EFFECT OF CHRONIC NOISE STRESS ON MANTLE ZONE OF LYMPHOID FOLLICLE OF SPLENIC TISSUE IN ALBINO RATS

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ABSTRACT

Background:

Noise is an ever growing problem in modern society. Apart from its effect on hearing, behavior, & mood it also has a potent effect on immune system. Therefore the present study was designed to asses effect of chronic noise stress on mantle zone (MnZ) of lymphoid follicle (LF) of spleen in albino rats.

Material & Methods:

Two groups of twenty rats, each group labeled A&B were used; serving as control (no noise) and noise stress (30 days) respectively. Group B was exposed to noise stress of 100 dB for 30 days (3-4hrs/day). Animals were sacrificed at the end of study, spleen was removed prepared, sectioned & stained with CD79a.

Result:

A highly significant decrease in mean no of cell in MnZ of LF was observed (15747±87.82/mm²) in noise stressed group when compared with MnZ of control animals (16567±106/mm²). There was also a highly significant increase in mean perimeter of nuclei observed in MnZ of stressed group (2.74±0.009µm) when compared with rats of control group (2.68±0.01µm).

Conclusion:

Our study concludes that noise stress induced release of glucocorticoid can significantly decrease number of B lymphocytes in MnZ of LF of spleen in albino rats.

Keywords:

Lymphoid follicle (LF), Mantle zone (MnZ), Noise stress, B lymphocytes, CD79a

INTRODUCTION

Stress is simply a fact of nature and an unavoidable consequence of life. Stress can be provoked by any stimulus called stressor, which produces a demand for adaptation. These stressors could be social, emotional, institutional, psychological, traumatic or environmental. Among these innumerable stressors, environmental stress is the most commonly encountered stress to which almost all individuals are exposed to a variable extent. Noise is a significant environmental problem and has potential to cause stress reaction.

Noise is a class of sound that is considered unwanted and can be produced by different sources. Noise level exceeding maximum permissible limits (60db day & 50dB night) causes several physical ailments as well as social and emotional problems.

Noise is the greatest threat to the people of large metropolitan cities in Pakistan. Several researches conducted in Pakistan reveals that many of the areas in metropolitan cities of Pakistan were identified where noise levels were greater than maximum permissible level.

Noise acts as a physical stressor on animals that can lead to behavioral, physiological and anatomical responses. Exposure to intense noise can trigger a cascade of neuro-endocrine events reminiscent of a stress response including activation of Hypothalamic-Pituitary-Adrenal (HPA) axis. Thus noise...
causes the release of different stress hormones (e.g. Corticotrophin releasing hormone: CRH; Adrenocorticotrophin hormone: ACTH) even while person is sleeping.

Stress is a potent modulator of immune system. Recent studies indicates that neuroendocrine-immune interaction can cause sufficient immunosuppression which adversely affect human health and had described the relationships between stress related hormones or neurotransmitter and immune functions. Receptors for these molecules are present on immune cells. The majority of the well characterized effects of adrenal steroids on immune system are produced by the type II receptor agonist, RU28362. RU28362 decreases the number of all lymphocyte subsets (T-cells, B-cells and natural killer cells) to very low absolute levels. The largest relative decrease was seen in B-cells. In stress, glucocorticoids type II receptor is a major mediator of the stress induced changes in the blood lymphocytes and monocytes distribution, both type I and type II receptors are expressed in spleen.

This study was designed to evaluate effect of noise stress on B lymphocytes in mantle zone of lymphoid follicle of splenic tissue in albino rats.

**MATERIAL & METHOD**

This study was conducted in department of anatomy BMSI, JPMC Karachi and microscopy was done in department of anatomy KMDC, Karachi. Twenty normal, adult and healthy male albino rats of ages (90 - 120 days) weighing 220-350 grams were selected. The animals were kept under observation for one week prior to the commencement of the experiment for assessment of their general state of health on the basis of weight gain or loss. Furthermore, this was also essential to acclimatize the animal to the environment of experimental room. All the animals were kept on standard laboratory diet and under closely controlled environmental conditions of 12 hour light / 12 hour dark cycle at room temperature.

