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Comparison of Serum Adrenocorticotropic Hormone (ACHT) and Cortisol Level Between Subjects Living In a Desert and Non-Desert Area of *Cholistan,* Punjab: A Cross Sectional-Analytical Study

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ABSTRACT: *The objective of this study was: "To assess the pituitary and adrenal cortical functions in natives of Cholistan desert". The study design was analytical cross-sectional, conducted in Cholistan desert and in the Physiology and Cell Biology Department of UHS Lahore. The sample size was one hundred (100) through convenience sampling. Blood samples were drawn from the subjects. Serum was separated from the venous blood immediately to avoid the effect of hemolysis on serum K+ levels. The serum isolated was then transferred to two vials for ACTH and Cortisol level measurements. The data was entered and analyzed using SPSS 18. The mean ± SD value for serum cortisol of the exposed and un-exposed group were14.54 ± 4.30 µg/dl and 15.56±4.90 µg/dl respectively. But the difference between the two groups was not statistically significant (p=0.273).The environmental heat present at all levels of biological organization and to restore and adjust constant body temperature, fluid balance, and energy metabolism as a survival mechanism in the demanding environment. The serum ACTH levels were decreased in this study in the exposed group as compared to the un-exposed group; although this difference was not statistically significant with the p-value of 0.66. Acute exposures to heat have led to an increase in ACTH levels while the chronic acclimatization has been associated with relatively lower levels. Chronic exposure led to decreased levels of ACTH. The cortisol hormone values were also comparatively decreased in the exposed group and it was also not statistically important with p= 0.273. Reasons for different responses to acute and chronic stress are unknown but may be related to altered adrenal metabolism.*

KEYWORDS: Serum adrenocorticotropic hormone (ACHT), cortisol level, hot weather, assimilation

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INTRODUCTION

The Cholistan is a desert area located in the province of Punjab, Pakistan, disbursing around almost 16ooo $km^{2.}$ The *Cholistan* desert locally known as '*Rohi'* is a barren desert. It occupies the land of about 16,000 square Kilometer and spreads up to the Thar desert of India. The people of *Rohi* live under harsh conditions and move from place to place in quest of water and feed for their cattle. The average annual rainfall varies between 100-250 mm and average temperature during summer is more than 50°C (Khan, Younas & Asif). Its hot weather makes life difficult for both humans and animals. The exposure of long duration to stressful environmental conditions cause marked physiological changes called acclimatization. The purpose of these changes is to minimize the physiological strain which is produced due to stressors. In human, cardinal changes of heat acclimatization decrease in heart rate and core body temperatures (Wenger & Hardy, 1990).
In mammals, physiological responses to thermal-stress in order to maintain homeostasis include increased sweating and respiratory rate, while decreased heart rate and food intake (Lenis, Cabrera & Morales 2016). Along with these adjustments, a low resting metabolic rate is common physiological adaptation for desert life. Moreover, those physiological adaptations under thermal stress of either short or prolonged duration alter the metabolism of absorbed nutrients (Das, Sailo, Verma, Bharti & Saikia, 2016).

Hypothalamic-pituitary-adrenal axis:

The hypothalamic-pituitary-adrenal axis is a central actor in endocrinology. Through it, the fine-tuning of corticosteroids secretion is maintained, from fetal to adult life, under basal and stressful conditions, with immediate and or long-term consequences (Bertagna 2017). As the endocrine system is involved in coordination of metabolism, it is not surprising that thermal stress results in alteration of hormone level in blood. Hormones involved are prolactin, growth hormone, thyroxine, glucocorticoids, antidiuretic hormone (ADH) and aldosterone. Some of these, such as ADH and aldosterone are associated with homeostatic regulation of specific nutrients i.e water and sodium respectively (Beede & Collier, 1986). It has been reported that different types of stress such as acute illness, trauma due to surgery or accident and shock cause elevation of ACTH and glucocorticoids levels (Hale, Sayers, Sydnor, Sweat & Van Fossan, 1957; Vardas etal, 2014).

