ORIGINAL ARTICLE

RESPONSE OF HYPOTHALAMO-PITUITARY-ADRENAL AXIS AND IMMUNE SYSTEM TO CHRONIC RESTRAINT STRESS IN MALE SPRAGUE DAWLEY RATS

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Background: During stress, hypothalamic pituitary adrenal axis (HPA) is activated as an adaptive 'fight or flight' response in order to maintain homeostasis of body function and alter immune response. **Method:** This study was conducted at National Institute of Health (NIH) Islamabad. Sixty healthy male Sprague Dawley rats (age=65±5 days; weight=250±50 gm) 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 mesh-wire restrainer for 6 hours daily for 15 days. The total leukocyte (TLC) and lymphocyte count, serum cortisol level and immunoglobulins (IgG, IgA, IgM and IgE) levels were estimated for comparison between the two groups. **Results:** Serum cortisol levels were found significantly (p<0.001) raised in rats exposed to chronic restraint stress. TLC, Lymphocyte count and serum IgA, IgE, IgG and IgM levels significantly (p<0.001) decreased in rats exposed to chronic restraint stress as compared to the rats which were not exposed to stress. **Conclusion:** Chronic restraint stress compromises immune status by decreasing the levels of immunoglobulins and lymphocyte count with concomitant increase in serum cortisol.

Keywords: Stress, cortisol, immune status, Immunoglobulins, lymphocyte count, Sprague Dawley rats

INTRODUCTION

A bi-directional relationship exists between the immune system and the Hypothalamo-pituitary adrenal axis (HPA axis). Following immune system activation, there is an increase in the production of cytokines (e.g., interleukins) that stimulate HPA axis activity. Glucocorticoids have immunosuppressive actions and their release following HPA axis activation by immune challenge modulates the immune response.[1] However, the interactions between the immune system and the HPA axis are not always deleterious. It seems relevant to distinguish between acute and chronic effects of stress. For example, acute stress enhances the trafficking of lymphocytes and macrophages to the site of acute challenge through HPA axis stimulation.[2] The effects of stress are probably beneficial. By contrast, repeated stress induces a decrease or a disruption of cellular immunity[3], a decrease of the different subsets of lymphoid cells in secondary lymphoid organs that correlates with a decrease of antibody levels[4], and/or a disruption of cytokine secretion[5].

Stress influences the plasma and tissue concentration of many hormones that bind specific receptors on the membrane or in the cytoplasm of cells of the immune system, including the cells that participate in the production of antibodies. 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.[6] The immune system protects the body from disease producing organisms and foreign bodies, (like antigens), by producing antibodies like IgG, IgM, IgA and IgE with the help of antigen specific T helper cells. Stress associated hormones having the principal effect on altering antibody production are the glucocorticoid (cortisol). Increased cortisol level causes the decrease in total lymphocyte and circulating B cells.[7]

Modes of stress used in different studies were different. Study conducted by Lookingland *et al* used two methods to impart restraint stress, one by keeping the rats immobilised in supine position for 20 minutes and the other by confining the rats in acrylic cylinder tube for 30 minutes.[8] Whereas a study conducted in 2008 by Klenerova *et al* imparted stress by both restraining the rats in acrylic cylinder tube and then immersing the rats in water for 30 minutes.[9] In the present study, mesh wire restrainer was used for chronic restraint stress, since it was relatively cheaper method.

This study was designed to evaluate the response of HPA axis and immune status of Sprague Dawley rats to chronic restraint stress by comparing lymphocyte count and levels of serum cortisol and immunoglobulins A, E, G, and M of rats exposed to chronic restraint stress with that of healthy control rats.

MATERIAL AND METHODS

This study was conducted at NIH Islamabad in collaboration with Centre for Research in Experimental and Applied Medicine (CREAM) at Army Medical College, Rawalpindi. Sixty male Sprague Dawley healthy rats (age 60±5 day, and weight 250±50 gm) were obtained. They were divided into two 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

mesh wire restrainer without food and water. Recommended duration for chronic stress is 5 hours per day for 14 consecutive days.[9] Body weight of all rats were taken at the beginning of the study, when rats were 13 weeks old. They were monitored for 4 weeks, before the stress group was exposed to chronic restraint stress 6 hours daily for 15 days.

The stress procedure was carried out at NIH, throughout the experimental period between 9.00 AM and 4.00 PM. Single sampling was done at 9.30 PM in each rat after exposure to chronic restraint stress. The mesh wire restrainer was comprised of stainless steel wire mesh restrainer hinged to the base. A restrainer with dimensions of 18 Cm (L)×8 Cm (B)×8 Cm (H) was used in this experiment. Intra cardiac sampling was done after two weeks exposure to restraint stress. At one time, 5 rats were placed in a closed chamber containing ether soaked cotton. It took 3 to 5 minutes to get the rats anaesthetised. Five ml blood was drawn with the help of 5 ml syringe, by intra cardiac puncture. Two ml blood of each sample was transferred into 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 ℃ and speed at 4,000 rpm. After cold centrifugation, serum was pippeted out. Approximately 1.5 ml of serum was obtained from each blood sample, transferred to serum tubes labelled and stored at -80 ℃ in deep freezer (Model DFU-446 CE, Operon, Korea) till the assay of immunoglobulin levels by using commercial kits. Different organs were also obtained for further study before their disposal.

