AGE-RELATED CHANGES IN HYPOTHALAMIC PITUITARY GONADAL AXIS IN MALES OF CENTRAL PUNJAB

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ABSTRACT

In males, there are alterations at all levels of the hypothalamic-pituitary-gonadal axis as a result of aging, including changes in GnRH pulse generator, the release of gonadotropins by anterior pituitary and steroidogenesis by the testis. There is a decrease in circulating testosterone levels with advancing age as a result of changes in feed-forward and feedback mechanisms involved in hormone synthesis of the HPG axis. The objective of the research is to study age-related trajectories of TT, FT, BAT, FSH, LH, SHBG in aging males of Central Punjab.A total of 98 subjects were selected aged 18 to 103 years. Subjects were divided into four age groups. Group I from 18 to 37.9 years, group II from 38 to 57.9 years group III from 58 to77.9 years, group IV is 78years onwards. Subjects with acute or chronic illness affecting the hypothalamic-pituitary-gonadal axis or taking any medication were excluded. Samples were drawn from the cubital vein. The serum was separated by centrifugation within two hours of sample collection.TT, SHBG, LH, FSH in the serum were analyzed.Collected data were entered and analyzed using IBM-SPSS (statistical package for social sciences), version 20. One way ANOVA test was used to compare variables between four groups.There was a highly significant difference between FT and BAT

between the four groups with a p-value of 0.000. The results for SHBG, LH, FSH are also significant with p-value < 0.005. These results strongly support the hypothesis that there is a progressive decrease in serum-free testosterone and bioavailable testosterone level with aging and progressive increase in SHBG, LH, and FSH. The TT shows a declining trend with advancing age.It is clear from the study that male reproductive hormones decline with an increasing age.

INTRODUCTION

Age is an issue of mind over matter, if you do 'not mind it doesn't matter. The population of world is progressing rapidly and the fact can be believed in instance of both long life expectancy and declining fertility rates. Survey of the World Health Organization (WHO) expects the population of the world will exceed double or more in over 60 years of time period, between 2015 and 2050 [1]. Aging comprises a set of physiological, biological, and psychosocial processes both independent and interactive. These changes result in inexorable loss of function that leads to frailty, increased vulnerability to disease, and disability. Testosterone the key sex steroid hormone of the male regulates sexual differentiation and development. In males, there is no abrupt decline in reproductive physiology with age. There is a noticeable linear decline in plasma testosterone levels and sperm count above the age of 40 years. With increasing age, there is an increase in SHBG, FSH, LH levels. Healthy men may remain sexually active and may reproduce even at the age of 80 years [2]. A condition defined as andropause brought about in a male's body at the age of forty years and above when they experience loss of physical agility, changes in mood and attitude, and sex drive [3].

There is an epidemiological relation between hyperandrogenemia and pathophysiology of osteopenia, sarcopenia, muscle weakness, erectile dysfunction, carotid artery wall thickness, insulin resistance, systolic hypertension, low HDL concentration, abdominal visceral fat deposition, depressive mood, decreased cognitive and executive function diminished working memory and impaired quality of life. In the human body, there are structural and functional changes in many organ systems with the passage of age. There is an adaptation of the human body to these changes like behavior, physical activity,

energy expenditure, and body composition **[4]**. Our aim is actually to evaluate the normal aging process as there are potential risks of cardiovascular mortality associated with the treatment of various pathophysiological conditions in the elderly. In aging adults exploring the natural changes in sex, steroids are very important for the characterization of the normal aging process. We initiated an across-sectional survey of 98 subjects selected randomly, aged between 18 to 103 years from central Punjab (a province of Pakistan) in April 2019. Currently, population of people over the age of 60 years are expected to be somewhere around 12 million and it is expected to increase to much larger ratio by 2050 **[5]**. The elderly population puts escalating demands on the quality of healthcare resources. The declining level of physical, cognitive, and neuromuscular function and comorbidities may severely curtail life quality and associated disability leads to dependency in the elderly. In case of testicular dysfunction levels of testosterone decreases under the action of hypothalamic-pituitary-gonadal axis **[6]**. In the aging male, there is decreased central responsiveness of the HPG axis. There are direct consequences of declining testosterone on quality of life, general health status cognitive and physical function with

