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EVALUATION OF OXIDATIVE AND GONADAL AXIS PROFILE IN AGING MALES FROM PUNJAB-PAKISTAN

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ABSTRACT

Fundamental bases of androgen deprivation in the aging male remain unknown despite their recognition biochemically almost 6 decades ago. Epidemiologically the testosterone (Te) deficiency has been associated with impaired quality of life. The main objective of the study is to evaluate the levels MDA, GSH, SOD, total testosterone, SHBG, FSH and LH in healthy aging males from Punjab-Pakistan. The individuals were divided into two groups, Group I from less than 40 years and Group II from more than 40 years. The levels of MDA, GSH and SOD were determined according to their respective protocols, while the levels of TT, FSH, LH and SHBG were measured by human diagnostic ELISA kit methods. The hematological profile was determined by automated analyzer method. The mean values of GSH (0.17±0.005 µmol/L vs. 3.56±0.56 µmol/L) and SOD (0.17±0.005 U/gHb vs. 0.11±0.002 U/gHb) were significantly increase in healthy males with group I (less than <40) as compared to group II (more than >40) respectively. On the other hand, the high level of MDA was observed in healthy male with age group II (4.15±1.26 nmol/ml) as compared to group I (3.015±0.23 nmol/ml). The mean values of SHBG (40.5±4.25 nmol/l vs. 29.0±6.26 nmol/l), LH (26.25±6.65 mIU/ml vs. 3.9±3.26 mIU/ml)

and FSH (76.92±8.15 mIU/ml vs. 2.28±4.56 mIU/ml) were significantly increase in healthy group with age group II (more than >40) as compared to group I (less than <40). While, the increased levels of total testosterone was recorded in-group I (500.12±25.58 ng/dl) as compared to group II (7.14±1.44 ng/dl) of healthy males from Punjab-Pakistan. This study indicated a significant descent in the levels of testosterone with an advancing age. It is further supported by the evidence that the failure in the hypothalamic-pituitary unit in response to decreasing levels of testosterone is the primary defect of advancing age.

Key words: MDA, GSH, SOD, FSH, LH, SHBG

INTRODUCTION

Sexual hormones produced by the gonads, including testosterone in males and estradiol in females, are fundamental hormones responsible for the regulation of development and sexual differentiation. Moreover, they influence many physiological processes not linked specifically to sex, it includes the metabolism of blood glucose and lipids, bone mineral density and mood **[1]**. The concentrations of sex hormones in serum are frequently associated with metabolic disorders as well as with mortality. Aging which is considered as a physiological process related with structural and functional modifications in several organs of the human body and complemented by adaptations in physical activity, behavior, composition of body, energy expenditure and the endocrine environment **[2]**. In males specifically, these adaptations are frequently confused with clinical features known to be initiated by hypogonadism in younger age. Precisely characterizing standard aging is significant for the reason that the potential risks related to the treatment in older males predominantly in context to risks of cardiovascular morbidity as well as mortality were well appreciated. To explore the natural modifications in sex related hormones in aging adult males is a noteworthy part of portraying the regular aging process **[3]**.

A dynamic system of hypothalamic-pituitary-gonadal axis helps in regulating the sexual hormones. Hypothalamic gonadotropin-releasing hormone (GnRH) releases pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH); gonadotropins, comprising LH and FSH, stimulate testicular testosterone (Te) synthesis in males and estradiol (E2) secretion in women. The concentrations of E2 and Te are responsible for negative feedback mechanism on GnRH as well as signaling of LH FSH, resulting in the

formation of a closed loop **[4]**. The fundamental bases of androgen deprivation in the aging male remain unknown despite their recognition biochemically almost 6 decades ago. Epidemiologically the testosterone (Te) deficiency has been associated with impaired quality of life, sarcopenia, skeletal muscle weakness, diminished physical stamina, osteopenia, insulin resistance, erectile dysfunction, systolic hypertension, carotid artery-wall thickness, increased abdominal visceral-fat mass, reduced HDL concentrations, depressive mood, diminished working memory, postprandial somnolence, and last but not least deteriorated executive–cognitive function **[5]**. One meta-analyses show the correlation of low testosterone availability with decreased grip strength, reduced lean-body mass and increased adiposity of viscera, which are markedly overturned by Testosterone supplementation. It is not established yet whether the androgen replacement is beneficial in old aged men without frank hypogonadism. Additionally, the different mechanisms that facilitate the development of hypoandrogenemia in elderly male population are only partially assumed. Reproductive function changes vary during the life process in humans. Immaculate synchronization of the hypothalamic-pituitary-gonadal axis is essential for normal testicular functionality in the males that includes normal production of testosterone and fertility in males.