Cortisol estimation was done by using Immulite 1,000 analyser (Catalog No. LKCO1) which is a solid-phase, competitive chemiluminescent enzyme immunoassay.[10] Estimation of immunoglobulins A, E, G and M levels were done by ELISA using Immunoperoxidase assay kits of Immunology Consultants Laboratory Inc. USA.[11] Total lymphocyte counts was done by fully automated haematology analyser Sysmex KX-21.[9] The absolute lymphocyte count was calculated by applying the following formula:

Absolute count of Lymphocytes=

$$\frac{\text{TLC}\times\%\text{Lymphocytes}}{100}$$

Data were entered into SPSS-15. The results were compared by applying Student's *t*-test, which reflected the statistical significance between the differences of means of various parameters.

RESULTS

The stress was assessed by plasma cortisol levels on the sampling. The levels were higher than in the control group with high statistical significance ($p<0{\cdot}001$). The comparison of weight gain amongst two groups revealed that there is a significant decrease in body weight of rats during two weeks (13^{th}, 14^{th}) of exposure to chronic restraint stress as compared to rats of the control group (Table-1). The comparison of serum cortisol and immunoglobulin levels amongst the two groups has been presented in Table-2.

Serum cortisol level in healthy control group was 21.4±0.92 ηg/ml, that significantly ($p<0.001$) increased in the stress group as 34.75±1.47 ηg/ml. Total leukocyte count in the control group was 8,024±44/µl, and in stress group the count significantly ($p<0.001$) decreased to 6785±78/µl. Total lymphocyte count in control group was 7194.90±107.74/µl and in stress group it was significantly decreased ($p<0.001$) to 6210.70±115.43/µl.

Comparison of immunoglobulin levels between the two groups in Table-2 was also found to be significantly ($p<0.001$) decreased in rats exposed to chronic restraint stress as compared to the healthy rats.

Table-1: Comparison of body weight between control and stress groups of Sprague Dawley rats

Weeks	Control (n= 30)	Stress (n= 30)	p
13^{th}	313.83±6.78	306.83±7.13	< 0.001
14^{th}	320.17±6.83	309.50±7.58	< 0.001

Table-2: Comparison of serum cortisol, IgA, IgE, IgG, IgM and TLC and absolute lymphocyte count between control and stress group

Variables	Control (n=30)	Stress (n=30)	*p*
Cortisol (ηg/ml)	21.4±0.92	34.71±1.45	<0.001
TLC (cells/µl)	8020±44	6785±78	<0.001
Lymphocytes (cells/µl)	7194±107	6210±115	<0.001
IgA (ηg/ml)	103±4.2	64.5±3.8	<0.001
IgE(ηg/ml)	22.26±1.75	13.1±1.5	<0.001
IgG (ηg/ml)	607±5.3	528±7.7	<0.001
IgM (ηg/ml	739±11.96	618.8±12.4	<0.001

DISCUSSION

This study highlights the fact that chronic stress may have important deleterious effects on both cellular and humoral immunity. The immune system, once thought to be autonomous is now known to respond to signals from many organs or systems of the body especially the nervous system and endocrines. Our study supports this view and has manifested a decline in immune status on exposure to chronic stress. However, different studies conducted on human beings had manifested the relationship between chronic stress and elevation of cortisol levels and changes in immune status. The present study was planned on Sprague Dawley rats to have the standardized stress and to determine the response of HPA axis and immune status to chronic restraint stress. Our results provide new insight into the modulation of immune response in context of chronic restraint stress.

Significant reduction in weight was seen in rats of the stress group as compared to the control group ($p<0.001$). Results of the study conducted on rats by Santos support our findings that chronic stress decreases the weight gain[12], while Dallman *et al* documented the contradictory results and postulated that chronic stress resulted in increase in weight gain. It could be due to the reduction in growth hormone secretion, linear growth,

and sympathetic neural outflow along with reduced fat mobilisation.[13]

There was a relationship between chronic restraint stress and immune status. Serum cortisol levels were found significantly raised compared to that of rats in control group. The study conducted by Schulz *et al* on subjects exposed to chronic stress, revealed more enhanced and prolonged increase in cortisol levels after awakening (38.11±1.12 ηg/ml).[14]

Our study confirmed the down regulation of TLC, and lymphocyte counts in rats following two weeks exposure to chronic restraint stress. In rats lymphocyte count is 72–94% while neutrophil count is 5–25%. In healthy rats differential lymphocyte count ranges 72–94% while neutrophil count is 5–25%. The total lymphocyte count in our study reduced from 7,197 to 6,210/µl in rats exposed to chronic restraint stress. The study conducted by Zager *et al*[15] on rats manifested the decrease in lymphocyte count on exposure to stress (6819±101/µl). Dhabbar *et al* showed an increase in lymphocyte count probably due to redeployment of lymphocytes in the tissues (8910±89.24/µl).[16]

In the present study since total lymphocyte count got significantly decreased on exposure to chronic stress, therefore immunoglobulin levels were also expected to the reduction in immune cells. Comparison of different immunoglobulins A, E, M and G between the two groups revealed that types of immunoglobulin levels significantly (p<0.001) decreased in the stress group than the healthy control rats. A number of studies have supported that chronic stress led to decreased levels of IgA, IgM and IgGs. A study conducted by Ling *et al* showed the effect of emotional stress on the immune status of Wister rats and found a significant reduction in immunoglobulin levels.[17] The human based study of Hucklebridge *et al* has documented the decreased levels of IgA on exposure to chronic stress.[18] In their study only IgA was evaluated. Herbert *et al* evaluated all the major types of immunoglobulins and found that IgA and IgM levels were decreased on exposure to stress.[19]

CONCLUSION

Chronic restraint stress compromises immune status by decreasing the total lymphocyte count and immunoglobulin levels with concomitant rise in serum cortisol.

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