

Morphological Changes after Chronic Heat Exposure on Zona Fasciculata of Adrenal Gland of Albino Rats

1. Surriyya Sarwat 2. Muhammad Rafique 3. Rais Ahmed

1. Asstt. Prof. of Anatomy, SMU, Karachi 2. Assoc. Prof. of Anatomy, DMC, DUHS, Karachi 3. Assoc. Prof. of Anatomy, SMU, Karachi

ABSTRACT

Objective: To compare the microscopic changes in zona fasciculata of adrenal gland of rats between control and chronic heat exposed groups.

Study Design: Experimental Study

Place and Duration of Study: This was conducted in IBMS, DUHS, Karachi from June 2010 to May 2011.

Materials and Methods: Forty eight male wistar rats were selected for the study and divided into two groups A and B, each group consisted of 24 rats. Group A served as control and subdivided into three groups. Group B was taken as chronic heat exposed and subdivided into three groups on period of exposure (B1, B2, B3). All rats were acclimatized at temperature of $22 \pm 2^\circ\text{C}$ for two weeks. Group B exposed to $35 \pm 2^\circ\text{C}$ for 2 hour daily for 2 weeks, 4 weeks and 6 weeks, and sacrificed on completion of respective period of heat exposure along with their respective control. After dissection the gross changes and weight of each adrenal gland was observed, gland was fixed in Bouin's fluid and embedded into paraffin wax. After tissue blocks made, $5\mu\text{m}$ thick sections were obtained; sections were stained with Haematoxyline and Eosin stain to observe the light microscopic changes. Statistical analysis was done by using the software SPSS version 16, t-test, Mann Whitney, ANOVA and Kruskal Wallis were applied for the comparison between the group variables.

Results: The changes in the total cortical thickness in chronic groups were shown by the P-value <0.0001 . Thickness of zona fasciculata and changes between all chronic and their control groups showed the P-value = <0.0001 ; The other significant changes were also observed on microscopic micrometry.

Conclusion: The significant increase in the total thickness of cortex and zona fasciculata of the gland was seen, as increased secretory vesicles were observed after chronic exposure to heat stress. After the completion of this study, it is suggested that more investigations should be carried out to see the genetic influences and the depth of the whole process.

Key Words: chronic stress, heat, adrenal gland, zona fasciculata.

INTRODUCTION

Stress is defined as "the non-specific response of the body to any demand made upon it".¹ It is the integral part of our life in this modern era. Activation of the Hypothalamic-Pituitary-Adrenal axis by the stress is known to be the main stress system in both human and experimental animals.² Stress may be acute or chronic. Chronic stress is state of continuous physiological arousal.³

It is noticed that heat is an environmental as well as the occupational hazard.⁴ The populations at risk to the thermal stress are those working in the fields.⁵ Heat stress causes significant morbidity and occasionally mortality in certain population who are at risk including farmers, athletes, fire fighters, children, elderly and disabled population.^{6,7} Increasing heat in our surrounding is the result, of not only the destruction of ozone layer, but it is produced by daily usage of electrical home appliances and advance electrical technologies. The rise in surrounding temperature can occur due to the emission of the radiation from the signaling towers.⁸

The HPA is the major endocrine axis to regulate the stress process and adaptation during the stressful events. Beside this neuronal connection the genetic factors also play very important role in the production and regulation of stress hormones released from adrenal gland after the stressful stimuli.⁹ Stress responses occurred in both sex of human as well as in rats and it is proven from the study that these events are genetically influenced by the various genes found in male and female rats.¹⁰

The morphological studies on the adrenal cortex of rats after the application of different types of stress have been conducted and each study has given the different morphological findings. Chronic environmental stresses affects the morphology of adrenal gland and these changes were observed in cortex. In a study after the application of single exposure to the cold stress, significant reduction in the lipid content of cortex was observed¹¹, but chronic stress may affects differently on the morphology of adrenal gland. The study conducted by Pellegrini and Slodani showed that after the chronic exposure to the industrial noise, causes marked reduction in the cells of zona fasciculata.¹²

Study conducted by Milošević showed that, continuous exposure of light stress in female rats increased the volume and cell in the zona fasciculata.¹³ There are different overlapping mechanisms activated during chronic exposure that may results in increase of volume of zona fasciculata, while reduction in another zone. In the Gordienko's study, the persistent immobilization stress in the rats shown the increased lysosomal degradation and reduction in the cell volume of zona fasciculata under high magnification micrometry.¹⁴ The stress causes increased production of cortisol hormone, under the influence of Adenocorticotropin hormone (ACTH) from the zona fasciculata, that result in the rise of glucocorticoid level. Increased ACTH secretion exerts tropic and trophic effects on the zona fasciculata.¹⁵

In our study we compared the effect of chronic heat exposure on the morphology of adrenal gland especially in zona fasciculata.