The biosynthesis of pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) is stimulated by pulsatile secretion of gonadotropin-releasing hormone (GnRH) that, sequentially, sustain testosterone production in gonads leading to the process of spermatogenesis **[6]**. The adequate levels of testosterone control the negative feedback mechanism which in turn is responsible for reduction in hypothalamic GnRH secretion into the portal circulation and release of gonadotropin from the pituitary gland into the bloodstream. Various congenital as well as acquired conditions lead to a failure in synthesis or action of hormones at any level of the axis leading to the development of hypogonadism clinical syndrome. Hypogonadism may also be initiated either by a primary testicular pathology or by a secondary (or central) origin (e.g, a pituitary or hypothalamic disorder). In case of acquired hypogonadism, the use of medications and comorbidities are considered to be the common etiologies of low testosterone levels and must be figured out before making the specific diagnosis **[7]**. Regardless of the etiology, the end organ

replacement therapy while using natural testosterone is recommended for long term use but, with the already known general stipulation that this treatment does not lead to any progress in fertility. Despite the fact, the medications that lead to the stimulation of hypothalamic or pituitary function are successful, these agents have to be consumed for many month intervals to promote spermatogenesis; conversely, their long-term consumption requires further research by lab investigations **[8]**. Besides, various novel therapeutic agents have been in consideration which can stimulate both the testosterone and spermatogenesis. To understand hypo-thalamic-pituitary-gonadal axis physiology is the initial step for the accurate diagnosis and management of hypogonadism, a common disorder distressing the quality of life and a root cause of various other comorbidities together with osteoporosis in males **[9]**.

MATERIALS AND METHODS

The present study is based on a total of hundred male subjects aged 20 to 103 years. Data was obtained from our cross-sectional prospective study which examined the natural alterations in hypothalamic pituitary gonadal axis in aging men in south east Asia to establish normative age trends for serum levels of testosterone, LH, FSH and SHBG to test whether the general health status affected the age trends .The study protocol was approved by Institutional review board and research ethical committee The University of Lahore and sample was collected from Gujranwala division Punjab-Pakistan. The subjects selected were those who do not have any acute or chronic disease effecting hypothalamic pituitary gonadal axis and did not take any exogenous supplements or medication known to affect body growth and metabolism. The subjects with severe communication problem, acute illness and unwillingness to participate were excluded .we excluded participants who were missing Lab or questionnaire data and those who were younger than 18years. The participants were provided with written informed consent prior to data collection.

PHYSICAL MEASUREMENTS AND BLOOD COLLECTION

Clinical and anthropometric measurements were taken and a questionnaire was used to collect information on demographic characteristics, medical history, life style risk factors and smoking history. Body weight, height, head circumference and blood pressure was measured by using standard methods.BMI was calculated as weight in Kg divided by height in meter squares. A 6ml sample was drawn from cubital vein between 9:00am to

1.30pm according to standard protocol with specific tools and methods used, centrifuged within two hours of sample collection. Random blood sugar and complete blood count analysis was performed same day and remaining sample was stored at -80˚C until used. Complete blood count was done by CBC counter Boule Medonic AB manufactured by MERCK SWEDEN. Blood sugar analysis was done by Techno 786 serial no E 113991 manufactured by GMI.

ASSAYS

All assays were performed according to manufacturer's recommendations by skilled technical personal .Total serum testosterone, FSH, LH and SHBG were measured from frozen serum aliquots using competitive chemiluminescence enzyme immunoassay machine Alinity Ci , a compact immunoassay system that maximizes throughput utilizing CHEMIFLEX chemiluminescence technology which is manufactured in Chicago USA. For hormonal assays ABBOTT kits were used for testosterone Lot no [107850P00] and reference no [07D68-22], for LH Lot no[90017UI00] and reference no [7P91-20], for FSH Lot no [91274UI00] and reference no [07P49-30] and for SHBG Lot no[00231L818] with reference no [09P38-20] were used. The results received and entered in excel sheet.

