if any.

3.5.2 Recording of Ocular vestibular evoked myogenic potentials (o-VEMP)

The o-VEMP was recorded using ER-3A insert ear phones from the bio-logic navigator pro evoked potential system. The participant was seated on a

comfortable chair in an upright position and following steps were followed while recording o-VEMP.

Steps:

- Identification of inferior oblique muscle: inferior oblique ocular muscle was identified by palpating and finding the stiff part when the participant was looking straight upright at an angle of 30 degree or more.
- Electrode placement: Electrode placement site was prepared using a skin preparation gel. Surface disc (Ag Cl) electrodes were used for recording. Using a single-channel surface electrode montage, the inverting electrode was placed 1cm inferior to the lower eyelid and the non-inverting electrode was placed 1cm below the inverting electrode. Absolute electrode impedances were maintained below 5 kΩ and inter-electrode impedances was maintained below 2 kΩ. The stimulus and acquisition parameters used for recording of cervical vestibular evoked myogenic potentials are given in Table 3.2

Table 3.2

Shows stimulus and acquisition parameters for recording of o-VEMP

Stimulus parameters		Acquisition parameters	
Stimulus type	Tone burst	Analysis Time	64 ms. (10ms pre-stimulus)
Frequency	500 & 1000 Hz.	Filter Setting	0.1 to 1000 Hz
Intensity	125 dB pe SPL	Averages	200 per recording
Gating	Blackman Window	Amplifies Gain	30000 times
Stimulus duration	2-1-2 ms.	No. of channels	1
Onset Phase	Alternating	Electrode	Non inverting: 1 cm
Rate	5.1/s	montage	below contralateral eye.
Mode	Monaural		Inverting: 2 cm below
Transducers	Insert earphones		non inverting electrode
			Ground : Forehead

3.6 Waveform interpretation and Analysis

3.6.1 Labelling of o-VEMP waveform

The first initial negative peak in the region of 7msec-13msec was taken as n1 and the subsequent positive peak in the 13 msec-19 msec region was taken as p1 (Todd, Rosengren, & Colebatch 2003). After the labelling of peaks, the latency of n1 peak, p1 peak and amplitude of n1-p1 complex was calculated for both smokers and non-smokers.

Latency calculation

The time interval between the onset of the stimulus to the first negative peak (n1) on the time axis was considered as the onset latency of the n1 peak as shown in the figure 3.1

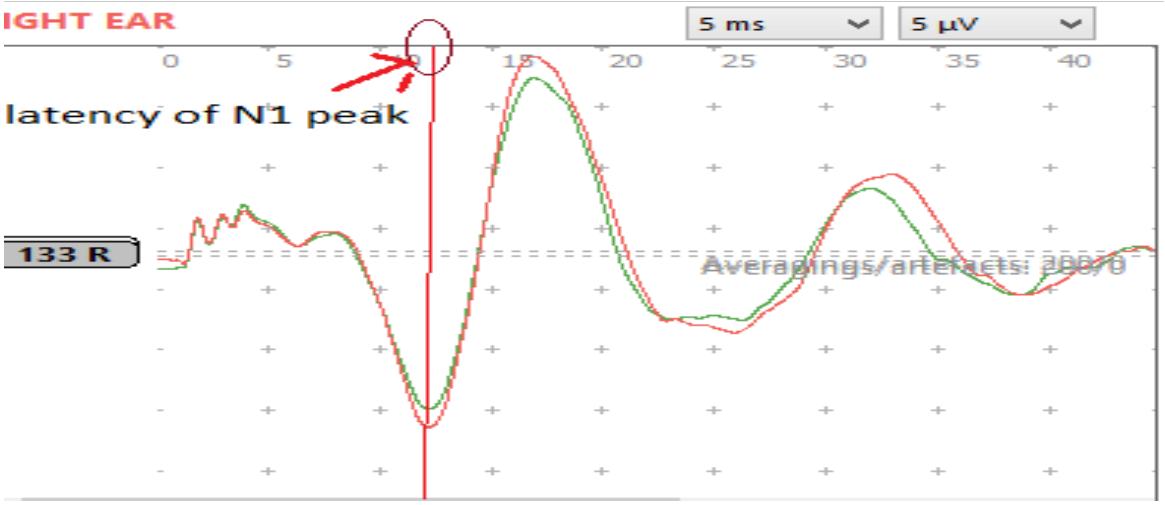

Figure 3.1: Shows schematic representation of the latency of n1 peak of the o-VEMP.

Similarly, the time interval between the onset of the stimulus to the first positive peak (p1) on the time axis was considered as the onset latency of the p1.

Amplitude calculation

The amplitude difference between n1 peak and p1 peak on the amplitude axis in microvolt was considered as peak to peak amplitude as shown in the figure.3.2

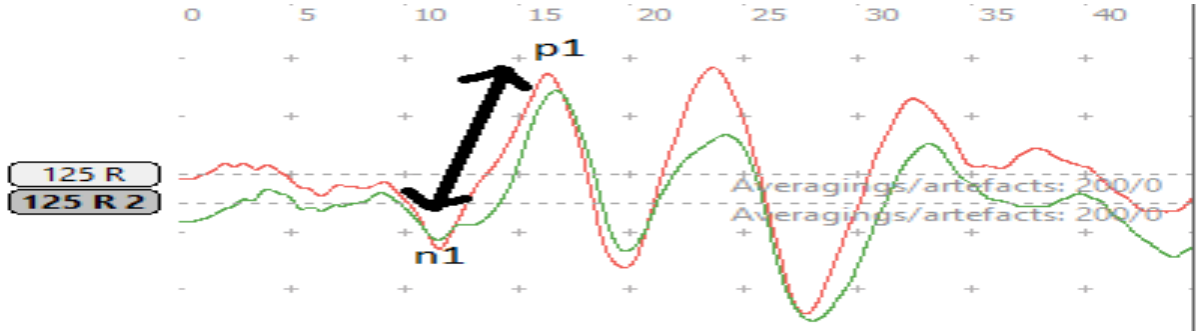

Figure 3.2: Shows calculation of the amplitude of n1-p1 complex in the o-VEMP waveform.

