KERATINIZATION OF SULCULAR EPITHELIUM IN LOW FREQUENCY NOISE EXPOSED MICE

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OBJECTIVE: The study was conducted to evaluate the histomorphological effects of low frequency noise on the periodontium of mice.

METHODOLOGY: Thirty BALBc mice, both male and female, were selected and divided into three equal groups having equal number of male and female animals. Control group A was exposed to normal environment of animal house, experimental group B was kept in silent condition and experimental group C was exposed to low frequency noise of 200 Hz for three months. The results of the experimental groups were compared with the control, and with each other. Statistical analysis was done using chi-square test at a confidence level of 95 percent and p value of <0.05 was considered as statistically significant. Results: Presence of keratinization of sulcular epithelium was statistically significant when group A was compared with group B and group B was compared with group C. Although there was no statistically significant difference in mean thickness of sulcular epithelium among the three groups but it was comparatively less in group C.

CONCLUSION: It was concluded that low frequency noise significantly increases the keratinization of sulcular epithelium.

KEY WORDS: Low frequency noise, keratinization, periodontium, Sulcular epithelium


INTRODUCTION

Our environment is full of different types of pollution of which noise pollution is an important component. It is most prevalent and almost beyond the individuals control. This problem is not new. It has been present since ancient Roman and German eras and special laws were formulated at that time according to prevalent circumstances. The use of iron wheeled wagons and horse riding was prohibited to avoid effects of noise on general public. But the time has changed and magnitude and intensity of sound has increased significantly through the ages.

In the present day environment, the noise has become a major concern for urban community of most of the developing countries. It is being further increased due to increasing industrialization and development of large infrastructure and heavy duty mobile sources of transportation. In the present era of power shortage, use of generators has increased this problem even more.

On physical terms, like source, propagation and perception, there is no difference between sound and noise. Noise is defined as unwanted or undesired sound. The frequency and intensity are also different. Frequency is defined as number of vibrations in one second and denoted as hertz while intensity is the loudness of sound and expressed as decibel. The audible frequency of sound ranges from 20 to 20000 Hz. Infrasound is the frequency below 20 Hz and ultrasound is above 20000 Hz. The term low frequency noise (LFN) is attributed to frequency of > 500 Hz, although some authors claim it to be between 20 to 250 Hz. Low frequency noise is an essential part of frequencies generated in daily life by automobiles, heavy machinery, fans, generators and aircrafts.

Periodontium is a group of structures responsible for holding the tooth in the mandible or maxilla. These include cementum, periodontal ligament, alveolar bone and gingiva. The gingiva is anatomically divided into the unattached (marginal), attached and interdental gingiva. The marginal gingiva forms the coronal border of the gingiva which surrounds the tooth, but is not adherent to it. Gingiva is composed of epithelium and connective tissue. Epithelial component comprises of gingival epithelium, sulcular

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epithelium and junctional epithelium. The gingival epithelium is stratified squamous keratinized epithelium that lines the gingiva. The basal cell layers of all 3 types of gingival epithelia are composed of rapidly proliferating cells that migrate toward the outer surface of the tissue. The keratinocyte is the main cell type of the gingival epithelium. Other cells found in the epithelium include Langerhans cells, Merkel cells, and melanocytes. The keratinization process includes flattening of the cuboidal cells, production of keratohyaline granules, and disappearance of the nucleus. The gingival sulcus is lined with a non-keratinized stratified squamous epithelium that is referred to as sulcular epithelium. The gingival sulcus is bound by the junctional epithelium apically and the tooth and the sulcular epithelium on the sides. Sulcular epithelium lacks rete pegs. It is suggested that the non-keratinized nature of the sulcular epithelium is the result of the local irritation and inflammation within the gingival sulcus. Gingival connective tissue consists of collagen fibres (type I and III), fibroblasts, nerves, blood vessels, lymphatics, macrophages, eosinophils, neutrophils, T and B lymphocytes, and plasma cells. This connective tissue is called lamina propria with a superficial papillary layer and deeper reticular layer. Gingival sulcular fluid is secreted by the sulcular epithelium. It contains numerous defense cells, electrolytes and antibodies providing protective effect.