STATISTICAL ANALYSIS

The significance of the difference between the groups was analyzed in SPSS by using ANOVA (F-test)t and the correlation between variables of interest was noted. The p-value < 0.05 was considered statistically significant.

RESULTS

The data analysis in **Table 1, 2** and 03 clearly demonstrate hematological, gonadal hormonal profile and oxidative profile of two age groups. The overall hematological parameter for group age less than 40 years were significantly different from the group with age more than 40 years. Significant differences were recorded between group I and group II. The data analysis in table 01 depicts the hematological profile in healthy population with two different age groups. The mean values of Hb, eosinophils, RBCs, platelets and HCT were significantly decreased in Group I (10.3±0.87 g/dl, 2±0.02 %, 3.2±0.16 million/mm^3, 198±24.11 10^9 /L and 26.6±2.43 mg/dl) as compared to Group II (13.2±1.2 g/dl, 2±0.09 %, 4.6±0.99 million/mm^3, 324±43.21 10^9 /L and 35.3±2.11 mg/dl) respectively. The data interpretation in table 02 explains the significant difference between group I (less than <40) and group II (more than >40). The

mean values of GSH (0.17±0.005 µmol/L vs. 3.56±0.56 µmol/L) and SOD (0.17±0.005 U/gHb vs. 0.11±0.002 U/gHb) were significantly increase in healthy males with group I (less than <40) as compared to group II (more than >40) respectively. On the other hand, the high level of MDA was observed in healthy male with age group II (4.15±1.26 nmol/ml) as compared to group I (3.015±0.23 nmol/ml). The data explanation in **Table 3** signifies the gonadal axis profile of healthy males with two different age groups. The mean values of SHBG (40.5±4.25 nmol/l vs. 29.0±6.26 nmol/l), LH (26.25±6.65 mIU/ml vs. 3.9±3.26 mIU/ml) and FSH (76.92±8.15 mIU/ml vs. 2.28±4.56 mIU/ml) were significantly increase in healthy group with age group II (more than >40) as compared to group I (less than <40). While, the increased levels of total testosterone was recorded in-group I (500.12±25.58 ng/dl) as compared to group II (7.14±1.44 ng/dl) of healthy males from Punjab-Pakistan.

Table 1: Hematological Profile of Healthy Population (Males) From Punjab-Pakistan

VERIABLES	GROUP I Less Than <40	GROUP II More Than >40
Hb (g/dl)	13.2±1.2	10.3±0.87
TLC mg/dl	5±0.89	8.3±0.65
Neutrophils %	49±3.22	63±4.32
Lymphocytes %	46±2.43	21±1.33
Monocytes %	3±0.31	4±0.4
Eosinophils%	2±0.09	2±0.02
RBC (million/mm^3)	4.6±0.99	3.2±0.16
Platelets 10^9 /L	324±43.21	198±24.11
HCT mg/dl	35.3±2.11	26.6±2.43
MCV mg/dl	75.3±5.87	81.7±5.44
MCH mg/dl	28.1±2.9	32.1±1.76
MCHC mg/dl	37.3±3.11	39.3±2.76
SBP (mmHg)	127±10	140±12
DBP (mmHg)	83±7	85±9.01
BMI (m2)	17.90±2.11	21.91±1.23

Table 2: Antioxidative Profile of Healthy Population (Males) From Punjab-Pakistan

VERIABLES	Group I less than <40	Group II more than >40	*P value*
GSH (µmol/L)	4.51±1.07	3.56±0.56	0.437
SOD (U/gHb)	0.17±0.005	0.11±0.002	0.393
MDA (nmol/ml)	3.015±0.23	4.15±1.26	0.121