3.6.2 Labelling of c-VEMP waveform

The first initial positive peak in the region of 10msec-15msec was taken as p1/p13 and the subsequent negative peak in the 20msec.-25msec. region was taken as n1/n23. This labelling was done on the basis of waveform labelling conventions given by Akin and Murnane (2001). After the labelling of peaks, the latency of p1/p13 peak, n1/ n23 peak and amplitude of P13-N23 complex was calculated for both smokers and non-smokers.

Latency calculation

The time interval between the onset of the stimulus to the first positive peak (p1/p13) on the time axis was considered as the onset latency of the p1/p13 peak. Similarly, the time interval between the onset of the stimulus to the first negative peak (n1/n23) on the time axis was considered as the onset latency of the n1/n23 peak.

Amplitude calculation

The amplitude difference between p1 peak and n1 peak on the amplitude axis in microvolt was considered as peak to peak amplitude.

Chapter-4
Results

The objective of the study was to compare the peak to peak amplitude and absolute latency difference in ocular vestibular evoked myogenic potential (o-VEMP) and cervical vestibular evoked myogenic potential (c-VEMP) between the smokers and non-smokers. To investigate the objectives of the study, peak to peak amplitude and absolute onset latency of the vestibular evoked myogenic potentials (both ocular & cervical) were considered. The study was conducted on 18 smokers and 22 non-smokers. Descriptive statistics was done to find the mean & standard deviation (SD) value of absolute latency and peak to peak amplitude of vestibular evoked myogenic potential (both ocular &cervical) in smokers and non-smokers.

The data was subjected to Shapiro-Wilk test to check whether it follows normal distribution or not. The test results showed that the data is following normal distribution ($p > 0.05$) and hence parametric statistical tests were performed to compare the mean latency and mean peak to peak amplitude between the smokers and non-smokers group. In the present study multivariate analysis of variance(MANOVA) was administered to compare the peak to peak amplitude and absolute latency values of both c-VEMP and o-VEMP between smoker and non-smoker groups among the various dependent variables (right p1, right n1, left p1and left n1 for both c & o-VEMP).

The results of the present study are presented in the following headings:

- Latencies and amplitude of Cervical vestibular evoked myogenic potentials in smokers and non- smokers.

- Latencies and amplitude of Ocular vestibular evoked myogenic potentials in smokers and non- smokers.
- Comparison of latencies for both cervical and ocular vestibular evoked myogenic potentials between smokers and non- smokers.
- Comparison of amplitude for both cervical and ocular vestibular evoked myogenic potentials between smokers and non-smokers.

4.1 Cervical vestibular evoked myogenic potentials in smokers and non-smokers

4.1.1 Absolute Latency of c-VEMP

Descriptive statistics was done to find the mean absolute latency (in msec) and the standard deviation (SD) for p1 latency, n1 latency of cervical vestibular evoked myogenic potentials for smokers and non-smokers group in each ear individually. The mean absolute latency and SD of p1 and n1 of cervical vestibular evoked myogenic potentials obtained for both right ear and left ear are represented in table 4.1

Table 4.1

Mean and standard deviation of c-VEMP p1 and n1 absolute latencies in smokers and non-smokers.

p1 and n1 latency of c-VEMP in smokers and non-smokers	Total number of participants(n)	Mean value of latencies (msec)	SD value of latencies (msec)
p1 latency of non-smokers (Right ear)	22	13.63	0.48
n1 latency of non-smokers(Right ear)	22	23.53	0.46
p1 latency of non-smokers(Left ear)	22	13.68	0.42
n1 latency of non-smokers(Left ear)	22	23.65	0.28
p1 latency of smokers(Right ear)	18	15.66	0.60
n1 latency of smokers(Right ear)	18	26.18	0.42
p1 latency of smokers(Left ear)	18	15.79	0.49
n1 latency of smokers(Left ear)	18	26.10	0.42

From the table 4.1 it can be seen that the mean absolute latency of p1 and n1 peak of smokers for both right and left ears were more when compared with non-smokers. The similar result is also clearly depicted in the figure 4.1 and 4.2

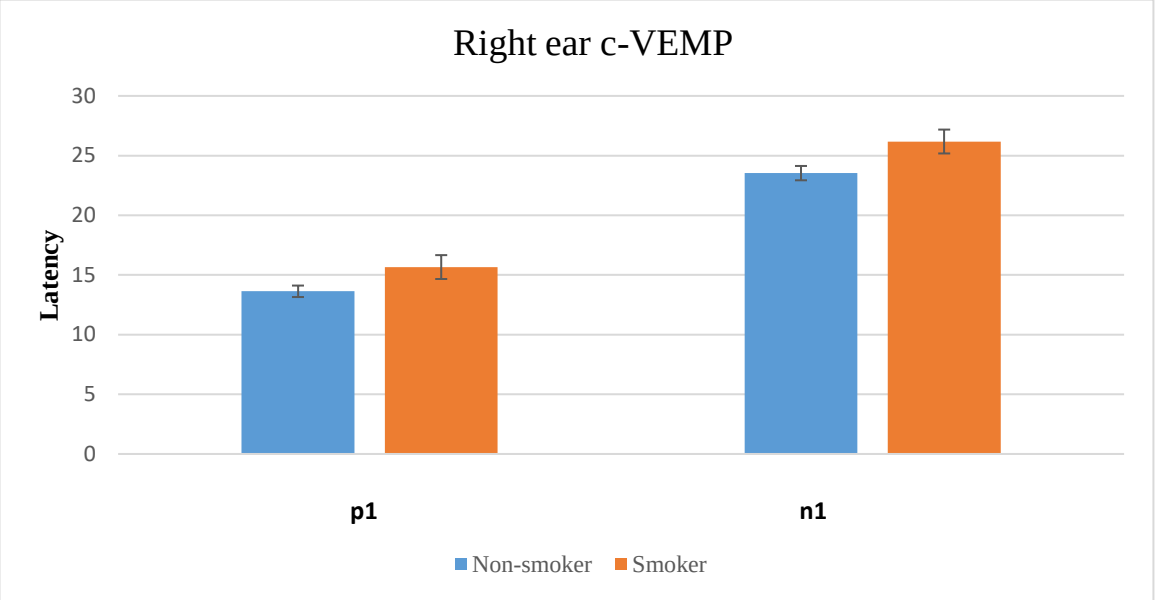

Figure 4.1: Mean and standard deviation of right ear c-VEMP p1 and n1 absolute latencies in smokers and non-smokers.

