Andropause phenomenon by measurement of serum free testosterone concentration in Iraqi healthy men

Age related study

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ABSTRACT

الأهداف: دراسة ظاهرة إياس الذكور في الأفراد الأصحاء في العراق عن طريق تقييم تركيز هرمون تستوستيرون وعلاقته مع العمر.

الطريقة: أجريت دراسة مقطية في قسم الكيمياء الحيوية، كلية الطب، جامعة بغداد خلال الفترة من فبراير 2012م حتى اكتوبر 2012م. اشتملت الدراسة على 251 فرد عراقي تتراوح أعمارهم من 82–20 عام. وتم تقسيم الأفراد إلى المراحل العمرية التالية مجموعة الأولى (40–20) عام العدد=16، المجموعة الثانية (60–40) عام العدد=165، المجموعة الثالثة (82–60) عام العدد=70، مجموعة A–I (أكبر من 50 عام، العدد=137)، ومجوعة A–I I (أكبر من 50 عام، العدد=114)، ومجموعة B–I (أقل من 60 عام، العدد=215) ومجموعة B–II (أكبر من 60 عام، العدد=36). كما تم قياس تركيز هرمون تستوستيرون باستخدام تقنية أليزا.

النتائج: أظهرت الدراسة ارتباط إحصائي عكسي بين تركيز هرمون تستوستيرون والعمر لدى أفراد الدراسة (p=0.0001, r= -0.231). انخفض معدل تركيز هرمون تستوستيرون في المجموعة الثالثة وذلك عند مقارنتهم بالمجموعة الأولى (p<0.009) والمجموعة الثانية (p=0.031) ومجموعة BII من مجموعة BI (p=0.043).

خاتمة: أظهرت الدراسة انخفاض مستوى تركيز هرمون تستوستيرون كما أن التحليلات لظاهرة إياس الذكور لدى الأشخاص الصحيين وتغيرات العمر المصاحبة تنخفض بشكل إحصائي في عمر 60 عام وأكثر.

Objectives: To investigate the andropause phenomenon in Iraqi healthy subjects by evaluating serum free testosterone (FT) concentrations in association with age.

Methods: This study was carried out at the Biochemistry Department, College of Medicine, University of Baghdad, between February 2012 and October 2012. This cross sectional control subject's study included 251 healthy Iraqi men with an age range of 20-82 years. Subjects were divided into variant age groups, group 1 (20-40 years, n=16), group 2 (40-60 years, n=165) and group 3 (60-82 years, n=70), group A-I (<50 years, n=137) and group A-II (≥50 years, n=114), and group B-I (<60 years, n=215) and group B-II (≥60 years, n=36). Serum FT concentrations were measured by using enzyme-linked immunosorbent assay technique.

Results: The results revealed significant negative correlation between serum FT concentrations and the age values of the studied subjects (r= -0.231, p=0.0001). The mean (±SEM) value of serum FT concentrations was significantly decreased in group 3 when compared with that of group 1 (p<0.009) and group 2 (p=0.031) as well as in group BII than in group BI (p=0.043).

Conclusions: This study found significant decline in serum FT level, the gold test for andropause phenomenon, in healthy male subjects in age-related changes and the cutoff at which such significant decrease occurred is at 60 years of age and above.

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Testosterone is the main androgen secreted by the Leydig cells of the testes, and its production increases during puberty. Testosterone circulates in plasma either free (approximately 2-3%) or bound to plasma proteins. The androgen binding proteins involved mainly the specific sex hormone-binding globulin (SHBG) and nonspecific proteins such as albumin and prealbumin. Testosterone contributes to development and maintenance of male secondary sex characteristics,

muscle bulk, bone mass, libido, and sexual performance in men.[1] Andropause or 'male menopause' has gained significant attention more recently. Andropause means the decrease in gonadal function in males with advancing age and is revealed by slow but steady reduction of the serum testosterone concentration. It is often referred to as late onset hypogonadism, male menopause, male climacteric andropause or viripause.[2] As men age, there is a gradual reduction in the serum levels of androgen hormones, mainly the testosterone and may experience decreased libido, with or without sexual dysfunction, as well as low muscle strength, psychological changes, and increased risk of osteoporosis.[3,4] The reproductive changes that occur in the aging male are more subtle and prolonged throughout many years of mature life than the profound modifications in gonadal function that occur in women.[5] With advancing age, the male reproductive capability is preserved (namely older men can still father a child), even though there is a steady decline in sexual performance capacity as well as the libido.[2] Nonetheless, the phenomenon of andropause remains controversial, in part because of difficulty in differentiating the effects of age-related confounding variables, such as obesity, medication intake, acute and chronic illness, from aging per se, on the hand. In addition, most studies on andropause phenomenon have been conducted in Caucasian populations, and data from other ethnicities are very few or even lack.[6,7] Moreover, it has been stated that measurement of free (or bio-available) testosterone levels are currently considered the gold standard and the most accurate indicator of androgenicity and hence the preferred laboratory test in the diagnosis of andropause.[1,8] There is no previous studies that investigate the androgenicity in Iraq and the around Arabic countries. Therefore, the aim of this study is to investigate the andropause phenomenon or age-related changes of serum free testosterone (FT) concentrations and to define a cut-off of age for the diagnosis of androgen deficiency in healthy Iraqi men.