The animals were divided into two groups A&B. The group A served as control and group B was exposed to 100 dB noise stress 4 hrs / day for 30 days. The animals were numbered, weighed and kept in proper cages, under natural environment. Diet and water were supplied ad libitum. All the experimental procedures were performed between 8:00 am to 12:00 pm in order to avoid circadian variations.

General appearance, i.e. activity, behavior and their food intake were checked daily. The intensity of the sound was measured by precision sound level meter (TES -1351) Noise level was set at 100 dB uniformly through out the cages.

All the animals were weighed at the commencement of the study and at the time of sacrifice. The animals exposed to chronic noise were sacrificed at the end of day 30th by prolong ether anesthesia in a glass container. After dissection blood samples were collected by intra-cardiac puncture to detect the hormonal levels of Corticosterone and ACTH in the plasma. Spleen was removed and cleared from fatty tissues. It was weighed by Sartorius electronic balance, washed by normal saline and then fixed in buffered neutral formalin (with pH 7.0) for 24 hours.

After fixation of the sections on the slides, they were stained with routine Haematoxylin and Eosin (H&E) staining technique to observe the general morphology of the tissue under the light microscope.

For immunohistochemistry 4-µm thick sections were cut and placed on poly-L-lysine coated glass slides. All the slides were routinely dewaxed in xylene and hydrated in graded alcohol. Slides then kept in tap water till the procedure of antigen retrieval.

Later slides were incubated with primary antibody CD 79a (Genetex, USA) in 1:600 dilution, diluted in standard primary antibody diluent (Genetex, USA) for one hour in a humidity chamber. The slides were then mounted with resin based mount-
ing medium after dehydration and clearing se-
quences.

Plasma ACTH was measured by ACTH, RIA kit purchased from Immunotech, France. Standard acylated sample was added to ACTH antibody coated tube and then was incubated for 1 hour at room temperature with shaking. The assay sensitiv-
ity was 1.2pg/ml.

Plasma corticosterone was measured by rat corticosterone EIA kit purchased from Diagnostic System Laboratory, USA.

All the results were analyzed statistically by using SPSS version 12, by applying student t test.

RESULT

Microscopic Examination:

The spleens of the two groups were observed under light microscope, stained by routine haematoxylin & eosin and immunostain (CD79a).

Control Animals:

The section of spleen in control animals was observed under light microscope, stained by routine haematoxylin & eosin and immunostain (CD79a).

Control Animals:

The section of spleen in control animals was observed under light microscope, stained by routine haematoxylin & eosin and immunostain (CD79a).

B Lymphocyte Count In Mantle Zone:

CD79a positive cells (B lymphocytes) were counted in mantle zone of splenic nodule in animals of the two groups with the help of a counting reti-
cule. Cells were counted in randomly selected 10 different fields in mantle zone. Their results and comparisons are summarized in table 1 and figure 1.

A highly significant decrease in B lymphocytes count was observed in animals exposed to 30days noise stress (15747±87.82/mm²) when compared with control animals 16567±106/mm² (P<0.0001).

Perimeter Of Nuclei In Mantle Zone:

The perimeter of nuclei was measured with the help of eye piece micrometer in 10 different fields in mantle zone of each splenic nodule. Perimeter of twenty randomly selected nuclei was measured in each field. The result and their comparison are mentioned in figure 2 and table 2.

The mean perimeter of nuclei in mantle zone in animals exposed to chronic noise stress of 30 days was 2.74±0.009µm. This showed highly significant increase when compared with mean perimeter of nuclei in mantle zone of control animals 2.68±0.01µm (P <0.0001).

Plasma acth concentration:

Plasma ACTH concentration in the two groups was analyzed by using radioimmunoassay kit. Their results and comparison of results are summarized in table 3 figure 3.

A highly significant increase was observed in mean plasma concentration of ACTH in animals exposed to chronic noise stress for 30days (131.50±9.07pg/ml) in comparison to mean plasma
ACTH concentration of control animals 53.80±5.75pg/ml (P <0.0001).

Plasma Corticosterone Concentration:

Plasma corticosterone concentration was analyzed in animals of the two groups by enzyme immunoassay kit and their observation with comparisons are summarized in table 4, figure 4.

