

Effect of Chronic Restraint Stress on Immune Status of Male Sprague Dawley Rats

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ABSTRACT

Objective: To determine the effect of chronic restraint stress on immune status of Sprague Dawley rats by comparing total leukocyte count (TLC), total lymphocyte count and levels of serum immunoglobulins A, E, G and M of the rats exposed to chronic restraint stress with that of healthy control rats.

Study Design: Quasi-experimental study.

Place and Duration of Study: Centre of Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, in collaboration of National Institute of Health (NIH), Islamabad, from June 2008 to June 2009.

Methodology: Sixty healthy male Sprague Dawley rats were obtained from NIH. Rats were divided into two groups, each having 30 rats. The rats of group-I were not exposed to chronic restraint stress, while rats of group-II were exposed to chronic restraint stress in meshwire restrainer for 6 hours daily for 15 days. Estimation of total lymphocyte count and serum immunoglobulins (IgG, IgA, IgM and IgE) was carried out and compared between the groups.

Results: TLC, lymphocyte count and serum IgA, IgE, IgG and IgM levels were found significantly lower in rats exposed to chronic restraint stress as compared to the rats which were not exposed to stress ($p < 0.001$).

Conclusion: Chronic restraint stress compromises immune status of rats by decreasing the levels of immunoglobulins and lymphocyte count.

Key Words: Stress. Immune status. Immunoglobulins. Lymphocyte count. Sprague Dawley rats.

INTRODUCTION

Acute and chronic stress can have both the short and long-term consequences, either protective or damaging.¹ During stress hypothalamo-pituitary adrenal axis (HPA) is activated as an adaptive response in order to maintain homeostasis. Glucocorticoids regulate catecholamine bio-synthesis in the adrenal medulla and catecholamines stimulate adrenocorticotropin release from the anterior pituitary.

Long-term stress can have a detrimental effect on the body that may lead to serious disease and debilitation.² Stress can alter antibody production through behavioural and neurobiological pathways.³ Conflicting reports of decrease in lymphocyte count due to stress,⁴ and an increase in lymphocyte count due to re-deployment of lymphocytes in the tissue have been published.⁵

The immune system is a complex network of cells, proteins, tissues, and organs that work together to

protect the body against infectious diseases or other insults. Stress has been documented to produce a profound effects on the immune system. Stress influences hormones that bind specific receptors on the membrane or in the cytoplasm of cells of the immune system, including various cells that participate in the production of antibodies. Experimental studies report heterogenous findings in relation to stress and the immune mechanisms.⁶

Hucklebridge and Clow compared acute stress with chronic stress and found that the secretory immunoglobulin A (sIgA) measured in saliva was down regulated during periods of chronic stress. In contrast, the response to an acute stress challenge is a transient increase in immunoglobulins.⁷ Pregnenolone, the precursor of all steroidal hormones, is diverted from the stress hormones; cortisol and cortisone under the states of the chronic stress.⁸

The aim of this study was to determine the total leukocyte count (TLC), differential leukocyte count (DLC) and serum immunoglobulins A, E, G and M, in rats exposed to chronic restraint stress and compared them with that of healthy control rats, in order to confirm the deteriorating effect of chronic restraint stress.

METHODOLOGY

It was a quasi-experimental study conducted at Centre of Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, in collaboration of National Institute of Health (NIH),

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Islamabad, from June 2008 to June 2009. All experiments were approved by animal experimentation ethical committee of Army Medical College.

The inclusion criteria were healthy male rats weighing 250 ± 50 grams, aged 60 ± 5 days. Exclusion criteria were female rats (due to estrous cycle), diseased rats or developing disease during the course of the study.