It has also been described the suprarenal gland react in a different way in response to numerous demanding conditions in spite of the similar feedback in the hypothalamic-pituitary part of the HPA axis(Koko, Djordjeviæ, Cvijiæ & Davidoviæ, 2004; Pacak & Palkovits 2001). Recently it has been confirmed that sudden exposure of heat stress also disturbs the morphology of suprarenal gland. In rats, acute exposure to high temperature (38°C) for 60 minutes caused a decrease in adrenal gland mass, which was mainly due to fibrosis and reduction of lipid droplets especially in the zona fasciculata (ZF) of the adrenal cortex (Koko, Djordjeviæ, Cvijiæ & Davidoviæ, 2004). In a research, it has been noted that in man, too, the adrenal cortex is affected by continuous exposure to a tropical climate (Sundstroem, 1927)

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Glucocorticoids

Glucocorticoids secreted from the adrenal cortex are steroid in nature and are essential for life, playing an important role in the regulation of cardiovascular, metabolic, immunologic and homeostatic functions. Glucocorticoids inhibit many of the initial events in an inflammatory response. Also profoundly affect the function of immune cells (Coutinho & Chapman, 2011). Most important metabolic functions of glucocorticoids are increased gluconeogenesis and mobilization of amino acids from extrahepatic tissues, as well as stimulation of lipolysis (Stewart, 2003). It has been studied that there is a seasonal decrease in glucocorticoid secretion from winter to summer in animals. However, little information is available regarding the effects of heat stress on the adrenal corticosteroid response in man. The few plasma determinations suggested that acute heat stress increases the level of glucocorticoids in human blood (Vingren, 2016; Collins & Weiner, 1968). In a study conducted in the arid desert revealed that cortisol and ACTH levels were higher in soldiers stationed in a desert area when compared with soldiers working in urban areas (Tao, Zhang, Song, Tang & Liu, 2015).

Our information regarding the influence of environmental temperature on pituitary and adrenal functions is mainly confined to experiments where human or animals were exposed to high environmental temperatures for a short period. Little evidence is available on possible adaptations to desert life in natives who have been living under extremely high environmental temperature and the shortage of water. The present investigation evaluated the pituitary-adrenal functions under chronic hot climatic conditions. We measured serum ACTH, cortisol values in residents of the Cholistan desert and compared these values with those obtained from subjects living in a non-desert area of Pakistan. We tried to find out any adaptive changes in the pituitary-adrenal functions in desert dwellers**.**

HYPOTHESIS: Significant differences exist in the levels of hormones (ACTH and Cortisol) of the pituitary-adrenal axis between subjects living in the desert and non-desert areas.
The objective of this study was: "To assess the pituitary and adrenal cortical functions in natives of Cholistan desert"

MATERIALS & METHODS

The study design was analytical cross-sectional, conducted in *Cholistan* desert and in the Physiology and Cell Biology Department of University of Health Sciences, Lahore. The study was approved by the Ethical Committee of the University of Health Sciences, Lahore. Duration of the study was one year (2008). The sample size **was** one hundred (100), calculated by using the formula;

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$$n = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2 + (\delta_1^2 + \delta_2^2)}{(\mu_1 - \mu_2)^2}$$

Level of Significance = $\alpha = 0.05$ and Power of study = $\beta = 99\%$

Mean Difference between Aldosterone Level = $\mu 1 - \mu 2 = 33.24 - 30.15$, Expected Standard deviation of Aldosterone level in Non Desert Group $\delta 1 = 4.65$ng/100ml and Expected Standard deviation of Aldosterone level in Desert Group $\delta 2 = 5.97$ng/100 ml.[17] Sample technique was convenience and sample size for the study was 100 and divided into two groups of 50 healthy subjects living in desert areas and 50from non-desert areas.

Data Collection methods

Written informed consent was applied before data collection. Demo biographic data was collected. The data about general physical examination such as weight, height, blood pressure were also recorded.

Blood Sample Collection:

Blood samples were drawn from the subjects between 7-8:30 am after an overnight fast of 8-12 hours. Serum was separated from the venous blood immediately to avoid the effect of hemolysis on serum K+ levels. The serum isolated was then transferred to two vials for ACTH and Cortisol level measurements.

Estimation of Serum Cortisol:

The first aliquot of each sample was thawed and serum cortisol values were assessed by using commercially available quantitative immuno-assay kit purchased from DSL, USA. Conferring to the manufacturer's recommendations wash solution was prepared by diluting 100 milliliters of wash concentrate with 900 milliliters of distilled water.

Estimation of serum ACTH Levels by ELISA:

Commercially available ELISA kit for ACTH from DSL, USA was used to find the ACTH levels in the serum samples from the subjects

DATA ANALYSIS

The data was entered and analyzed using SPSS 18. The numeric data like age, aldosterone level, cortisol level, ACTH etc are presented in the form of mean ± SD and the qualitative data like smoking status, diabetic/ hypertensive history etc is presented in the form of frequency and percentages.

