

ADVERSE EFFECTS OF LOW FREQUENCY NOISE ON ALVEOLAR BONE OF MICE, AN UNSEEN DILEMMA

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ABSTRACT

Objective: To observe the destructive effects of low frequency noise on periodontium with special emphasis on the alveolar bone.

Study Design: Laboratory based randomized control study.

Place and Duration of Study: The study was conducted at Anatomy department of Army Medical College, Rawalpindi, in collaboration with animal house of National Institute of Health, Islamabad. The study continued for one year, from Sep 2012 to Aug 2013.

Material and Methods: Thirty adult BALB mice were divided into three equal groups. The animals from group A (Control) were exposed to routine outside environment of animal house with normal daily activity going on. The animals from experimental group B were kept in an environment having complete silence with no exposure to external environment. The animals from group C (Experimental) were exposed to low frequency noise of 200 Hz continuously for a period of three months. This sound was generated by an analogue frequency generator having uninterrupted power supply (UPS). At the end of the study, animals were sacrificed and histomorphological changes were observed on the alveolar bone. The data was collected at the end of study and analyzed using SPSS version 21. The results with p -value <0.05 were considered to be statistically significant.

Results: There was significant distortion of normal architecture of the alveolar bone. A few detached bony fragments were also found scattered among fibers of periodontal ligament in specimen from group A and C. No significant change was found in specimen from experiments group B who were kept in silent environment.

Conclusion: Exposure to low frequency noise has a significant destructive effect on alveolar bone of experimental animals.

Keywords: Low frequency noise, Periodontal ligament, Periodontium.

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INTRODUCTION

Any unwanted, undesirable or unpleasant sound is termed as noise¹. The frequency range that is audible for a normal human ear, without damaging it, lies between 20 to 20000 Hz. With growing urbanization and industrialization, the level of noise in our environment is increasing on a day-to-day basis. With introduction of modern machinery, noise levels have shifted from high to rather moderate levels. These levels are dominated by the presence of low frequency noise which is typically characterized to lie within a frequency range of 20 to 200 Hz. It is a

silently growing form of pollution that though unnoticed, can cause deleterious effects on the human body and therefore, is a pressing concern that needs to be addressed². Though low frequency sounds are within an audible hearing range, they produce vibration that is responsible for aggravating annoyance and other harmful effects caused by it. Of these effects, annoyance, fatigue, headache and irritation are the most commonly reported ones that greatly decrease a person's working capacity³. Environmental noise can also result in noise-induced hearing impairment that affects both genders equally, tinnitus, reduction in REM sleep which causes sleep disturbance, cardiovascular changes like increase in blood pressure and mental health disturbance. It has been proven that one of the most adverse health effects of low frequency

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noise is on the alveolar bone. Alveolar process is the specialized bone structure that contains the alveolus or sockets of tooth. It provides support to the teeth. The loss of this bone has significant effects on the survival of teeth, as observed in millions of patients with periodontal diseases. Mainly, it is composed of two parts, the alveolar bone proper or lamina dura and supporting bone. The main functions of the bone include protection, support, shock absorption and attachment to the Sharpey's fibers. Alveolar bone consists of three layers; from outside in; cortical plate, spongiosum and the bone that lines the alveolus. To the innermost layer, is attached the periodontal ligament. Factors like stress, smoking, drugs and alcohol adversely affect the alveolar bone and so does low frequency noise because it results in extracellular matrix proliferation that ultimately leads to fibrosis, albeit without any evidence of inflammation. Due to exposure to low frequency noise, the cementum in the alveolar bone disappears and the surface of the bone gets irregularly eroded with some evidence of bone necrosis. Moreover, the periodontal ligament is also affected, resulting in decreased fiber anchorage. These changes are due to the combined effect of the low frequency noise and vibration or may be attributable indirectly to low frequency noise as a result of the stress or vascular changes it brings about, or a combination of all these⁴. Moreover genetic factors like WNT are also found to have effects on remodeling of alveolar bone and periodontal ligament⁵. We are living in an environment rich in different chemical as well as physical harmful stimuli to health. So much work has been done to study different chemicals causing untoward effect on human health but very little emphasis is given to different physical stimulants causing health hazards. One of the most important physical factors is the noise to which normal human being is exposed most of time of his life. The objective of this study was to see the effects of the low frequency noise on the histomorphology of the periodontium in mice.

MATERIAL AND METHODS

The study was laboratory based randomized control trial and of one year duration. It was carried out in the department of Anatomy, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. The experiment was carried out with the permission of ethical committee of center for Research in Experimental and Applied Medicine (CREAM), of the Army Medical College, Rawalpindi from September 2012 to August 2013.

Thirty adult BALB/c mice, weighing 26 ± 0.23 grams were used in the experiment and were kept in standard environment of Animal house of National Institute of Health, Islamabad. Mice were divided into three equal groups by using simple random technique ($n=10$ animals). Control group A (kept in normal environment of animal house), Experimental group B (kept in complete silence with no external sound) and Experimental group C (exposed to low frequency noise of 200 Hz continuously for three months). Low frequency noise (LFN) was produced by an analogue frequency generator. Uninterrupted power supply (UPS) was ensured with the help of DC adaptor with standby battery. The frequency and intensity of sound were confirmed with the help of different devices (Thurlby Thunder, TF 830, 1.3 GHz digital counter, Hitachi VC 6155 oscilloscope and RadioShack analogue 33-4050 sound level meter). Frequency and intensity of sound were recorded at the start, the middle and the end of the experiment. Animals having diseased teeth were excluded from the study.