Table 3: Gonadal Axis Profile of Healthy Population (Males) From Punjab-Pakistan

VERIABLES	Group I less than <40	Group II more than >40	*P value*
TTeng/dl	500.12±25.58	7.14±1.44	0.123
SHBG nmol/l	29.0±6.26	40.5±4.25	0.315
LH mIU/ml	3.9±3.26	26.25±6.65	0.245
FSH mIU/ml	2.28±4.56	76.92±8.15	0.015

DISCUSSION

Aging is a condition with progressive decline in normal physiological functions of organs that is accompanied with development of age related comorbidities. Aging is manifested depletion of anabolic hormones and increased fragility of body. The bases of aging remain unidentified, perhaps being related to a multifactorial process **[10]**. Current study analyzed the hematological profile of elderly men with age greater than 40 selected without known comorbidities and compared them with a group of young adults that were also healthy. In this study mild decrease in hemoglobin levels was observed in the old age group. Statistically the Hb levels were different but this did not have clinical significance and so it was not identified as an aging–related hematological abnormality **[11]**. Study by Yamada *et al* indicated a physiological reduction in normal hematologic profile as age increases. Study by Nilsson–Ehle also demonstrated a decline in Hb level in elderly, more so among men as compared to women **[12]**. Significant difference between differential and total leucocyte count between apparently healthy elderly males and young adults was observed in this study and this study was concurrent with the findings of Nilsson *et al*. **[13]** stated that differences in WBCs counts defined in some reports may be accredited to the individual selection criteria used. In the current study, mean values of MCV and MCHC were higher in the elderly men compared to young, while HCT was lower. A tendency of increased values of MCV was reported in another study. They found a tendency of higher levels of MCV and MCHC in the old individuals **[14]**. Another study comparing hematological indexes between adults and elderly individuals showed significant differences in MCV, MCHC and RDW, with MCV and RDW being higher **[15]**. The blood results regarding age related variation in antioxidant activities of enzymes are still under debate. In current study, there seems no significant differences in the oxidative stress biomarkers and antioxidants between two age group. The physical activity lowers the lipid peroxidation caused due to ROS and improves antioxidant defenses in both the adult and aged individuals **[16]**. The increased aged people that are physically active have been shown to have antioxidant and oxidative stress activity similar to the young inactive individuals highlighting the significance of regular physical exercise to minimize the effects of impairment process related to age **[17]**. Mecocci *et al* **[18]** demonstrated that erythrocytic activity of

SOD augmented with the age and was significantly raised in 81 to 99- years old people groups in comparison with the younger groups having age less than 60 or 61 to 80 years. Besides this, the activity of SOD and GPx were similar in different age groups as report by Polidori *et al* **[19]**. The elder male that are physically inactive practiced oxidative stress induced adaptations. The adaptive increase in the antioxidant activities was insufficient to prevent the oxidative stress, because in older inactive group, the biomarkers of oxidative stress including isoprostanes and protein carbonyls were raised as compared to respective younger old group while the MDA concentration is almost equal that contradicts the finding of Ozbay and Dulger, 2002 examining men and women with the age ranging from 9 to 71 years showing the levels of MDA increasing with age **[20]**.

CONCLUSION

This study indicated a significant descent in the levels of testosterone with an advancing age. It is further supported by the evidence that the failure in the hypothalamic-pituitary unit in response to decreasing levels of testosterone is the primary defect of advancing age. Moreover, the increased sensitivity of negative feedback of testosterone has also been noticed. Different analysis also indicated the increased levels of LH and FSH with aging and almost half of the subjects were presented with the elevated levels of FSH at the end of the study. These results confirmed the failure of sertoli cells along with the decrease in inhibin thus inhibiting FSH secretion. Gonadotropins such as LH and FSH both are secreted by the action of gonadotropin releasing hormone, hence, they are correlated with each other. The decreasing levels of testosterone associated with aging fails to increase LH secretions properly. This study postulated the statement that older men are more likely to develop secondary hypogonadism because of impaired function of hypothalamus and pituitary gland. Several interventional studies suggested a decrease in upper body strength, change in bone metabolism and decrease in hematocrit as functional consequences of gonadal failure. However, the cross sectional study by field et al reported the elevated levels of SHBG along with the levels of free and bioavailable testosterone that were found even more decreased as compared with the total testosterone levels.

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