MATERIALS AND METHODS

This was an experimental study. The experimental work was done in the animal house of DUHS, Karachi. The work was completed during the period of 10 months (June 2010-May 2011). The study was done on 48 male wistar albino rats of weight range $200\text{gm} \pm 50\text{gm}$.

The animals were divided into two large groups A and B, each group consisted of 24 animals. Group A and group B were subdivided into 3 subgroups, each subgroup consisted of 8 animals. Group A was taken as a control group consisting of A1, A2 and A3. Group B was experimental exposure group and further subdivided into B1, B2 and B3 according to the experimental exposure.

All the animals were acclimatized to the temperature of $22^\circ\text{C} \pm 2^\circ\text{C}$ for the period of two weeks with the adequate supply of food and water. All the animals of group A were kept at same environmental condition during the period of experiment. Group B1, B2, and B3 were exposed to the temperature of $35^\circ\text{C} \pm 2^\circ\text{C}$, 2 hours daily for the period of 2 weeks, 4 weeks and 6 weeks respectively.

All animals were scarified after their respective exposure along with controls. The animals were dissected through midline thoraco-abdominal incision. Thoracic cavity was exposed after the dissecting the sternum. Both the glands were freed from the surrounding fat and fascia. Left Adrenal gland was removed, weighed immediately, fixed in Bouin's fluid. $5\mu\text{m}$ thick sections were made. Staining with hematoxylin and eosin (H&E) was then done. The slides were examined under microscope for morphological changes in the cortical zone of the adrenal gland especially in the zona fasciculata after chronic heat exposure.

Micrometry was done by using the stage and ocular micrometer¹⁶. t-test and ANOVA were applied for the parametric variables while Mann Whitney and Kruskal Wallis were applied for the non-parametric variables to observe the differences between the control and exposed groups and also the differences between the different exposure groups.

RESULTS

The effects of experimental exposure of chronic heat stress in zona fasciculata of adrenal gland of rats were observed and compared.

Table No 1: Total Cortical thickness of Adrenal Gland.

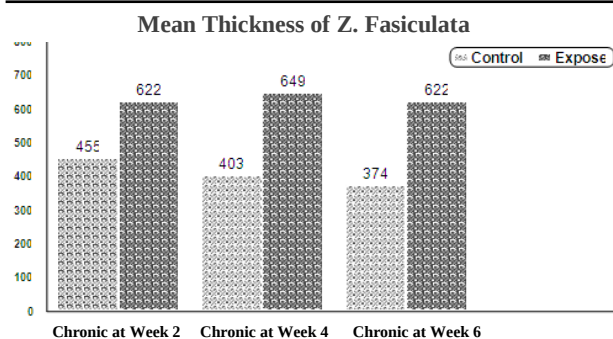
Variables	At Week 2			At Week 4			At Week 6		
	Control (N = 8)	Chronic I (N = 8)	P Value	Control (N = 8)	Chronic II (N = 8)	P Value	Control (N = 8)	Chronic III (N = 8)	P Value
	Mean $\pm$ SD	Mean $\pm$ SD		Mean $\pm$ SD	Mean $\pm$ SD		Mean $\pm$ SD	Mean $\pm$ SD	
Cortical Thickness of Gland	667.58 $\pm$ 41.83	928.49 $\pm$ 102.15	<0.0001	634.41 $\pm$ 84.51	938.88 $\pm$ 66.53	<0.0001	656.18 $\pm$ 50.68	906.33 $\pm$ 91.01	<0.0001
Thickness of Z. Fasiculata*	455.33 $\pm$ 28.33	621.76 $\pm$ 101.14	<0.0001	403.33 $\pm$ 74.07	648.62 $\pm$ 81.38	<0.0001	373.70 $\pm$ 16.77	621.99 $\pm$ 86.50	<0.0001

Total Cortical thickness of Adrenal Gland:

The total cortical thickness of adrenal gland of control was compared with the respective exposed group at the magnification of 4X and 10X objectives of microscope and 8X of ocular micrometer.