Methods. This cross-sectional healthy male subjects study was conducted in the Biochemistry Department, College of Medicine, University of Baghdad, and in the Teaching Laboratories, Baghdad Hospital, Baghdad, Iraq, between February 2012 and October 2012. It included 251 healthy Iraqi men recruited from different regions of Iraq with an age range of 20-82 years.

The exclusion criteria included those subjects who had primary and secondary causes of infertility, ischemic heart complications, diabetes mellitus, hyperlipidemia, androgens and glucocorticoides drugs intake, renal dysfunction, and obesity. Formal consent was taken from each subject. We received ethical approval from the Scientific Committee of the Biochemistry Department, College of Medicine, University of Baghdad, Baghdad Iraq.

Men were divided into 3 groups depending on their age: Group 1 (age range 20-40 years, n=16), Group 2 (40-60 years, n=165), and Group 3 (60-82 years, n=70). Moreover, men were subdivided into: Group AI, (<50 years, n=137) and Group AII (≥50 year, n=114) as well as Group BI (<60 year, n=215) and Group BII (≥60 year, n=36). Serum investigation involved measurement of FT concentration between 8-9 am, in all enrolled subjects using enzyme-linked immunosorbent assay (ELISA) technique.[9] Free testosterone kit was provided by Demeditec Diagnostics GmbH, Lise-Meitner-StraBe 2, D-24145 Kiel, Germany. The Demeditec Free Testosterone ELISA kit is a solid phase enzyme-linked immunosorbent assay, based on the principle of competitive binding. The ELISA study was performed using Biotek Instrument, Highland Park, USA. The body mass index (BMI) was also measured by equation of kg/m^2.

We used the Statistical Package for Social Sciences (SPSS Inc., Chicago IL, USA) version 15, and Minitab analysis programs (Minitab Inc, version 15, PA, USA) for all statistical studies. We used Analysis of Variance and Student's t-tests to test for statistical significance. Linear regression was utilized to test for correlation between different studied parameters, and the significance of the r-value was assessed by related t-test. P-values of less than 0.05 were considered significant.

Results. This study found that 90 Iraqi men out of the 251 studied subjects had serum FT concentrations below the cutoff value of 5 ng/ml (35%; 90/251 subjects). Important results of this study was the significant negative correlation between serum FT concentrations and age values of the entire studied subjects (r= -0.231, p=0.0001) and Group 2 (r= -0.159, p=0.041).

Table 1 shows the mean (±SEM) values of serum FT concentrations of Group 1 (20-40 years), Group 2 (40-60 years), and Group 3 (60-82 years). The mean

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Table 1 - The mean (±SEM) values of age and serum free testosterone concentrations in groups 1-3.

Parameter	Group 1 (20-40 years) n=16	Group 2 (40-60 years) n=165	Group 3 (60-82 years) n=70	*P*-value Group 3 & Group 1	*P*-value Group 3 & Group 2
Age (years)	30.63±1.38	47.30±0.45	68.0±0.77		
Free testosterone (ng/ml)	6.84±1.19	5.22 ±0.32[NS]	3.92±0.38	0.009	0.031

NS - No significant difference between group 2 and group 1 in serum FT concentration (p=0.207).

Table 2 - The mean (±SEM) values of age and serum free testosterone concentrations of group AI, group AII, group BI, and group BII.

Parameter	Group AI (<50 years) n=137	Group AII (≥50 years) n=114	*P*-value	Group BI (<60 years) n=215	Group BII (≥60 years) n=36	*P*-value
Age (years)	42.86±0.5	63.0±0.77		48.47±0.63	73.17±0.75	
Free testosterone (ng/ml)	5.39±0.37	4.45±0.32	0.056	5.13±0.28	3.96±0.49	0.043

values of age were 30.63±1.38 years for Group 1, (47.30±0.45 years), Group 2, and (68.0±0.77 years) Group 3. The mean (±SEM) value of serum FT concentrations of Group 3 (3.92±0.38 ng/ml) was significantly decreased compared with that of Group 1 (6.84±1.19 ng/ml, p=0.009) and Group 2 (5.22±0.32 ng/ml, p=0.031). However, there was no significant difference in serum FT concentration between Group 2 and Group 1(p=0.207).