The effects of LFN on histomorphology, physiology and biochemical parameters have been studied on different organs and systems of body. Vibroacoustic disease (VAD) is defined as pathology involving whole body in response to chronic exposure to LFN. It is characterized by proliferation of extra cellular matrix without inflammation. The cause of injury can be direct vibrational effect, stress, vascular involvement or combination of these factors.

Chronic exposure to LFN affects different body systems including endocrine system, cardiovascular system, immune system, reproductive system, vestibular system, respiratory system, central nervous system, digestive system and oral cavity. Periodontium can be affected by stress, smoking, drugs, alcohol and low frequency noise.

The objective of this study was to see the effects of the low frequency noise on the histomorphology of the sulcular epithelium in mice. 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. The study was laboratory based randomized control trial and of one year duration.

METHODOLOGY

Thirty adult BALB/c mice, half male and half female, with initial body weight of 25-28 grams were used in the experiment and were kept in controlled environment of Animal house of National Institute of Health, Islamabad. They were fed with standard NIH laboratory diet for three months. The mean final body weight was 42.00±1.224gm. Mice were randomly divided into three groups (n = 10 animals, half male and half female, in each group). The mice in group A (Control) were kept in normal environment of animal house for three months. The mice in group B (Experimental) were kept in silent conditions for three months and mice of group C (Experimental) were exposed to low frequency noise of 200 Hz continuously for three months.

Exposure to Low Frequency Noise

Low frequency noise (LFN) of 200 Hz was produced by analogue frequency generator purchased from local market. Power supply was with DC adopter with stand by battery of nine volts for uninterrupted power supply. The frequency output was confirmed with the help of digital universal frequency counter (Thurlby Thunder, model No TF 830, 1.3 GHz) and oscilloscope (Hitachi VC 6155). The intensity of sound was measured with sound level meter (RadioShack analogue model 33-4050). Frequency and intensity of sound were recorded at start, middle and end of the experiment. It was placed outside the cages.

Sacrifice and Dissection

At the end of 90 days, the animals were euthanized by placing ether soaked cotton in the jar. The skin was removed by scalpel. Mandible was dislocated from the temporomandibular joint and dissected out. Tongue and surrounding muscles were removed. Care was taken to protect the buccal and lingual mucosa from any injury during the procedure (Fig 1).

Fig 1: Photograph showing dissected mandible
Tissue processing

Hemisected mandibles were fixed with 10% formalin and embedded in liquid paraffin. The blocks were allowed to solidify on cold plate. 5µm thick sagittal slices of animal tissue were made using rotary microtome (Leica rm 255). The observations on each of the parameters were recorded. The lining epithelium was assessed for any apparent keratinization. One slide per specimen was observed under high power objective and keratinization was recorded as present or absent.

Statistical analysis

The data was entered for analysis in the computer software SPSS version 18. Mean and standard deviation were calculated for quantitative data using ANOVA statistical test. Chi-square tests was applied for qualitative data to measure the level of significance for analysis at a confidence level of 95 percent and p value of <0.05 was considered as statistically significant.

RESULTS

The histological sections of periodontium showed all the components under H&E stain. In control group A, sulcular

![Graph showing comparison of keratinization of sulcular epithelium among groups A, B, and C.]

Fig 2: Comparison of keratinization of sulcular epithelium among groups A, B, and C.

![Photomicrograph of animal from group B showing non-keratinized sulcular epithelium (SE). CT: Connective tissue, MEp: Marginal epithelium. H&E 40X.]

Fig 4: Photomicrograph of animal from group B showing non-keratinized sulcular epithelium (SE). CT: Connective tissue, MEp: Marginal epithelium. H&E 40X.

![Photomicrograph of animal from group C showing keratinized sulcular epithelium (SE). H&E 40X.]