advancing age. There are three basic subdivisions of male reproductive function including spermatogenesis, male sexual act performance, and secretion of the various hormone. There are number of reproduction associated dysfunctions that can be observed among the population that includes effects of male sex hormones over the growth, cellular metabolism, sexual organs and other motor-associated functions of the body. The male reproductive system is comprised of genital ducts, glands, pair of testis and penis. The physiology of the male reproductive system is controlled by testosterone and its metabolite dihydrotestosterone (DHT) **[7]**. Testosterone controls the gonadotropin secretion from anterior pituitary necessary for spermatogenesis and differentiation of sex during fetal and embryonic development. Dihydrotestosterone (DHT) is believed to act over number of reproductive organs and related tissues during the period of maturation especially puberty causes changes in physiological patterns including hair growth, broadening of muscles etc. The genital ducts and accessory glands produce secretions and aided by smooth muscle contractions, propel spermatozoa towards the exterior. Spermatozoa get their nutrition from these secretions **[8]**. Hypothalamus is the part of diencephalon present in the brain,

weighing only about 4 grams lies between optic chiasm and caudal part of mammillary bodies. Hypothalamic releasing and inhibitory factors are released by the nerve ending in the median eminence and then transported to the anterior pituitary gland. The neuronal cell bodies that give axons to median eminence are located in discrete areas of the hypothalamus [9]. Hypothalamic releasing factors include thyrotrophin-releasing hormone (TRH), said hormone is a peptide comprised of three amino acids which stimulates the release of thyroid stimulating hormone (TSH). While, Gonadotropin-releasing hormone (GnRH) is a single chain of ten amino acids that stimulates follicular stimulating hormone (FSH0 and luteinizing hormone (LH). Corticotrophin-releasing hormone(CRH) which is a single chain of 41 amino acids, stimulates the release of ACTH by the corticotropes. Growth hormone-releasing hormone (GHRH) is a single chain of forty-four amino acids that stimulates the somatotropes to release growth hormone. The inhibitory hormones include somatostatin a single chain of 14 amino acids, it inhibits the release of GH from somatotropes. Prolactin inhibitory hormone is Dopamine it acts on lactotrophs and inhibits the release of prolactin [10].

MATERIAL AND METHODS
SOURCE OF DATA

The present study is based on a total of ninety-eight male subjects 18 to 103 years of age, randomly selected from a population of central Punjab. The study protocol was approved by the ethical committee of the University of Lahore. The sample collection was conducted with the permission of the Principal of Gujranwala Medical College and its teaching affiliate, the DHQ Hospital Gujranwala. Subjects with any chronic or infectious disease (including chronic liver and thyroid diseases) or on long term medication such as steroids, were excluded from the study. The subjects with severe communication problems or intellectual disabilities were also excluded. Signed written informed consent was obtained in each case. The subject's family and medical history were recorded in a specifically designed form.

STUDY DESIGN

This is a prospective cross sectional study. Subjects were grouped according to age, as follows:

Group I: 18 to 37.9 years
Group II: 38 to 57.9 years
Group III: 58 to 77.9 years
Group IV: 78 or >78 years

PHYSICAL MEASUREMENTS AND BLOOD COLLECTION

Clinical and anthropometric measurements were taken and a questionnaire was used to collect data relating to their medical history, lifestyle demographic factors and other possible risk factors. Bodyweight, height, head circumference, and blood pressure was measured by using standard methods. BMI was calculated as weight in kg divided by height in m^2. Six ml sample was drawn from the cubital vein between 9:00 am and 12.00 noon and was centrifuged a 4000 rpm for 10 minutes to separate serum. Random blood sugar and complete blood count analysis were performed the same day and the remaining sample was stored at -80°C until used for hormone analysis. Complete blood count was performed by the CBC counter (Boule Medonic AB, Merck, Sweden). Blood glucose was estimated by using an automated system (Techno 786. GMI, serial no E 113991).

HORMONE ANALYSIS

All assays were performed according to the manufacturer's recommendations by skilled technical personnel. Serum FSH, LH, testosterone, and SHBG were measured by competitive chemiluminescence enzyme immunoassays using Alinity Ci (Abbott Diagnostics, Chicago, IL, 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 [0231L818] 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 (t-test) and the correlation between variables of interest was noted. The p-value < 0.05 was considered statistically significant. All calculations were performed on IBM, SPSS version 20. For each assay FT and BAT, levels were calculated from values of TT and SHBG according to the method described by **[11]** using computer programs available at the website www.issam.ch/freetesto.htm.

RESULTS

DETERMINATION OF PHYSICAL AND CLINICAL CHARACTERISTICS

The physical characteristics, random blood glucose and hemoglobin levels in the four age groups of subject have shown in figure 01. The mean BMI in groups I, III, and IV was ≤25 (normal range) whereas, in Group II, mean BMI (28.1±1.6) of subjects showed a tendency towards overweight.