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The numeric data satisfying the parametric assumption is analyzed with Student t-test. The qualitative data is analyzed using the Chi-Square test. P-value of less than 0.05 is considered significant.

RESULTS

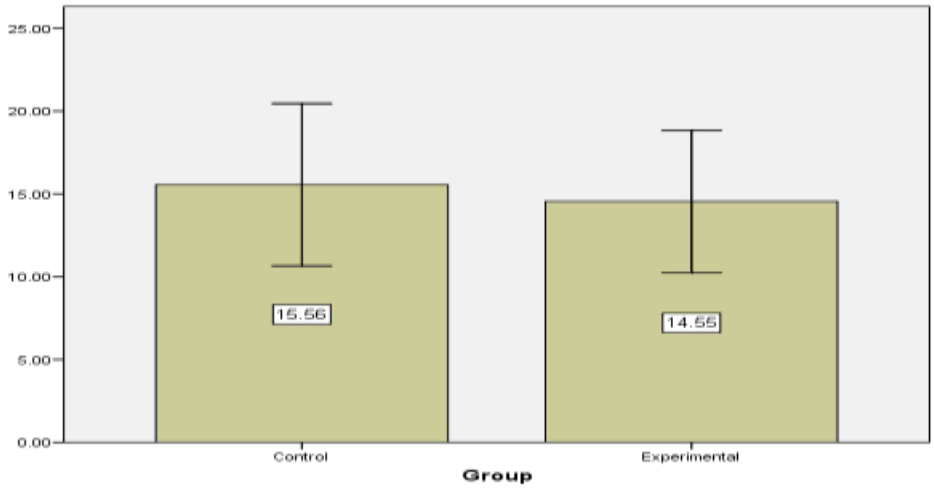
The mean ± SD value for serum cortisol of the exposed and unexposed group was 14.54 ± 4.30 µg/dl and 15.56±4.90 µg/dl respectively. But the difference between the two groups was not statistically significant (p=0.273) as shown in table 02.

Experimental group n = 50	**Control group n = 50**	**p –Values**
11.84 ± 7.70 pg/dl	12.51 ± 8.10 pg/dl	0.66†

Table 1: Comparison of (mean ± SD) serum ACTH concentration in experimental and control group

Experimental group n = 50	**Control group n = 50**	**p –Values**
14.54 ±4.30 µg/dl	15.56 ± 4.90 µg/dl	0.273ł

Table 2: Comparison of (mean± SD) serum cortisol concentration in experimental and control group Fig no 2: Comparison of mean ± SD serum cortisol concentration in exposed and non-exposed groups



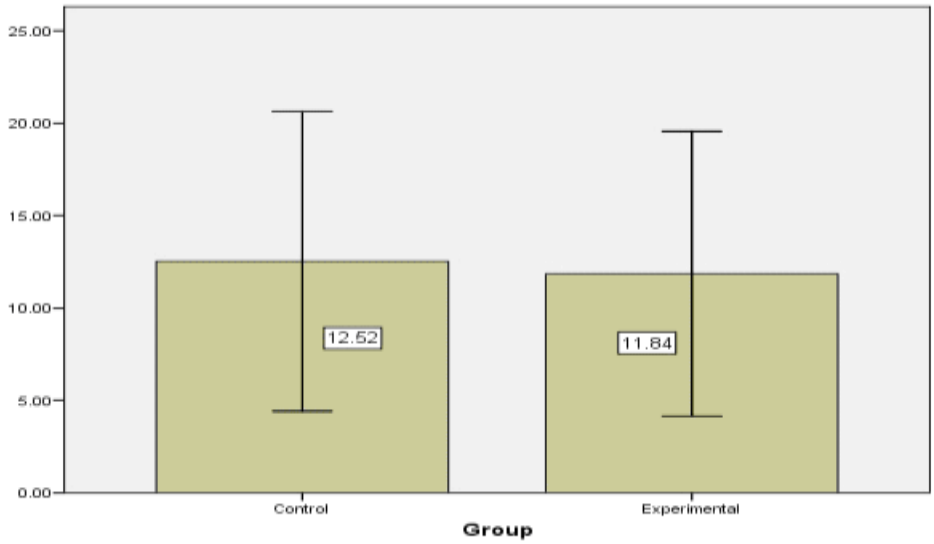


Fig no 4: Comparison of mean ± SD serum ACTH concentration in exposed and unexposed groups