Sixty healthy male Sprague Dawley rats were taken from National Institute of Health (NIH), Islamabad. They were divided into two main groups. Group-I was the healthy control group of rats which were not exposed to chronic stress. Group-II (stress group); comprised of rats exposed to chronic restraint stress, 6 hours daily for 15 days by keeping them in a meshwire restrainer without food and water. The stress procedure was carried out at NIH, throughout the experimental period between 9 a.m. and 4 p.m. The meshwire restrainer had stainless steel wire mesh restrainer hinged to the base. A restrainer with dimensions of 18 cm (L) x 8 cm (B) x 8 cm (H) was used in this experiment. Intra-cardiac sampling was done after 2 weeks exposure to restraint stress. At one time, 5 rats were placed in a closed chamber containing ether soaked cotton. It took 3 – 5 minutes to get the rats anaesthetized. Five ml of blood was drawn with the help of 5 ml syringe, by intra-cardiac puncture. Two ml of each sample was transferred to an EDTA tube, while 3 ml of blood was transferred into a plain tube and allowed to clot. Blood containing plain tubes were centrifuged for 15 minutes in cold centrifuge machine (Model 5810R; Eppendorf, Germany). Temperature of centrifuge was adjusted at 4°C and speed at 4000 rpm. After cold centrifugation, serum was pipetted out. Approximately 1.5 ml of serum was obtained from each blood sample, transferred to serum tubes (Eppendorf, Germany) which were labeled and stored at -80°C in deep freezer (Model DFU-446 CE, Operon, Korea) till the assay of immunoglobulin levels by using commercial kits.

Estimation of immunoglobulins A, E, G and M levels were done by enzyme linked immunosorbent assay (ELISA) using immunoperoxidase assay kits of Immunology Consultants Laboratory Inc. U.S.A.¹¹ Estimation of total lymphocyte counts and absolute counts of other leukocytes were done by fully automated haematology analyzer Sysmex KX-21,¹² by first estimating the total and differential leukocyte counts, by applying the formula:

$$\text{Absolute count of lymphocytes} = \frac{\text{TLC} \times \% \text{Lymphocyte}}{100}$$

Absolute counts of other leukocytes were also determined by applying the same formula.

Data was entered into Statistical Package for Social Sciences (SPSS) version 15. Mean and standard deviation was calculated for numerical variables and frequencies with percentages were calculated for categorical variables where required. The results were

Table I: Comparison of total and differential leukocyte counts between rats exposed to chronic restraint stress and healthy control using student t-test.

Groups Parameters	Control (n = 30)	Stress (n = 30)	p-value
TLC / μl	8024 \pm 44	6785 \pm 78	< 0.001
Lymphocyte / μl	7194.90 \pm 107.74	6210.70 \pm 115.43	< 0.001
Monocyte / μl	50.10 \pm 1.71	58.97 \pm 1.85	< 0.001
Eosinophil / μl	70.37 \pm 1.73	54.33 \pm 2.48	< 0.001
Basophil / μl	10.17 \pm 0.95	3.93 \pm 0.69	< 0.001
Neutrophil / μl	501.23 \pm 11.51	628.12 \pm 10.14	< 0.001

All values are presented as mean \pm SD

Table II: Comparison of serum immunoglobulin levels between rats exposed to chronic restraint stress and healthy controls using student t-test.

Groups Parameters	Control (n = 30)	Stress (n = 30)	p-value
IgA (ng/ml)	103.57 \pm 4.28	64.50 \pm 5.19	< 0.001
IgE (ng/ml)	22.27 \pm 1.75	13.12 \pm 1.51	< 0.001
IgM (ng/ml)	739.13 \pm 11.96	618.83 \pm 12.42	< 0.001
IgG (ng/ml)	607.50 \pm 5.37	528.20 \pm 7.79	< 0.001

All values are presented as mean \pm SD

compared by applying student t-test, which reflected the statistical significance between the differences of means of various parameters. P-value < 0.05 was considered significant.

RESULTS

The comparison of lymphocyte between the two groups has been presented in Table I. Total leukocyte count in control group was $8024 \pm 44/\mu\text{l}$ (mean +SD), and in stress group the count decreased to $6785 \pm 78/\mu\text{l}$. Total lymphocyte count in control group was $7194.90 \pm 107.74/\mu\text{l}$ and in stress group it was $6210.70 \pm 115.43/\mu\text{l}$. Comparison amongst two groups revealed that all the parameters except neutrophils and monocytes were found significantly lowered in rats which were exposed to chronic stress ($p < 0.001$).

Comparison of immunoglobulin levels between the two groups have been presented in Table II. Immunoglobulin levels were also found to be significantly decreased in rats exposed to chronic restraint stress as compared to healthy rats ($p < 0.001$).

DISCUSSION

The immune system, once thought to be autonomous is now known to respond to signals from many systems of the body especially the nervous system and endocrine system. The present study supports this view.