Mean blood pressure values in all the four age groups were within the normal limits in relation to the age of subjects. As expected, with advancing age the blood pressure showed a gradual increase from 119/80 in Group I to 138/85. However, in the most aged group of subjects, (Group IV) mean systolic and diastolic blood pressure recorded a slight drop compared to that of Group III. The average blood glucose levels (random) were minimal (77±3 mg/dl) and maximal (151±19 mg/dl) in subjects included in age Groups I and III, respectively. Age-related changes in blood hemoglobin were unremarkable - the mean values for the four age groups, varied from 11.7±0.3 to 13.0±0.3 g/dl.

## MEASUREMENT OF AGE-RELATED CHANGES IN CIRCULATING GONADOTROPINS AND ANDROGENS

Mean serum FSH and LH concentrations in the four age groups are given in figure 01. Levels of both gonadotropins showed an increase with advancing age. A discernible rise in FSH levels was already evident in Group II and mean FSH levels increased by almost 3-fold in subjects of age Group IV compared with the mean concentrations in the youngest group of individuals (Group I) (16.3±2.8 *vs* 5.9±1.6 mlU/ml, respectively).

Mean serum LH levels first tended to increase in Group III and were 2-fold in Group IV compared to corresponding mean levels in 18 to 37.9-year-old subjects (Group I).

ANALYSIS OF AGE-RELATED CHANGES IN ANDROGENS AND SEX HORMONE-BINDING GLOBULIN (SHBG)

Serum testosterone and steroid hormone-binding globulin (SHBG) concentrations are summarized in figure 01.Circulating total testosterone (TT) levels concerning age were unremarkable although mean TT levels in the four age groups exhibited a discernible decrement with advancing age (415.5 vs 323.2 in Groups I and IV, respectively). On the other hand, sex hormone-binding globulin (SHBG) levels showed a steady rise across the four age groups. Consequently, a significant decline in bioavailable testosterone (BAT) and free testosterone (FT) were registered with advancing age. A significant decrement in of BAT and FT were recorded in age Groups II and III. Furthermore, a pronounced decline in the levels of BAT and FT were reported in subjects of 80 year or more of age, presumably not only due to increased SHBG levels but also owing to lower serum TT secretion.