DISCUSSION

The environmental heat present at all levels of biological organization a stress that brings into play in the homeothermic animals a complex of nervous, endocrine, neurohumoral, and motor functions combining to restore a constant body temperature and to adjust body fluid balance, energy metabolism and behavior to the needs concomitant with survival in the new environment. On the physiological responses in the native of deserts, little latest information is available.Very little is known of the effects of heat stress on the adrenal corticosteroid response in man, largely because of the inadequacy of the analytical methods commonly employed in early investigations. The present analytical cross-sectional study which is based on 100 subjects provides normative values of adrenal gland secretions from a sample of Cholistan desert and non-desert area subjects.

The serum ACTH levels were decreased in this study in the exposed group as compared to the unexposed group; although this difference was not statistically significant with a p-value of 0.66. This is also supported by the various studies that have shown that acute exposures to heat have led to an increase in ACTH levels while the chronic acclimatization has been associated with relatively lower levels. A study conducted used the exposure to sauna bath and its effect of various metabolic profiles and hormonal levels. And it was observed that it led to an increased level of ACTH which was significantly higher in cases exposed to hot sauna bath as compared to the unexposed group. Interestingly it was observed that when they underwent continuous exposure to heat in sauna bath, the levels of ACTH, at the 7th bath were lower as compared to the first bath; hence reinforcing the belief that the continuous and longer exposure leads to relative acclimatization and decreased hormonal response(EI-Nouty, Elbanna, Johnson, 1978; Pilch, Szygula, Torii, 2007). This was

further strengthened by the evidence from other studies who also suggest that ACTH and glucocorticoid output are not 34 increased during acclimatization to heat. This supports the data of our cases which are residents of *Cholistan* and hence chronic exposure led to decreased levels of ACTH (Streeten, 1960).

The cortisol hormone values were also comparatively decreased in the exposed group and it was also not statistically important with p= 0.273. Studies on the metabolism of cortisol under hot conditions indicate that the enhanced secretion of glucocorticoids that occur when body temperature is raised above 38°C is also accompanied by an increase in the rate of removal of cortisol from plasma (Collins & Weiner, 1968). Few studies on rats, sheep, and cattle suggest that adrenal glucocorticoid activity is reduced during heat acclimatization and with the seasonal increase in environmental temperature. It has been reported that glucocorticoid concentration was elevated in acute but not during chronic thermal stress (Streeten, 1960; Collier, Beede, Thatcher, Israel & Wilcox, 1982). Reasons for different responses to acute and chronic stress are unknown but may be related to altered adrenal metabolism. Lower glucocorticoid release to ACTH challenge was noted in studies in chronically thermal-stressed animals. However, these animals had much higher progesterone release in response to ACTH than cortisol because progesterone is a precursor of cortisol. The lower glucocorticoid and higher progesterone concentrations in heat-stressed animals may be related to reducing the conversion of progesterone to cortisol (Roman-Ponce, Thatcher, Collier & Wilcox, 1981). In experiment three of five subjects who experienced heat stress in combination with hypoxia in a heated decompression chamber exhibited a significant increase in the concentration of peripheral plasma 17-hydroxycorticosterone levels after three hours of heat exposure (Hale, Sayers, Sydnor, Sweat & Van Fossan, 1957)

Conclusions

There was a statistically non-significant difference in terms of ACTH & cortisol hormone levels in both exposed and unexposed groups. In the present study serum cortisol levels were lower in the exposed group (14.54 ± 4.30 μg/dl) when compared with the unexposed group (15.56 ± 4.90 μg/dl). Similarly, serum ACTH levels were also lower in the exposed group as compared with control. A non-significant difference in hormone levels between two groups might indicate physiological adjustment (acclimatization) in subjects who are living in desert areas.

Limitations of Study

The study is conducted on a relatively smaller number of subjects. In order to impart generalization to results, it is required that the study is conducted on a larger sample size that may reflect the difference in results obtained.

All the study subjects belonged to the rural area. The results may not be projected on the urban population as there are vast differences in terms of literacy levels, about the lifestyle, work habits, food availability, and water supply.

Recommendations

Based on the findings of this study certain recommendations are provided as follows:

Studies on larger sample size are required that can impart generalization to the results over the whole population living in the desert.

Genetic-based studies that can further explore the pituitary, adrenal morphology and functions are suggested. That might explain adaptive changes that occur in subjects living in the desert.

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