At the end of 90 days, the animals were euthanized by placing ether soaked cotton in the jar 6. Hemisected mandibles of mice with intact teeth were fixed in 10 percent formalin. $5\mu\text{m}$ thick sagittal slices were made⁷ and stained with H&E. High power objective was used to see alveolar bone loss in animal specimens. It was assessed by irregular junction between periodontal ligament and bone margin and recorded as present or absent. The presence of broken pieces of necrosed alveolar bone scattered among fibers of

periodontal ligament was recorded as present or absent. The osteonecrosis was indicated by changes in architecture of the bone and blood supply leading to microfractures⁸.

The data was entered for analysis in the computer software SPSS version 21. Descriptive statistics were used to calculate means. Chi-square test was applied for comparison of morphological features between the groups and a p -value <0.05 was considered significant.

RESULTS

All the thirty animals remained healthy and active throughout the experimental period. The histological sections showed all the components of periodontium including gingival epithelium, cementum, periodontal ligament and alveolar

DISCUSSION

All the human beings are facing continuous threat from physical hazards including noise of different intensity. Very little work has been done on these hazards during the past few years. Most of the work belongs to the aeronauts and astronauts exposed to high frequency noise generated by the spaceships and huge planes although much of this hazard especially low frequency noise is an important component of our daily life. The magnitude and severity of this threat is on continuous rise due to multiple factors like change in life style, population outburst and urbanization. The results of the experimental groups B & C were compared with those of control group A, as well as with each

Table-I: Comparison of alveolar bone erosion among group A, B & C

Parameter	Group A (n=10)		Group B (n=10)		Group C (n=10)		p -value
	Finding	Frequency	Finding	Frequency	Finding	Frequency	
Alveolar bone erosion	Present	8 (80%)	Present	1 (10%)	Present	9 (90%)	0.000
	Absent	2 (20%)	Absent	9 (90%)	Absent	1 (10%)	

Table-II: Comparison of Bony particles in periodontal ligament among group A, B & C

Parameter	Group A (n=10)		Group B (n=10)		Group C (n=10)		p -value
	Finding	Frequency	Finding	Frequency	Finding	Frquency	
Bony fragments among PDL fibers	Present	3 (30%)	Present	1 (10%)	Present	9 (90%)	0.001
	Absent	7 (70%)	Absent	9 (90%)	Absent	1 (10%)	

bone under H&E stain.

Group A (Control)

Alveolar bundle bone showed evidence of erosions at multiple sites in 8 out of ten specimens with detached bony pieces scattered among the fibers of PDL (table-I & II, fig-1).

Group B (Experimental)

Alveolar bundle bone showed regular attachment of PDL fibers with smooth margins in contrast to findings in group A. No significant bone loss was found (table-I & II, fig-2).

Group C (Experimental)

Alveolar bone erosion was seen in 90% of specimens with pieces of detached bone from the alveolar bone seen among the fibers of PDL in 90% of specimens (table-I & II, fig-3). A p -value <0.05 was significant.

other. In present study, all animals remained healthy and active throughout the experimental period. This was same as previous studies conducted on LFN. The integrity of the bone can be assessed by different means like histopathology, conventional radiography and CT scan^{9,10}. Different measurements are taken from digitized radiographs by using softwares like adobe Photoshop or Image tool software etc¹¹. Alveolar bone loss, both horizontal and vertical, has been observed in different studies but horizontal loss is more common than vertical loss¹². In 2005, Serakides *et al*¹³ observed slight loss of trabecular bone in castrated female rats. The risk factors for alveolar bone loss include caries, food impaction, pulp pathosis, calculus formation and periodontal status. This loss can be seen in primary teeth also¹⁴, around dental implants and due to consumption of alcohol¹⁵

although bone loss is considered to be having an underlying pathology, it can occur in healthy population like obesity and hyperlipidemia^{16,17}. A significant evidence of alveolar bone loss has been found in age related macular degeneration in males¹⁸. In the present study, alveolar bone loss was assessed histologically in animal specimens. Most of the bone loss was found in specimen from experimental group C which was exposed to low frequency noise. The results were statistically high significant when group A was compared with group B (p -value 0.000), group B was compared with group C (p -value 0.000) (table-I). The common microscopic findings included irregular or jagged alveolar bone margins with Sharpey's fibers inserting irregularly into the woven bone. Fractured pieces of alveolar bone were found in 70% of specimen of LFN exposed group C. About 30% of specimen of experimental group A also had bony pieces. The results were highly significant (p -value 0.001) when the three groups were compared with each other (table-II). In 2015, Arabacı *et al*¹⁹ observed increased bone loss in response to Melatonin in experimentally induced periodontitis in experimental rats. Similarly in 2012, Aguirre and Akhter²⁰ observed significant bone loss in experimental animals fed on high sucrose diet. During same year, Verzeletti²¹ proposed a weak negative effect of body weight on progression of ABL. On the other hand, role of atorvastatin was found to be beneficial for reduction of ABL¹². Alikhani in 2012²² proposed that mechanical stimulation contributes to the health of alveolar bone when he exposed the teeth of experimental rats to high frequency acceleration. The results showed that the bone formation is increased by increasing frequency and acceleration in the absence of mechanical load. The synergistic effects of vitamin D3 and vitamin K2 on bone loss prevention have also been reported²³.

LIMITATION OF STUDY

This study has certain limitations. The number of study groups may be increased with increase in sample size. Similarly different fixed

frequencies may be used to see the differential effects.

CONCLUSION

It was concluded by this experimental study that low frequency noise has a significant role in destroying the normal alveolar bone morphology in experimental animals.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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