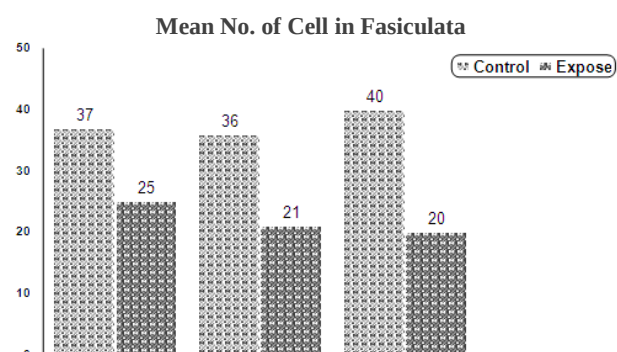
The mean $\pm$ S.D value of total cortical thickness of adrenal cortex in A1 group was $667.58 \pm 41.83 \mu\text{m}$ and in the B1 group was $928.49 \pm 102.15 \mu\text{m}$, and the P-value < 0.0001. The mean $\pm$ S.D value of total cortical

thickness of adrenal cortex in A2 group was $634.41 \pm 84.51 \mu\text{m}$ and in the B2 group was $938.88 \pm 66.53 \mu\text{m}$, and the P-value = 0.0001. The mean $\pm$ S.D value of total cortical thickness of adrenal cortex in A3 group was $656.18 \pm 50.68 \mu\text{m}$ and in the B3 group was $906.33 \pm 91.01 \mu\text{m}$, and the P-value < 0.0001 as shown in Table No.1 and Graph No.1. In Photomicrograph 1 and 2 microscopic changes in the cortical thickness and thickness of zone are observed.



Graph No. 1: Thickness of Zona Fasciculata of cortex

The graph no.1 showed the difference in the thickness of zona fasciculata in all the chronic exposure groups when compared with their control.



Graph No. 2: Number of cells in Zona Fasciculata (per unit area)

The graph No. 2 showed the comparative difference (decreased) in the cell count per unit area per field in between all chronic with their control groups.



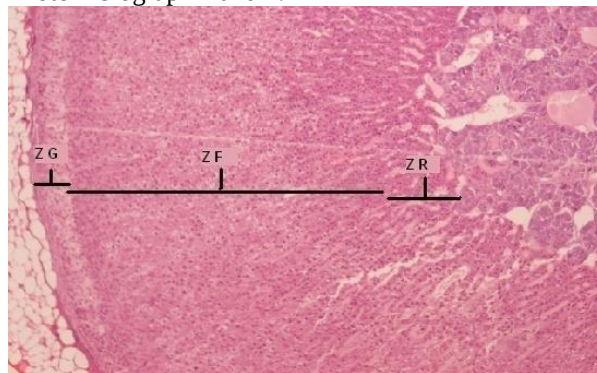
Photomicrograph No.1: Photomicrograph of 5µm thick section of tissue stained with H & E showing 3 zones of cortex of albino rats at 32X (4X objective x 8X ocular) in control group

Thickness of Zona Fasciculata of cortex:

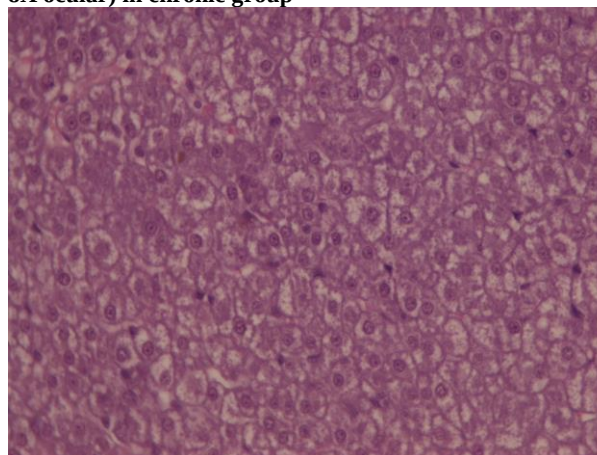
The thickest zone of adrenal cortex is zona fasciculata, where the cells are arranged in parallel cords. In the present study marked morphological changes were

observed in this zone in chronic heat exposed animals, when compared with their respective controls.

The mean $\pm$ S.D value of thickness of zona fasciculata in A1 was $455.33 \pm 28.33 \mu\text{m}$ and in B2 group was $621.76 \pm 101.14 \mu\text{m}$, P-value turned out to be <0.0001 . The mean $\pm$ S.D value of thickness of zona fasciculata observed in A2 was $403.33 \pm 74.07 \mu\text{m}$ and in B2 group was $648.62 \pm 81.38 \mu\text{m}$, P-value was turned out to be <0.0001 . The mean $\pm$ S.D value of thickness of zona fasciculata was in A3 was $373.70 \pm 16.77 \mu\text{m}$ and in the B3group was $621.99 \pm 86.50 \mu\text{m}$, P-value was turn out be <0.0001 as shown in Graph no.1 and Photomicrograph 1 and 2.