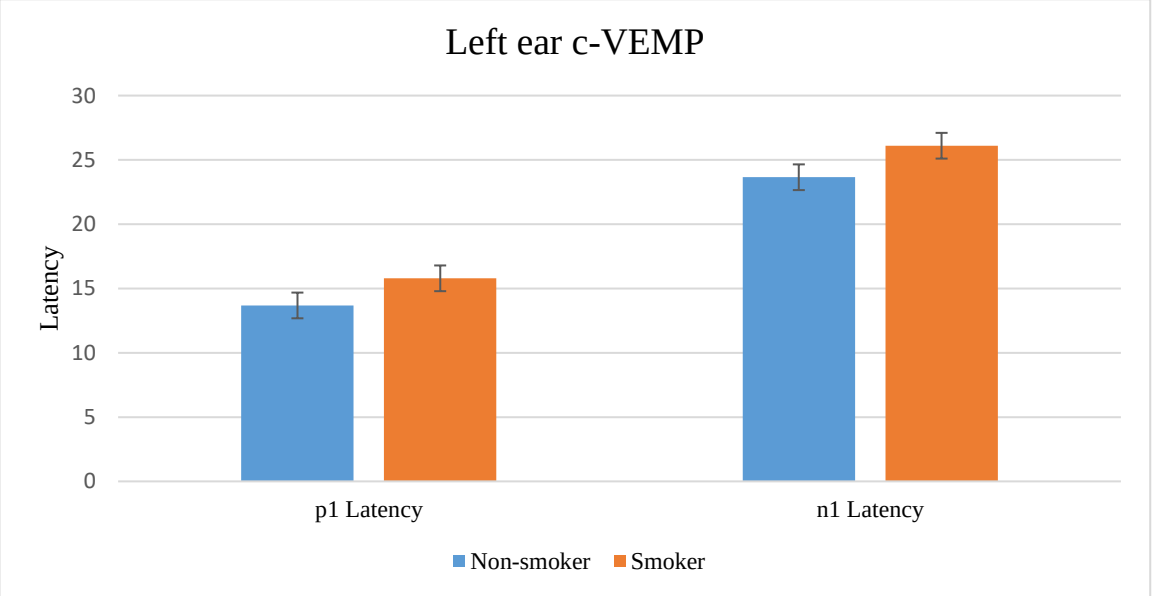

Figure 4.2: Mean and standard deviation of left ear p1 and n1 absolute latencies of c-VEMP in smokers and non-smokers.

4.1.2 Peak to Peak Amplitude of c-VEMP

Descriptive statistics was done to find Mean and standard deviation of cervical vestibular evoked myogenic potentials (c-VEMP) peak to peak amplitude in smokers and non-smokers group in each ear individually. The mean and SD of peak to peak amplitude of cervical vestibular evoked myogenic potentials obtained for both right ear and left ear are represented in table 4.2

Table 4.2

Mean and standard deviation of c-VEMP peak to peak amplitude in smokers and non-smokers

Peak to peak amplitude of c-VEMP in smokers and non-smokers	Total number of participants(n)	Mean value of amplitude (microvolt)	SD value of amplitude (microvolt)
c-VEMP amplitude in non-smokers (Right ear)	22	51.30	14.14
c-VEMP amplitude in non-smokers (Left ear)	22	52.88	14.17
c-VEMP amplitude in smokers (Right ear)	18	44.19	14.32
c-VEMP amplitude in smokers (Left ear)	18	44.11	13.21

From the table 4.2 it can be seen that the mean c-VEMP peak to peak amplitude in smokers for both right and left ears were reduced when compared with non- smokers. The similar trend is also clearly depicted in the figure 4.3