A — BMI

B — RBG

C — Total Testosterone
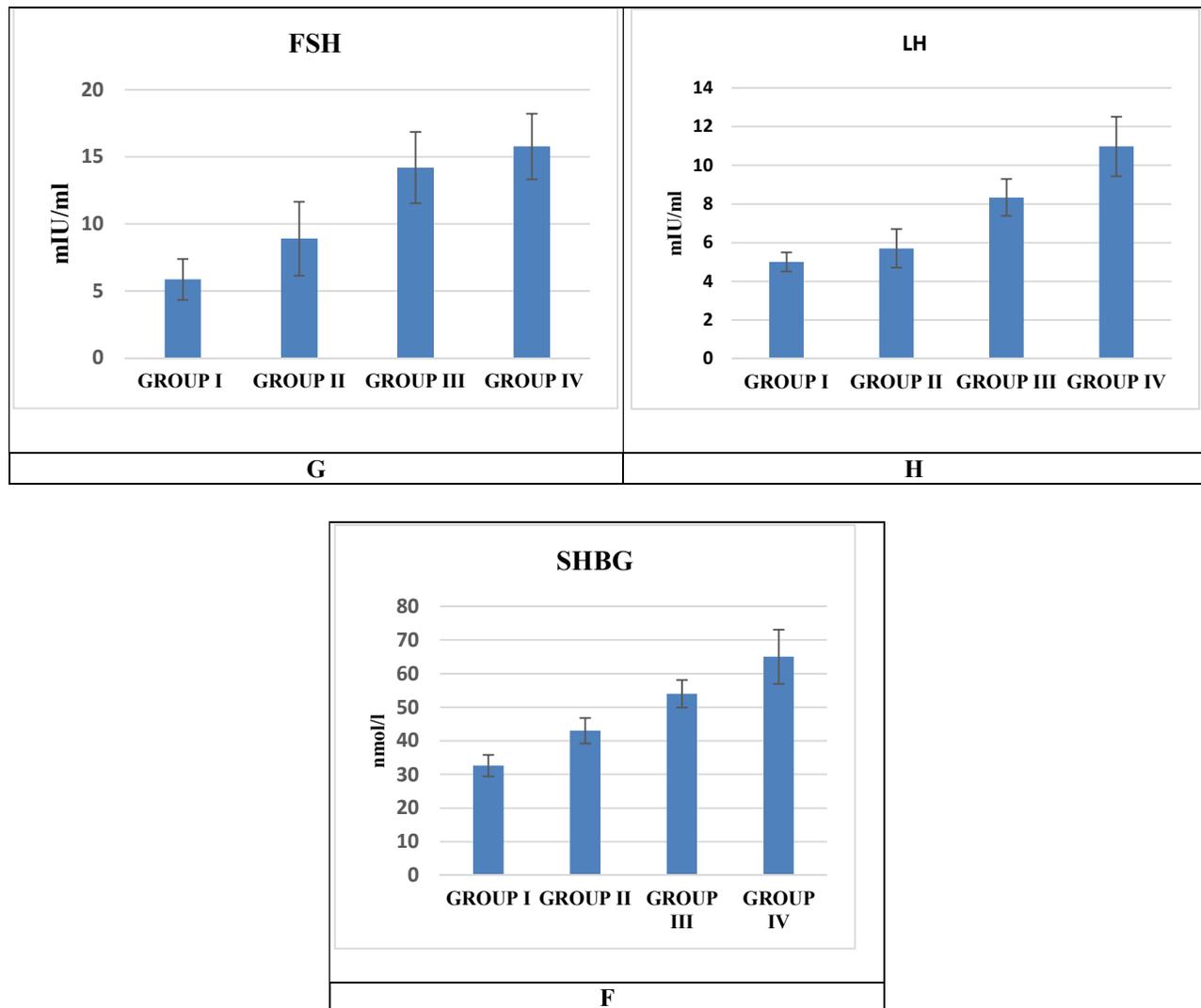
D — HB

E — FT

F — BAT
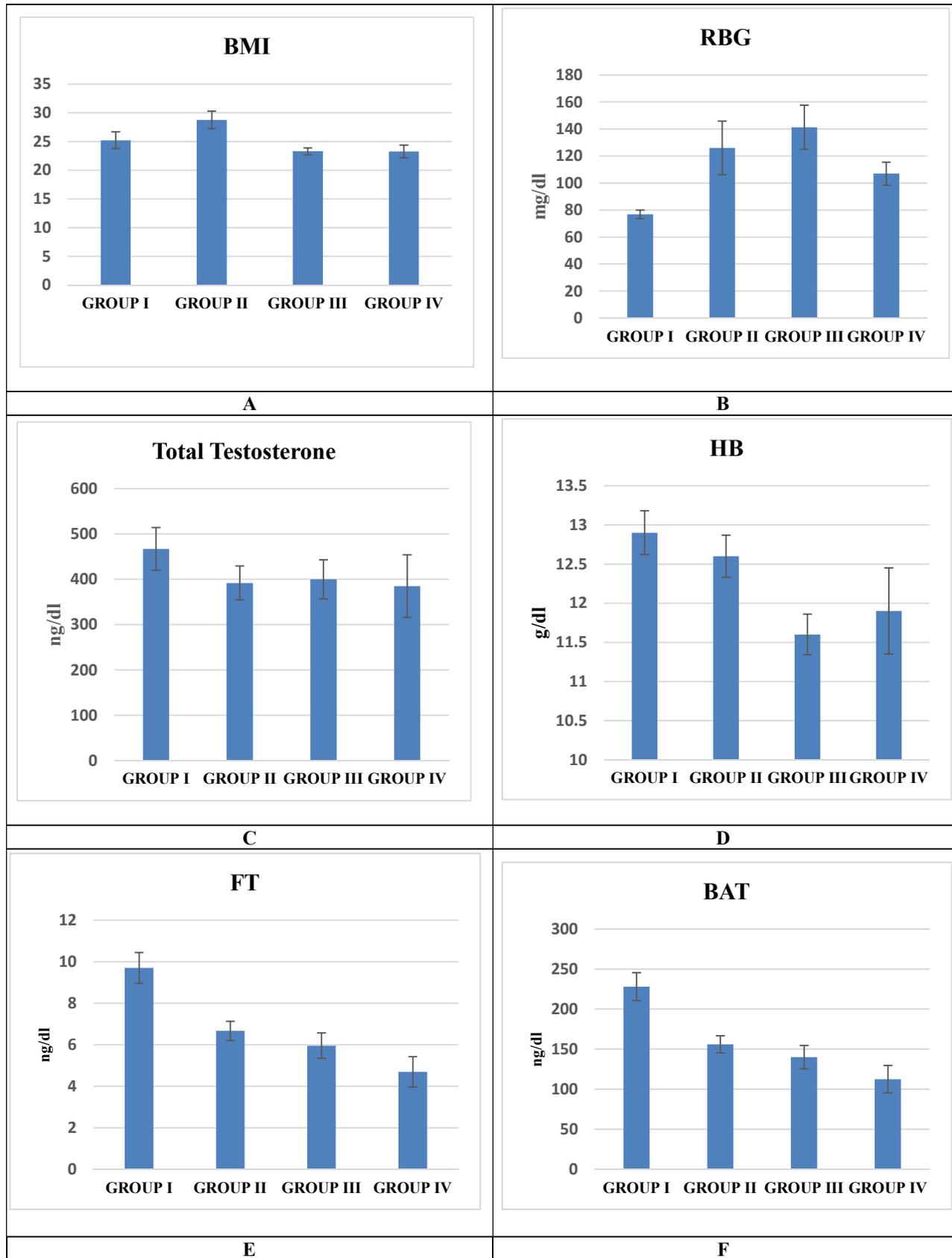
Figure 1: Graphic Representation of BMI, RBG, Hb and Sex Hormones of Aging Male