The mean plasma corticosterone concentration in animals exposed to 30days (chronic) noise stress was found to be 4.21±0.22µg/dl, which shows highly significant increase (P <0.0001) when compared with mean plasma corticosterone concentration of control animals (1.75±0.25µg/dl).

**TABLE 1**
MEAN NO OF CELLS IN MANTEL LAYER /MM²

<table>
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<th>Animal No</th>
<th>Control (A)</th>
<th>Chronic Noise Stress (B)</th>
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<tr>
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Mean 16567 15747

SEM 106 88

**TABLE 2**
MEAN PERIMETER OF NUCLEI IN MANTLE ZONE (µM)

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</table>

Mean 2.684 2.742

SEM 0.01 0.01

**DISCUSSION**

Noise is increasingly being recognized as physical factors in the environment that is injurious to health and it affects the body system at various levels. According to the environmental expert council of Germany, severe annoyance persistent over prolonged periods of time is to be regarded as causing distress.

In the present study we have observed the effect of high intensity noise level (100 dB) applied for longer duration 30 days (chronic) to observe
changes in the level of stress hormones and changes in the morphology of white pulp of spleen in albino rats.

It is now accepted that there are complex interactions among the central nervous system, the endocrine system, and the immune system. Studies in the field of psychoneuroimmunology have shown that stress, through hypothalamic pituitary adrenal (HPA) and sympathetic adrenal medullary (SAM) axis, can induce modulation of the immune system\textsuperscript{14}. Recent studies indicate that neuroendocrine immune interactions can cause sufficient immunosuppression to adversely affect human health\textsuperscript{8}.

Increase in plasma glucocorticoid concentration secondary to stress induced by injury causes splenic atrophy, splenic leucopenia & suppressed B lymphocyte functions\textsuperscript{15}. Marked changes were observed in our study in white pulp of spleen tissue in albino rats when exposed to noise stress. A marked decrease in B-lymphocyte in mantle zone was observed. Our finding is in conformity with Kainuma et al.,\textsuperscript{16} who observed decrease in number of plaque forming cells on exposure to glucocorticoids and with Zimecki et al.,\textsuperscript{17} who found stress induced decrease in antibody forming cells. Exposure of 4 week noise stress has shown to cause a reduction of splenic lymphoproliferation and decrease serum IgG concentration\textsuperscript{18}. The study reported here demonstrates that 30 days noise stress significantly decrease the number of B lymphocyte in mantle zone of lymphoid follicle.

Exposure of different concentration of glucocorticoid analogous to those produced during stress, had rapid but varying effects on the major classes of cells of immune system. Immature B-lymphocytes are more susceptible to stress induced rise in glucocorticoid level as glucocorticoids are potent inducer of apoptosis in B-cell line\textsuperscript{19}. A large amount of cell loss occurs at the intermediate stages of B-lymphopoiesis\textsuperscript{20}. Dexamethasone at physiologic induce rapid apoptosis of most B-lineage precursor\textsuperscript{21}.

Exposure of noise stress provokes cascade of reactions resulting in activation of HPA axis. Activation of HPA axis is the consequence of the convergence of stimulatory inputs from different region of brain into paraventricular nucleus of hypothalamus where the most important ACTH secreatourgues, Corticotrophin releasing hormone and Arginine vasopressin are formed. Plasma levels of ACTH and corticosterone are considered as good marker of stress.

In our study we have observed the highly significant rise in plasma ACTH and corticosterone concentration when animals were exposed to chronic noise stress. Our finding was found in agreement with the observation of Goshen et al\textsuperscript{22}. Glucocorticoids are the final hormone of HPA axis secreted from zona fasiculata of adrenal cortex. The hormones bind with intracellular glucocorticoid receptor present on almost all cells. Glucocorticoid has important anti inflammatory and immunosuppressive properties\textsuperscript{23}. In addition exposure of restraint stress increases the binding of glucocorticoid to cytosol of spleen cells\textsuperscript{24}. This increase in stress hormone has shown to modulate immune function as blockade of glucocorticoid by pretreatment of glucocorticoid antagonist has shown to reduce effects of stress\textsuperscript{25}.

Our study concludes that noise is a potent modulator of immune system by activating HPA axis.

REFERENCES