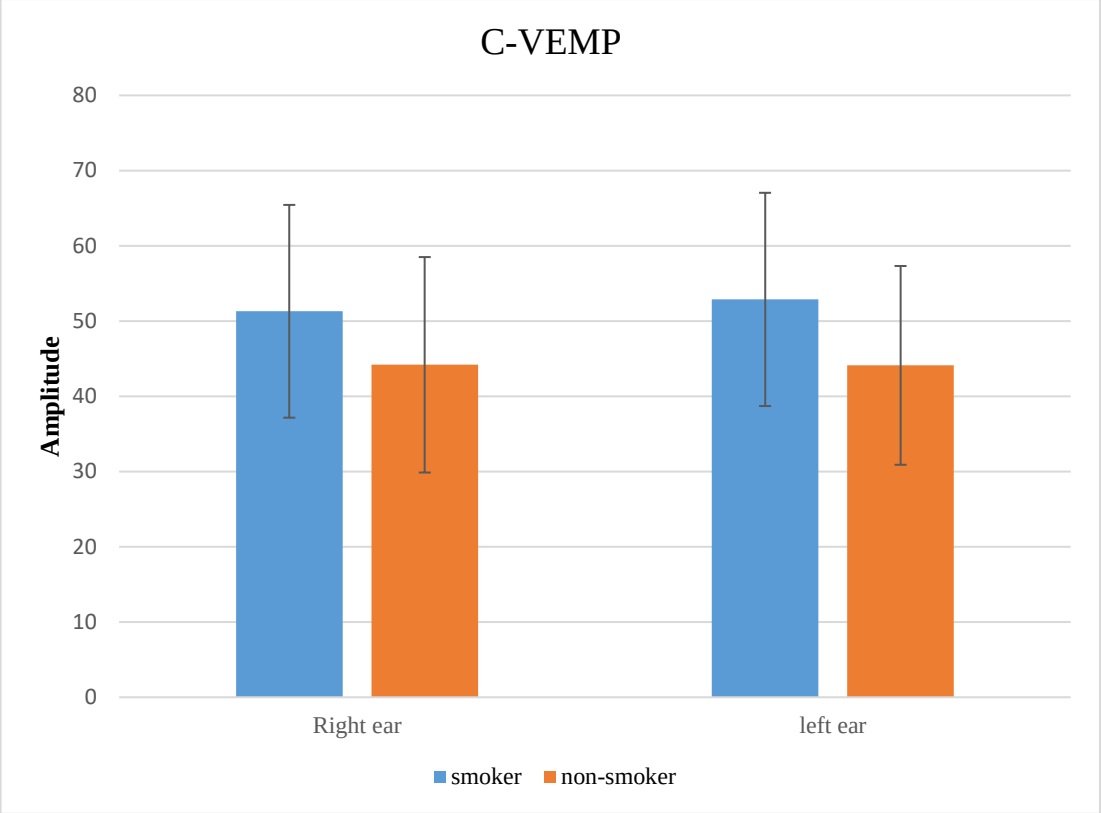

Figure 4.3: Mean and standard deviation of c-VEMP peak to peak amplitude in smokers and non-smokers.

4.2 Ocular vestibular evoked myogenic potentials in smokers and non- smokers

4.2.1 Absolute Latency of o-VEMP

Descriptive statistics was done to find the mean absolute latency (msec) and the SD for n1 and p1 latency of ocular vestibular evoked myogenic potentials for smokers and non-smokers group in each ear individually. The mean absolute latency and SD for n1 and p1 latency of ocular vestibular evoked myogenic potentials obtained for both right ear and left ear are represented in table 4.3

Table 4.3*:*

Mean and standard deviation of o-VEMP absolute latencies in smokers and non-smokers

n1 and p1 latency of o-VEMP in smokers and non-smokers	Total number of participants (n)	Mean value of latencies (msec)	SD value of latencies (msec)
n1 latency of non-smokers (Right ear)	22	9.94	0.30
p1 latency of non-smokers(Right ear)	22	13.61	0.44
n1 latency of non-smokers (Left ear)	22	9.98	0.35
p1 latency of non-smokers (Left ear)	22	13.68	0.49
n1 latency of smokers (Right ear)	18	11.78	0.44
p1 latency of smokers (Right ear)	18	16.03	0.43
n1 latency of smokers (Left ear)	18	11.66	0.39
p1 latency of smokers (Left ear)	18	15.94	0.32

From the table 4.3 it can be seen that the mean absolute latency of n1 and p1 peaks of o-VEMP of smokers for both right and left ears were prolonged when compared with non-smokers. The trend is also clearly depicted in the figure 4.4 and 4.5

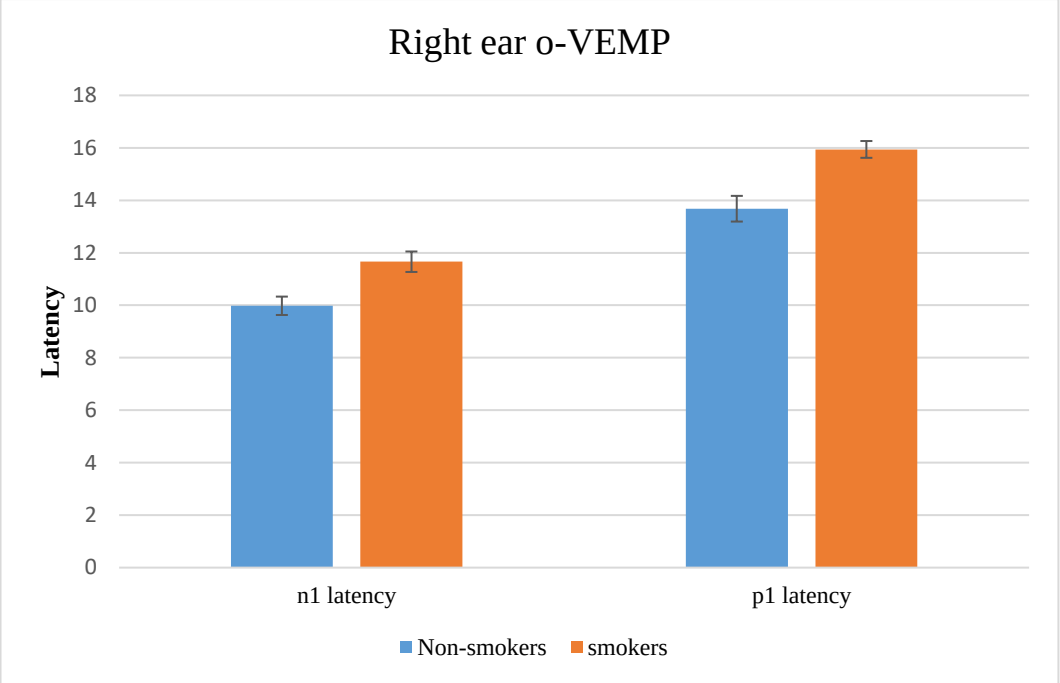

Figure 4.4: Mean and standard deviation of right ear o-VEMP absolute latencies in smokers and non-smokers.