Fig 5: Photomicrograph of animal from group C showing keratinized sulcular epithelium (SE). H&E 40X.

cells were cuboidal shaped and the top most layer having flattened nuclei (Fig 3). Only two out of ten specimens (20%), one male and one female, showed keratinization of sulcular epithelium (Fig 2). In experimental group C, the sulcular epithelium was composed of stratified squamous epithelium with keratinization. Keratinization of sulcular epithelium was seen in nine out of ten specimens. Of these five were female and four were male animals (Fig 5). Same findings were observed in group A but these were not so in group B which was exposed to silent environment.

DISCUSSION

Environmental noise pollution, a form of air pollution, is a continuous threat to normal health and well-being. It is becoming
increasingly severe and widespread than ever before, and it will continue to increase in magnitude and severity because of change in life style, increase in population, urbanization, and the associated growth in the use of increasingly powerful and highly mobile sources of noise. It is likely to continue due to sustained growth in highway, rail, and air traffic, which are the important sources of environmental noise.

In the normal tissues, sulcular epithelium is non-keratinized. In the current study, sulcular epithelium was found to be keratinized in 70% of animals in group A, 20% of animals in group B and 90% of animals in group C (Fig 2). ANOVA was approved as statistical test. Keratinization was statistically significant when group A was compared with group B (p-value<0.02) and highly significant when group B was compared with group C (p value<0.001). The degree of keratinization was almost same when group A was compared with group C and it was not statistically significant (p value > 0.05) (Table 1). No significant difference was found among the two genders. These findings are in agreement with findings observed by Cafesse et al 16 in two separate studies when they observed significant keratinization of sulcular epithelium in response to intensive antimicrobial therapy. In another study by same researchers in 1982, it was proposed that mechanical stimulation of sulcular epithelium plays a role in promoting its keratinization17. In our study, this mechanical stimulation may be elicited due to vibrational effect of low frequency noise. Changes in keratinization are also manifested in response to diabetes, antimicrobial therapy and smoking 18.

In another study done by Vogel in 1981, keratinization was induced in a group of subjects by intrasulcular tooth brushing. Biopsies revealed no effect of keratinization on permeability of sulcular epithelium 19. It has been suggested that in normal conditions, keratinization of sulcular epithelium is prevented by contact of epithelium to the tooth 20.

One of the major functions of sulcular epithelium is the secretion of gingival sulcular fluid. This fluid contains many protective agents. When sulcular epithelium is damaged due to any cause, this protection is lost leading to increase in the depth of gingival sulcus forming pockets for the deposition of invading organisms.

The results of the study show the change of non-keratinized sulcular epithelium to keratinized type in those groups of animals which were exposed to low frequency noise. The pattern of effect is almost same in groups A and C. These findings indicate that it is the low frequency component of the noise which is the culprit for change in the type of sulcular epithelium. This change in the characteristic of sulcular epithelium may be due to continuous direct insult caused to epithelium due to mechanical effect of low frequency noise thus protecting the deeper layers from this insult.

Study gave a good insight into the problem of noise pollution affecting the livings but it is recommended that future study can be conducted by performing the study on fixed frequency with different sound intensity or stopping the LFN exposure and observe whether these effects are reversible.

**CONCLUSION**

This study concludes that 1. Low frequency noise and normal animal house conditions induce keratinization of sulcular epithelium in mice.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Keratinization in sulcular epithelium</th>
<th>Mean and SD of keratin layer at a fixedpoint as seen on 10 X magnification</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7/10</td>
<td>23.99±1.16;24.11</td>
<td>0.000*</td>
</tr>
<tr>
<td>B</td>
<td>2/10</td>
<td>24.11±1.78</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9/10</td>
<td>19.49±2.00;19.49</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*p value <0.05 significant

Statistical test applied is ANOVA.
2. Damage to the sulcular epithelium causes disruption of its normal function of secretion of sulcular fluid leading to increased susceptibility to periodontal disease.

3. This change occurs irrespective of the gender.

REFERENCES