Photomicrograph No. 2: Photomicrograph of 5µm thick section of tissue stained with H & E showing 3 zones of cortex of albino rats at magnification 32X (4X objective x 8X ocular) in chronic group



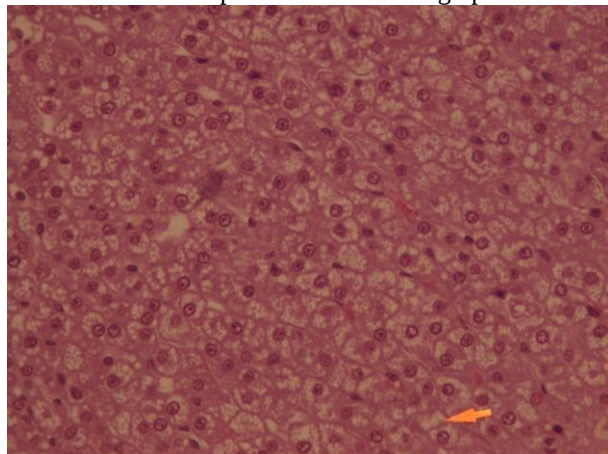
Photomicrograph No. 3: Photomicrograph of 5µm thick section of tissue stained with H & E showing cell appearance and count of zona fasciculata in the cortex of albino rats at 320X (40X objective x 8X ocular) in control group

Number of cells in Zona Fasciculata (per unit area):

In zona fasciculata the cells are arranged in parallel cords and filled with lipid droplets so as to give foamy appearance under microscope. In the present study the cell count was observed at the magnification of 40X with the reticule.

The mean $\pm$ S.D value of cell count per unit area of zona fasciculata in A1 was 36.60 ± 2.30 and in the B1 group was 25.20 ± 3.77 . When the comparison was

drawn between the two groups the calculated P-value was <0.0001 . The mean $\pm$ S.D value of cell count per unit area of zona fasciculata in A2 was 35.95 ± 2.98 and in B2 group was 21.21 ± 1.96 . When the comparison was drawn between the two groups, the calculated P-value was <0.0001 . The mean $\pm$ S.D value of cell count per unit area of zona fasciculata in A3 control was 35.81 ± 4.53 and in the B3 group was 19.58 ± 2.29 . When the comparison were drawn between the two groups the calculated P-value was <0.0001 as shown in Graph No. 2. and also compared in Photomicrograph 3 and 4.



Photomicrograph No. 4: Photomicrograph of 5 μ m thick section of tissue stained with H & E showing cell appearance and cell counts in the zona fasciculata of cortex of albino rats At 320X (40X objective x 8X ocular) in chronic group. Arrow showed the Fat Filled Empty Cells.

DISCUSSION

The adrenal gland responds immediately after the stress stimulus, whether the stress is acute or chronic. The gland responded differently to various types of stressors and these responses are also influenced by the duration of stress applied.¹⁷ There are different types of stressors but heat is an important environmental stress. It is very hazardous to human life and causes many ill-health effects, especially for those who working in hot and humid climate.

This increase in the weight of adrenal gland could be attributed to the continuous activation of HPA axis that resulted in increased synthesis of stress hormones by the cortical cells of adrenal gland and a corresponding increase in the cells size due to an increased number of secretory vesicles.¹⁸ The number of cells in the cortex was also increased, which resulted in the overall increase in the organ weight. These findings are also supported by the work done in the previous studies, although it was not clearly demonstrated that this was due to increased cell count or due to increased cell size.¹⁹ In future, further studies need to be done to find out exact mechanism of the process by expanding the work on cellular basis.

The chronic heat exposure showed morphological changes in the adrenal gland of rats. Belonging to all

three groups of chronic category, the mean cortical thickness was significantly increased in all chronic heat exposure groups as shown in Table no. 3 as well as in Photomicrograph 2. This increase was supposed to be due to the significant increase in the thickness of zona glomerulosa and zona fasciculata. As in the previous study of chronic stress resulted in prolonged activation of HPA pathway that also causes hypertrophy of this zone.²⁰

In the present study the increase in the cell size correlated to the decrease in the cell count per reticule per field although there was an overall increase in the cell number. The specific cell volume measurement is required at cellular level in future. At the higher magnification the increased number of fat droplets gave foamy appearance to most of the cells as shown in Photomicrograph 4. Fewer cells of the zona fasciculata were occupied by fat droplets to such an extent that the nuclei were pushed to the sides as in Photomicrograph No.4, that can be correlated to the study showing ultra structural changes after prolonged stress.