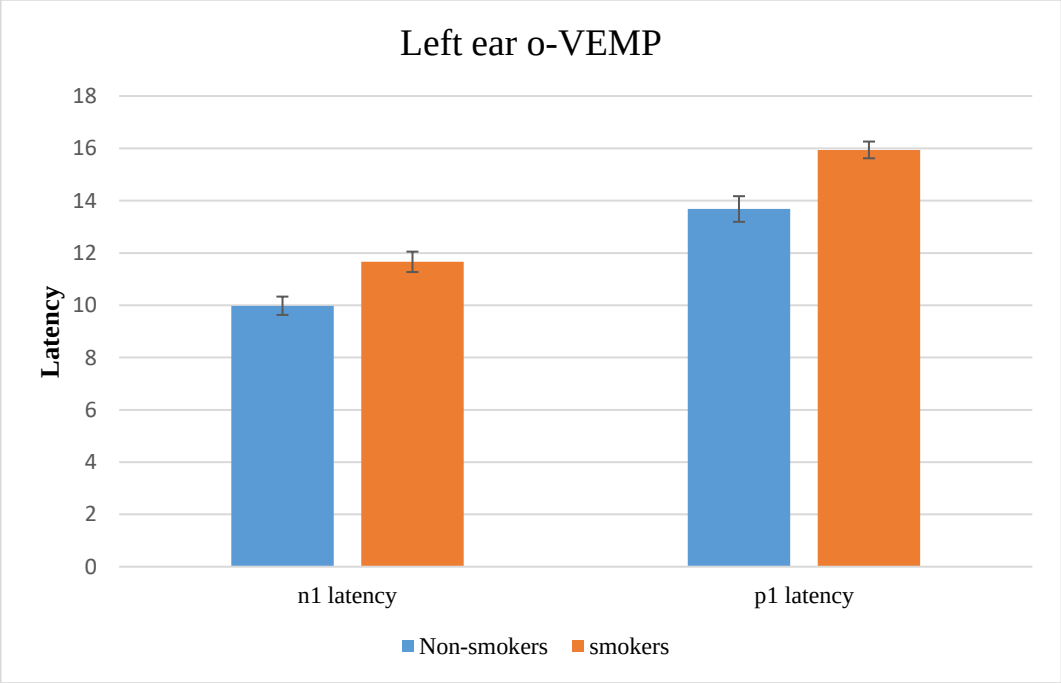

Figure 4.5: Mean and standard deviation of left ear o-VEMP absolute latencies in smokers and non-smokers.

4.2.2 Peak to Peak Amplitude of o-VEMP

Descriptive statistics was done to find the Mean and standard deviation of o-VEMP peak to peak amplitude of n1-p1 complex in smokers and non-smokers in each ear individually. The mean and standard deviation of peak to peak amplitude of ocular vestibular evoked myogenic potentials calculated for both right ear and left ear are represented in table 4.4

Table 4.4

Mean and standard deviation of o-VEMP peak to peak amplitude in smokers and non-smokers.

Peak to peak amplitude of o-VEMP in smokers and non-smokers	Total number of participants(n)	Mean value of amplitude(microvolt)	SD value of amplitude (microvolt)
o-VEMP amplitude in non-smokers (Right ear)	22	5.28	1.66
o-VEMP amplitude in non-smokers (Left ear)	22	5.55	1.57
o-VEMP amplitude in smokers (Right ear)	18	4.96	1.64
o-VEMP amplitude in smokers (Left ear)	18	4.94	1.28

From the table 4.4 it can be seen that the mean peak to peak amplitude of n1-p1 complex of ocular vestibular evoked myogenic potentials for both right and left ears were reduced in smokers when compared non- smokers. The similar trend is also clearly depicted in the figure 4.6

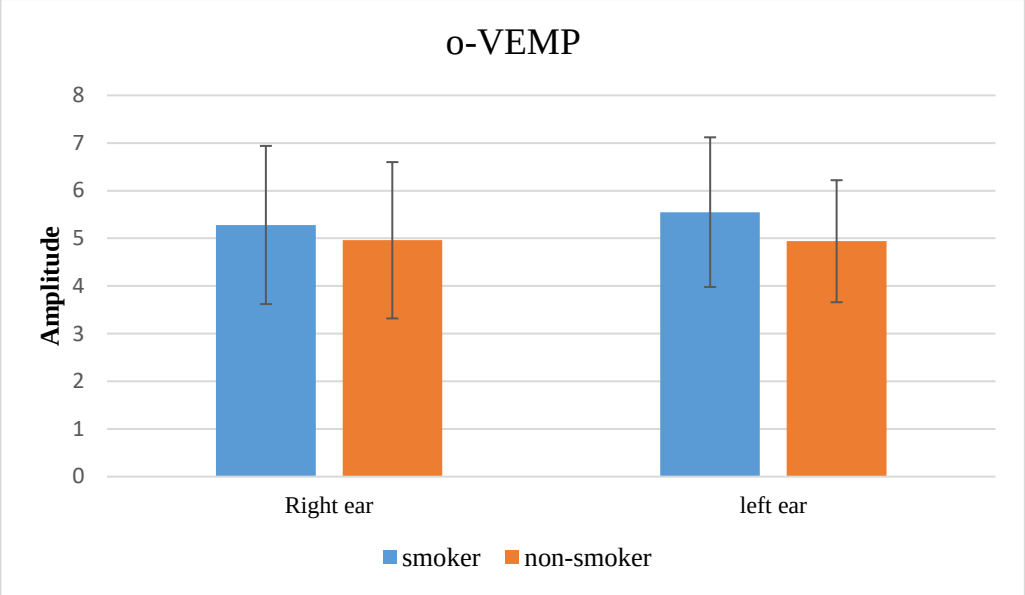

Figure 4.6: Mean and standard deviation of o-VEMP peak to peak amplitude in smokers and non-smokers.

4.3 Comparison of absolute latencies for both cervical and ocular vestibular evoked myogenic potentials between smokers and non- smokers

4.3.1 Comparison of absolute latencies of cervical vestibular evoked myogenic potentials between smokers and non-smokers

Descriptive statistics revealed that the mean value of absolute latency p1 & n1 peaks of cervical vestibular evoked myogenic potentials in smokers were more when compared to non-smokers. So in order to investigate if there exists significant difference between the smokers and non-smokers for the mean absolute latency of p1 and n1 peaks of cervical vestibular evoked myogenic potentials, MANOVA was performed. The MANOVA results showed that there was a statistically significant difference in mean absolute latency between the smokers and non-smokers, $F(4, 35) = 159.99$, $p < .0005$; Wilk's $\Lambda = 0.52$, partial $\eta 2 = 0.95$. The One way MANOVA test results obtained for comparison of n1 and p1 absolute latencies of cervical

vestibular evoked myogenic potentials between smokers and non-smokers are shown in table 4.5

Table 4.5

One way MANOVA test results for comparison of p1 and n1 absolute latencies between smokers and non-smokers.