First this study confirmed the effect of chronic restraint stress on total leukocyte count and lymphocyte distribution, in agreement with previous study.⁴ There was significant decrease in lymphocyte count. Lookingland *et al.* used two methods to impart restraint stress, one by keeping the rats immobilized in supine

position for 20 minutes and the other by confining the rats in acrylic cylinder tube for 30 minutes.⁹ Whereas Klenerova *et al.* imparted stress by both restraining the rats in acrylic cylinder tube and then immersing the rats in water for 30 minutes.¹⁰ In the present study, meshwire restrainer was used for chronic restraint stress, since it was a relatively economical method.

In normal healthy human beings, neutrophil count ranges between 50-70% and lymphocytes 20 – 40%. Whereas in rats, lymphocyte count is 72 – 94% while neutrophil count is between 5 – 25%.¹³ Differential analysis done by Doeing *et al.* showed lymphocytes as the predominant cell type present in the peripheral blood of both male and female mice, comprising 74% of the total leukocytes and 24% of neutrophil count.¹⁴ The present study also found the same percentage distribution of white blood cells in healthy rats i.e., lymphocytes were 89% whereas neutrophils were 7%.

Stress hormones are regarded widely as being immunosuppressive. The comparison of total leukocyte count amongst the two groups of present study revealed significant reduction in total leukocyte and lymphocyte counts following two weeks exposure to chronic stress. The studies conducted by Zager *et al.* on rats manifested the effect of chronic sleep deprivation on immune status of rats. In their study 24 and 96 hours sleep restriction (SR) for 21 days by the modified multiple-platform method, and their respective 24-hour recovery periods, affect immune activation in rats and found a significant decrease in not only total leukocyte and lymphocyte counts along with immunoglobulin levels.⁴

Nowland *et al.* determined the effect of prolonged fasting of 16 hours on the lymphocyte and neutrophil counts of male Sprague Dawley rats and found a significant decrease in lymphocyte count, with no change in neutrophil.¹⁵ Another study was conducted by Clougherty *et al.* on male Sprague Dawley rats. The immune markers like TLC, DLC was determined in responses to effect of fine ambient particles on respiratory tract. TLC and lymphocyte count was found to be decreased by 12% as compared to 14% in this study.¹⁶

In addition, eosinophil and basophil counts were also reduced, while neutrophil and monocyte counts got increased. Dhabbar *et al.* showed a decrease in lymphocyte count due to re-deployment of lymphocytes in the tissues following restraint stress to Sprague Dawley rats.¹⁷

The TLC and total lymphocyte count was found significantly decreased, immunoglobulin levels were also expected to be decreased as a result of chronic restraint stress. So, when levels of all the immunoglobulins A, E, M and G of the two groups were compared, all the immunoglobulin levels were found to

be significantly lower in the stress group than the healthy control rats ($p < 0.001$). Multiple studies have supported that chronic stress led to decreased levels of IgA, IgM and IgGs. An animal study conducted by Lin *et al.* studied the effect of emotional stress on the immune status of Wister rats and found a significant reduction in immunoglobulin levels.¹⁸ Rowland studied male Sprague Dawley rats to determine the effect of food and fluid restriction for 20 hours daily for 14 days on immunoglobulins E and A and found a significant decrease in levels.¹⁹ The human based study of Hucklebridge *et al.* has documented the decreased levels of IgA on exposure to chronic stress.⁷ In their study, only one immunoglobulin (IgA) was evaluated, however, Herbert *et al.* evaluated all the major types of immunoglobulins and found that IgA and IgM levels decreased on exposure to stress.²⁰

Stress causes lymphocytes and macrophages to be redistributed throughout the body. This trafficking is mediated in part by glucocorticoids.²¹ The great majority of psychopathological studies have examined immunological alterations associated with affective and anxiety disorders. There is substantial evidence that stress and anxiety enhance the production of pro-inflammatory cytokines, including IL-6 which has been associated with the lower levels of T and B lymphocytes.²² This might lead to the decrease in immunoglobulin levels as there was increase in the duration of stress.

CONCLUSION

Chronic restraint stress alters the immune status that can be detrimental to the health and may be responsible for the disease.

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