CONCLUSION

It is concluded that the morphological changes in adrenal gland after chronic heat exposure, we observed. It is required that more research work is done to find out the genetic influences and the ultra structural consequences of the process.

REFERENCES

1. Jone TL. Definition of stress. In: JJ Robert-McComb, editor. Eating disorder in women and children: prevention, stress management and treatment, Boca Raton FL: CRS Press; 2001.p. 89-100.
2. Vigas M, Kvetnansky R, Jurcovicova J, Jezova D and Tatar P. Comparison of catecholamine and adenopituitary hormone response to various stress stimuli are maintaining in man. In: Usdin E, Kvetnansky R, Axeelrod J, editors. Stress: the role of catecholamine and other neurotransmitters. Gordon and Breach: New York: 1984.p.865-82
3. Elizabeth SMS. Acute and chronic stress definitions. About com Health Disease and condition, Med Rev Board 2007.
4. Heat stress and public health: A critical review. Annu Rev Public Health 2008; 29: 41-55.
5. Bates GP, Miller VS, Jonbert DM. Hydration status of expatriated manual workers during summer in Middle East. Ann Occup Hyg 2010; 54: 137-43.
6. Corris EE, Ramieez AM, Van Durme DJ. Heat illness in athletes: the dangerous combination of heat, humidity and exercise. Sport Medicine 2004; 34: 9-16.
7. Glazer JL. Management of heat stroke and heat exhaustion. Am Fam Physiol 2005;71: 2133-40.

8. Stacy E, Desise W, Anna R, Kontantia Z, Riccardo R, Framasos, et .al. Short term exposure to mobile base station signaling increasing symptom who reports sensitivity to electromagnetic field. *Env Hea J* 2007; 115:1603-08.
9. Mormede P, Foury A, Barat P, Corceff JB, Terenina E, Marissal ARY. Molecular genetics of hypothalamus-pituitary adrenal axis and function. *Ann NYC Acad Sci* 2011; 1220: 127- 36.
10. Diccurziol DL, Goldwiz D. *Mamm Geome* 2011; 22: 2001- 24.
11. Ferrira M, Valenti VE, Cisternas JR, Ferreira C, Meneghini A, Filho CF. et al. Mamantine effects on liver and adrenal gland of rats exposed to cold stress. In *Arch Med* 2011; 4: 5.
12. Pellegrini A, Slodani P, Gesi M, Lenzi P, Natde G, Paparelli A. Effects of varying noise stress duration on rats adrenal gland on ultra structure study. *Tissue cell* 1997; 29: 597- 602.
13. Miloservic V, Trigonovic S, Sekulic M, Jurjui B, Filipovic B, Negic N, et al. Chronic exposure to contact light affects morphology and secretion of adrenal zone fasciculata cells in female rats. *Gen Physiol Biophys* 2005; 4: 299-309.
14. Gordien VM, Bienenova TI, Shvirst EM. Morphometric study of the ultra structure of zona fasciculata of adrenal in rats under stress. *Tisitologia* 1977; 19:131-36.
15. Pignatelli D, Pinto P, Azevedo M E, Magalhaes MM, Magalhaes MC. Acute stress effect on adrenal cortex in the rats: A biochemical and immunohisto chemical study. *End Res* 1996;22: 445-51.
16. Weibel ER, Gomez DM. A principle for counting tissue structures on random sections. *J Appl Physiol* 1962;17:343- 48.
17. Mazzochi G, Malenodoweiz LK, Rebuffat P, Robba C, Gttardo G, Nussdonfer GG. Short and long term effects of ACTH on the adrenal zona glomerulosa of rats. A couple stereological and enzymological study. *Cell Tissue Res* 1986;243: 303-10.
18. Aguilera G, Kiss A, Lu A, Camachoc. Regulation of adrenal steroidogenesis during chronic stress. *Endocr Res* 1996; 22(4): 433-43.
19. Thomas TC, Kumar VM. Effect of ambient temperature on brain temperature and sleep-awakefulness in medial preoptic area lesimed rats. *Ind J Pysiol Pharmacol* 2002; 46: 287-97.
20. Kodyshera EV, Luhnkova EL, Nepomnyashchikh LM, Termuev YV. Morphogenesis of adaptation and compensatory reaction in mouse during restitution after thermal exposure. *Bull Ex Bio Med* 2005; 140: 464- 7.

Address for Corresponding Author:**Dr. Surriyya Sarwat.**Assistant Professor Anatomy.
Sindh Medical University.
Karachi.