DISCUSSION

Aging is generally accompanied by a gradual loss of all physiological functions, which is reflected by a diminished feeling of well-being, reduced muscle mass and strength, decreased sexual activity, and weak cognitive functions [12]. This study provides an extensive exploration of age-related changes in the hypothalamic-pituitary-gonadal axis in aging males of the local population of Central Punjab. We divided the subjects into four groups based on an increasing age. All the biochemical and physical variables were compared between four groups and the trend of each variable with increasing age was studied. The present study depicts a decrease in body weight and BMI after the age of 58 in males. Different studies have shown a significant relationship of low testosterone with body composition

[13]. However, the exact mechanism of its link is still unclear. The effects of testosterone on mesenchymal stem cell differentiation regulation, muscle protein synthesis through activation of inflammatory pathways, androgen receptor-mediated pathways are the possible biological mechanisms that can justify the level of sex hormones and their effects on body composition with age **[14]**. The Framingham Offspring Study reported the association of low free testosterone levels and greater risk of mobility limitation in older men **[15]**. An interventional data demonstrated that the administration of testosterone improves body composition, including increasing lean mass and decreasing fat mass **[16]**. However, the evidence in support of testosterone, improving muscle strength and physical function is limited and remains controversial. An increase in blood pressure is an inevitable consequence of aging in modern society leading to hypertension **[17]**. The present study demonstrated an increase in blood pressure with age. It cannot be explained physiologically because we selected 'normotensive' subjects in our study. It is stated in a study that it can be due to structural changes in the arteries and the large artery stiffness **[18]**. Moreover, the depression in renin-angiotensin system, and depression in baroreflex sensitivity accompanied by aging can contribute in overall increase in blood pressure and heart health. In the data of Framingham Heart Study, systolic blood pressure shows a continuous rise during the age of 30 and 84 or above. However, the diastolic readings were inconsistent and showed varying values in aging **[19]**. Another study has suggested the testosterone direct effects on cardiac muscles and vascular reactivity. Furthermore, several cross-sectional studies have shown an inverse correlation between sex steroids and cardiovascular diseases **[20]**.

This study also shows a gradual decrease in hemoglobin with the subsequent increase in age. There is evidence that declining testosterone with increasing age influences erythropoiesis negatively. There is testosterone-induced increase in hemoglobin and hematocrit which is associated with the stimulation of erythropoiesis and reduced ferritin and hepcidin concentrations **[21]**. The people of old age groupare an important demographic population with a very high prevalence of anemia. However, the analysis of two databases (NHANES-III), third U.S National Health and Nutrition Examination survey found that normal values of hemoglobin remain unchanged for the aging population. WHO defined anemia as Hb<

13g/dl in males **[22]**. Causes of anemia in older individuals includes chronic inflammatory diseases, chronic renal insufficiency, and myelodysplastic disorders.

It is submitted by multiple studies that when no specific underlying cause of anemia is detected, in older individuals, along with decreased levels of TT in blood. In this case, low Hb was termed as unexplained anemia which improved with testosterone administration **[23]**.

CONCLUSION

The hormonal behavior of the male reproductive system is very complex and tedious to understand. There are multiple hormones of HPT axis interacts with one and other to initiate specific feedbacks that are involved in the disease progression. Based on this and other studies, a small and progressive decline in several sex hormones has been reported, in particular, testosterone, free testosterone, and BAT. Moreover, there is a subsequent increase in FSH, LH, and SHBG in the aging male. The importance of these changes is wide-ranging because of the predominant role of sex hormones in male physiology. Age-related decrease in male sex hormones has a direct effect on physical or cognitive function and overall quality of life.

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