Dependent variable	Degree of freedom (df)	F-value	p-value	error	Partial Eta Squared(η2)
Right _p1	1	139.17	0.00*	38	0.78
Right_n1	1	347.20	0.00*	38	0.90
Left_p1	1	210.50	0.00*	38	0.84
Left_n1	1	469.88	0.00*	38	0.92

* Level of significance ($p < 0.01$)

From the table 4.5 it can be seen that the p1 and n1 absolute latency value of right and left ear was significantly prolonged ($p < 0.05$) in smokers when compared non-smokers. There was a significant difference in absolute latency between the groups of right ear positive p1 peak absolute latency [$F (1, 38) = 139.17$, $p < 0.05$; partial $\eta2 = 0.79$], right ear negative n1 peak absolute latency [$F (1, 38) = 347.20$, $p < 0.05$; partial $\eta2 = 0.90$], left positive p1 peak absolute latency [$F (1, 38) = 210.50$, $p < 0.05$; partial $\eta2 = 0.85$] and left negative n1 peak absolute latency [$F (1, 38) = 469.88$, $p < 0.05$; partial $\eta2 = 0.92$].

4.3.2 Comparison of absolute latencies of ocular vestibular evoked myogenic potentials between smokers and non-smokers

Descriptive statistics revealed that the mean value of absolute latency of n1 & p1 peaks of ocular vestibular evoked myogenic potentials in smokers were more when compared non-smokers. So in order to investigate the significant difference

between the smokers and non-smokers for the mean absolute latency of n1 and p1 peaks of cervical vestibular evoked myogenic potentials MANOVA was performed. The results of MANOVA showed that there was a statistically significant difference in mean absolute latency between the groups, $F(4, 35) = 86.88$, $p < .0005$; Wilk's $\Lambda = 0.091$, partial $\eta 2 = 0.91$. The One way MANOVA test results for comparison of n1 and p1 absolute latencies of ocular vestibular evoked myogenic potentials between smokers and non-smokers are shown in table 4.6

Table 4.6

One way MANOVA test results for comparison of n1 and p1 absolute latencies of ocular vestibular evoked myogenic potentials between smokers and non-smokers

Dependent variable	Degree of freedom(df)	F-value	p-value	error	Partial Eta Squared(η2)
n1 latency(Right ear)	1	238.41	0.00*	38	0.86
p1 latency(Right ear)	1	303.22	0.00*	38	0.89
n1 latency(left ear)	1	199.90	0.00*	38	0.84
p1latency(left ear)	1	274.22	0.00*	38	0.88

* Significant level ($p < 0.05$)

From the table 4.6 it can be seen that the n1 and p1 absolute latency value of right and left ear was significantly prolonged ($p < 0.05$) in smokers when compared non-smokers. There was a significant difference in absolute latency between the groups of right ear negative n1 peak absolute latency [$F(1, 38) = 238.41$, $p < 0.05$; partial $\eta 2 = 0.86$], right ear positive p1 peak absolute latency [$F(1, 38) = 303.22$, $p < 0.05$; partial $\eta 2 = 0.88$], left negative n1 peak absolute latency [$F(1, 38) = 199.90$, $p < 0.05$; partial $\eta 2 = 0.84$] and left positive p1 peak absolute latency [$F(1, 38) = 274.22$, $p < 0.05$; partial $\eta 2 = 0.87$].

4.4 Comparison of peak to peak amplitude for both cervical and ocular vestibular evoked myogenic potentials between smokers and non-smokers

Descriptive statistics revealed that the mean peak to peak amplitude of p1-n1 complex of both cervical and ocular vestibular evoked myogenic potential for both right and left ears were reduced in smokers when compared to non- smokers. Further to investigate if there exists a significant difference between the smokers and non-smokers for the mean peak to peak amplitude of p1-n1 complex of both cervical and ocular vestibular evoked myogenic potentials, MANOVA was performed. MANOVA results showed that there was a statistically significant difference in the mean peak to peak amplitude of p1-n1 complex of both cervical and ocular vestibular evoked myogenic potential between the groups, $F(4, 35) = 3.06$, $p < .0005$; Wilk's $\Lambda = 0.74$, partial $\eta 2 = 0.26$. The One way MANOVA test results for comparison of VEMP peak to peak amplitude between smokers and non-smokers are shown in table 4.7.

Table 4.7

One way MANOVA test results for comparison of VEMP (both ocular and cervical) peak to peak amplitude between smokers and non-smokers

Dependent variable	Degree of freedom(df)	F-value	p-value	Error	Partial Eta Squared(η2)
Right c-VEMP amplitude	1	2.47	0.12	38	0.06
Left c-VEMP amplitude	1	4.02	0.05	38	0.90
Right o-VEMP amplitude	1	0.34	0.56	38	0.01
Left o-VEMP amplitude	1	1.75	0.19	38	0.04

From the table 4.7 it can be seen that there is no significant difference in the right ear p1- n1 peak to peak amplitude between the smokers and non-smokers of complex peak to peak amplitude of cervical [F (1, 38) = 2.470, $p < 0.05$; partial $\eta 2 = 0.061$], left ear p1- n1 peak to peak complex amplitude [F (1, 38) = 4.02, $p < 0.05$; partial $\eta 2 = 0.09$], right ear n1-p1 peak to peak complex amplitude of ocular vestibular evoked myogenic potential [F (1, 38) = 0.34, $p < 0.05$; partial $\eta 2 = 0.01$] and left ear n1-p1 peak to peak complex amplitude of ocular vestibular evoked myogenic potential [F (1, 38) = 1.75, $p < 0.05$; partial $\eta 2 = 0.04$].

In summary, test results of the present study showed that the absolute latency of the cervical and ocular vestibular evoked myogenic potential was significantly more in the smokers than non-smokers. However, the peak to peak amplitude value of both ocular and cervical vestibular evoked myogenic potential of the smokers was significantly less than the non-smokers.

Chapter 5

Discussion

The present study was conducted with the aim of assessing the effect of smoking on the ocular and cervical vestibular myogenic potentials. The objectives of the study were, to compare the amplitude and latency difference in ocular vestibular evoked myogenic potentials (o-VEMP) and cervical vestibular evoked myogenic potentials (c-VEMP) between the smokers and the non-smokers. The results of the present study show that the smokers had prolonged absolute latencies and reduced amplitude of both cervical & ocular vestibular evoked myogenic potentials.

5.1 Comparison of absolute latencies for both cervical and ocular vestibular evoked myogenic potentials between smokers and non- smokers

The mean values of both c-VEMP and o-VEMP p1 and n1 absolute latencies of smokers was prolonged as compared to the non-smokers i.e., smokers had prolonged c-VEMP and o-VEMP latencies. The p1, n1 latency of the cervical and ocular vestibular evoked myogenic potentials was significantly more in the smoker's group as compared to non-smokers.

The results of the present study show that the smoking is found to have hazardous effects on the vestibular peripheral receptor cells. Cervical vestibular evoked myogenic potential is a test for assessing the functioning of saccule and the vestibulo-spinal reflex pathway (Colebatch & Halmagyi,1992). The damage to the hair cells of the saccule and its neuronal pathway was evidenced by the increase in the latencyof thep1, n1 peaks of cervical vestibular evoked myogenic potentials in smokers compared non-smokers in the current study.

Ocular vestibular evoked myogenic potential is a test for assessing the functioning of the utricle and the vestibulo-ocular reflex pathway (Todd, Rosengren, & Colebatch2003). The damage to the hair cells of the utricle was evidenced by the increase in the absolute latencyof the n1, p1 peaks of ocular vestibular evoked myogenic potentials in smokers when compared with the control group.

These findings obtained from the current study agrees with the findings of Mustafa et al. (2016) study, where he had compared the amplitude of transient evoked otoacoustic emissions (TEOAEs) and latencies of vestibular evoked myogenic potentials (VEMPs) among non-smokers, cigarette smokers, water pipe smokers, mixed smokers and ex-smokers aged 20 to 40. Damage to the outer hair cells was evidenced by the reduced TEOAE amplitude in smokers and ex-smokers compared with the control group. Their study also showed that smoking had deleterious effects on the hair cells in the labyrinth. Harmful to the saccular hair cells is detected by the increased latency of the c-VEMPs which was significantly prolonged in smokers than non-smokers. He concluded that this prolongation in the latency is due to the damaging effects of the nicotine on the vascular dynamics of the venules and consequently on the blood capillaries of the inner ear. Smoking is found to cause endothelial dysfunction and vasospasm which eventually leads to impaired vasodilation. This impaired vasodilation is found to cause hearing impairment and peripheral vascular disorder (Wada et al, 2017).

The latency findings of c-VEMP and 0-VEMP in the present study can be attributed to the delayed release of the neurotransmitters from vestibular hair cells in smokers where, the free radicals in tobacco is found to damage the hair cells and lead to the delayed release of neurotransmitter which in turn lead to the prolonged latency (Cruickshanks et al. 1998). The prolonged latency obtained for smokers in the

present study can also be attributed to demyelination of the vestibular nerves in smokers because of toxic substance present ion cigarette. This vestibular nerve demyelination could cause delay in carrying/travelling the action potential which is generated in hair cells, which leads to prolonged recording of action potentials (Cruickshanks et al. 1998). Moreover, the improper activation of the nicotine receptors due to smoking at various nuclei of the vestibular system can also be reflected as prolongation of absolute latency of vestibular evoked myogenic potentials in smokers. There probably could be few or limited literature which have examined the effect of smoking on vestibular structures.

5.2 Comparison of peak to peak amplitude for both cervical and ocular vestibular evoked myogenic potentials between smokers and non- smokers

The mean value of cervical VEMP peak to peak amplitude of p1-n1 and ocular peak to peak amplitude of n1-p1 complex was reduced in smokers compared to non-smokers. The peak to peak amplitude of the p1-n1 complex of cervical and n1-p1complex of ocular vestibular evoked myogenic potentials was significantly low in smokers as compared to non-smokers.

The amplitude of the vestibular evoked myogenic potential is found to be depended upon the total number of functioning of the vestibular hair cells (Akin, Murnane, Tampas, & Clinard 2011). Thus reduced amplitude of vestibular evoked myogenic potential indicates the loss of hair cells of the peripheral vestibular apparatus (saccule & utricle).

The cochlea and the vestibular organs are found to share the same membranous labyrinth of the inner ear and hence the abnormality or the dysfunction of one part may lead to dysfunction of the other part too. This is due to similarities

seen in the vestibular hair cells and the cochlear hair cells and the blood supply to both the systems (Starr et al., 2003). Previous studies are also in the agreement that smoking causes dys-functioning of outer hair cells. Hair cell dysfunction /loss in these studies shown by reduction in the amplitude of distortion product otoacoustic emission (Rogha et al., 2015; Munjal et al. 2017,Gopal et al., 2009) or transient evoked otoacoustic emissions (Vinay et al 2010, Mustafa et.al , 2014).These authors attributed this reduction in the reduction of DP amplitude in the smokers group was due to the high amounts of carbon monoxide and nicotine which restricts blood circulation to the cochlea in turn damaging the outer hair cells. Similarly, the reduced amplitude of the vestibular evoked myogenic potential (both c-VEMP & o-VEMP) in the present study could be caused by the same mechanism.

The amplitude findings of c-VEMP and o-VEMP in the present study can be attributed to the damage of the vestibular hair cells in smokers due to the hypoxia because of the occlusion of blood vessels which supply to the inner ear (Zelman, 1973; Cunningham et al., 1983; Cruickshanks et al., 1998; Nakanishi et al., 2000). Damage of the hair cells can also occur due to nicotine content in cigarette which reduces the amount of oxygen and increase the carbon mono oxide in the inner ear (Negley et al, 2007). The reduced amplitude obtained in smokers can also be attributed hair cell loss because of toxicity caused by few of the ingredients present in the cigarette (Cruickshanks et al. 1998).

In summary, smoking has hazardous effects on the vestibular system as shown by the increase in the absolute latency and reduction in the peak to peak amplitude of the cervical and ocular vestibular evoked myogenic potentials. This can be attributed to the vestibular hair cells and vestibular nerve dysfunction caused by the smoking and adverse effect of nicotine.

Chapter 6

Summary and Conclusions

Smoking is well known risk factor of inner ear dysfunction and it ranges from loss of hearing and balance sensitivity to the hyperactive disorders like tinnitus. Studies in literature have shown that smoking leads to outer hair cell dysfunction of inner ear. Since there are very few studies conducted on the effect of smoking on the vestibular system and all these studies are done in western population. Hence the present study was taken to study the effect of smoking on peripheral vestibular system with respect to Indian context. The objectives of the study were:

- To compare the peak to peak amplitude of both ocular and cervical vestibular evoked myogenic potentials between the smokers and non-smokers.
- To compare the absolute latency in both ocular and cervical vestibular evoked myogenic potential between the smokers and non-smokers.

The study was conducted on 18 smokers and 22 non-smokers of age ranged from 24-40 years. Both the groups were having normal hearing sensitivity with no vestibular complaints. All the participants in both the groups underwent a detailed case history, pure tone audiometry, Immittance audiometry and vestibular evoked myogenic potential testing. Both ocular and cervical vestibular evoked myogenic potentials were recorded using 500 Hz tone burst stimuli presented at 125 dB SPL through ER-3A insert ear phones. For cervical vestibular evoked myogenic potentials, the inverting (negative / reference) electrode was placed at the sterno-clavicular junction, the non-inverting (positive / active) electrode at the upper one-third of the sternocleidomastoid muscle and the ground (common) electrode on the forehead. For ocular vestibular evoked myogenic potentials, the positive electrode

was placed 1cm below the eyes, negative electrode was placed 1cm below the positive electrode and ground electrode was placed on the forehead.

The data was subjected to Shapiro-Wilk test to check for normality and the test results showed that the data is following normal distribution ($p > 0.05$) and hence parametric statistical tests were performed see the significant difference in the mean absolute latency and mean peak to peak amplitude of c-VEMP and o-VEMP between the smokers and non-smokers group.

Descriptive statistics was done to find the mean & standard deviation value of absolute latency and peak to peak amplitude of c-VEMP and o-VEMP in the smokers and non-smokers. Results of descriptive statistics shows that the mean absolute latency and standard deviation (SD) of p1 and n1 peak of cervical vestibular evoked myogenic potentials in smokers were prolonged when compared with non-smokers. Similarly, the mean absolute latency and SD of n1 and p1 peak of ocular vestibular evoked myogenic potentials in smokers were prolonged when compared with non-smokers. The mean peak to peak amplitude and SD of p1-n1 complex of cervical vestibular evoked myogenic potentials in smokers was reduced when compared with non-smokers. Also, the mean peak to peak amplitude and SD of n1-p1 complex of ocular vestibular evoked myogenic potentials in smokers was reduced when compared with non-smokers.

Since, the data was following normal distribution ($p > 0.05$) multivariate analysis of variance (MANOVA) was administered to check for the significant difference in the mean peak to peak amplitude and absolute latency values of both c-VEMP and o-VEMP between smoker and non- smoker groups among the various dependent variables (right p1, right n1, left p1and left n1 for both c & o-VEMP).

Results obtained from parametric tests reveal that:

- There is a significant difference found in mean absolute latency of p1 and n1 peaks of both cervical and ocular vestibular evoked myogenic potentials between smoker and non-smoker.
- There is a significant difference found in mean absolute latency of n1 and p1 peaks of both cervical and ocular vestibular evoked myogenic potentials between smoker and non-smoker.
- The mean peak to peak amplitude of p1-n1 complex of cervical vestibular evoked myogenic potentials in smokers was significantly reduced when compared with non-smokers
- The mean peak to peak amplitude of n1-p1 complex of ocular vestibular evoked myogenic potentials in smokers was significantly reduced when compared with non-smokers.

Thus it can be concluded that, smoking has hazardous effects on the vestibular system as shown by the increase in the absolute latency and reduction in the peak to peak amplitude of the cervical and ocular vestibular evoked myogenic potentials. This can be attributed to the endothelial dysfunction caused by the smoking and adverse effect of nicotine receptors on various synaptic levels in the central vestibular system. Moreover, changes in blood viscosity and toxic substances in blood due to smoking can also lead to loss of hair cells.

6.2 Implications of the study

- The findings of the study can be used in creating awareness and counselling the general population regarding the hazardous effect of smoking on vestibular system.

- The results of the study suggest that smokers require a detailed vestibular assessment to show its impact on the vestibular system.
- The results of the study will add on information to the existing literature with regard to influence of smoking on vestibular system.

6.3 Future directions

- To investigate the correlation between different duration of smoking and its effect on vestibular system.
- To include tests which can assess the functioning of semicircular canals and central vestibular system to assess its involvement in smokers.
- Longitudinal studies can be done to investigate the relationship between smokers and nonsmokers with more number of individuals for more clear results.
- Study can be conducted on females to see the effect of smoking on gender on c-VEMP and o-VEMP.

6.4 Limitations of the study

- Number of individuals included in the study was less.
- The study includes only male population and hence effect of smoking on gender on c-VEMP and o-VEMP could not be studied.
- The effect of different duration of smoking on c-VEMP and o-VEMP was not compared.

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