Table 2 reveals the mean (±SEM) value of serum FT levels of Group AI (<50 years) and Group AII (≥50 years) as well as Group BI (<60 years) and Group BII (≥60 years). The mean (±SEM) value of serum FT concentrations of Group BII (3.96±0.49 ng/ml) was significantly lower in comparison with that of Group BI (5.13±0.28 ng/ml; p=0.043). The results also reveal that the mean value of serum FT levels of Group AII (4.45±0.32 ng/ml) was on borderline significant decrease compared with that of Group AI (5.39±0.37 ng/ml; p=0.056).

Discussion. The occurrence of andropause phenomenon as measured by decrease of serum FT level below 5 ng/ml, in age related manner in healthy Iraqi men was 35% (90/251). This percentage is higher than that found in healthy Brazilian men in whom 41 men (19%) were diagnosed with andropause phenomenon by laboratory criteria (calculated FT)[10] and to aging Taiwanese men in whom the prevalence of androgen deficiency was 24.1% based on the criterion of total testosterone (TT) and 16.6% based on the criterion of both TT and FT.[6] The latter authors stated that older age, obesity, and diabetes mellitus (DM) were independent risk factors for androgen deficiency and both obesity and DM should be prevented to maintain normal testosterone levels during aging in men.[6] Tenover[11] reported that approximately 30% of men in their 60 year, and more than 80% of men over 80 year may have a low FT index. The present study revealed significant decline in serum FT levels in Iraqi subjects aged 60-82 years compared with both those aged 40-60 years, and 20-40 years. Moreover, Iraqi subjects aged more than 60 years showed significant decline in their serum FT levels compared to those aged less than 60 years. Li et al[12] found that the levels of calculated FT gradually decline with aging and the prevalence of androgen deficiency was <15% before the age of 50 years, and approximately 30% thereafter, approaching 45% after the age of 70 years. It has been reported that the etiology of the decline of serum FT with aging is partly the result of testicular failure, and partly a result of diminished stimulation of the Leydig cell by decreased output of LH.[9] With aging, there is a decrease in the absolute number of Leydig cells, and the remaining cells shows a decline in testosterone production. The levels of SHBG increase with age, and increased binding of testosterone to SHBG results in lower levels of free, biologically active testosterone.[2] Hwang et al[13] found the decline in serum concentrations of FT and DHEAS and the increase in SHBG with age both in normal subjects and in patients with erectile dysfunction in Taiwan. Of importance result of the present study is the significant negative correlation between the serum levels of FT and the age of the studied healthy men (r=- 0.231,

p=0.0001) which reflect the declining of serum FT levels with age in Iraqi healthy individuals. Declining of FT concentrations in age dependent manner are primarily due to defects in the testes, which show reduction in the number of Leydig cells, the enzymes activities that contribute to testosterone synthesis, and the ability to increase testosterone production in response to gonadotropins stimulation, the follicle stimulating hormone and luteinizing hormone. Concentrations of these hormones increase with age, but do not increase sufficiently to overcome the reduction in testosterone levels.[1] In longitudinal data from the Massachusetts Male Aging Study,[14] total testosterone decreased at a rate of 1.6% per year, while bio-available testosterone decreased at a rate of 2-3% per year.

The limitation of this study is the difficulties of accurate data collection on the clinical characteristics of the involved subjects regarding the androgen deficiency such as the erectile dysfunction, cognitive function, impotence, and so forth.

This study concluded the significant occurrence of andropause phenomenon in Iraqi healthy men and the significant decline of androgen status, the serum FT concentration, with advancing age. The cutoff at which such significant decrease of FT occurred is at ≥60 years of age. Further studies involved measurements of serum free testosterone, SHBG, and DHEAS concentrations along with clinical characteristics of androgen deficiency are needed to confirm the results of the present study in order to allow for androgen supplement in healthy men of age ≥60 years.

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References

1. Gronowski AM. Reproductive Disorders. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Fundamental of clinical chemistry. 6th ed. St. Louis: Elsevier Saunders; 2008. p. 780-801.
2. Schwarz ER, Phan A, Willix Jr RD. Andropause and the development of cardiovascular disease presentation more than an epi-phenomenon. ***J Geriat Cardiol*** 2011; 8 :35-43.
3. Haren MT, Kim MJ, Tariq SH, Wittert GAA, Morley JE. Andropause: a quality-of-life issue in older males. ***Med Clin North Am*** 2006; 90: 1005-1023.
4. Mooradian AD, Korenman SG. Management of the cardinal features of andropause. ***Am J Ther*** 2006; 13: 145-160.
5. Krysiak R, Okopien B.. Pathogenesis and clinical presentation of andropause. ***Pol Merkur Lekarski*** 2012; 32: 70-73.
6. Liu CC, Wu WJ, Lee YC, Wang CJ, Ke HL, Li WM, et al. The prevalence of and risk factors for androgen deficiency in aging Taiwanese men. ***J Sexual Med*** 2009; 6: 936-946.
7. Schwarz ER, Phan A, Willix RD. Andropause and the development of cardiovascular disease presentation-more than an epi-phenomenon. ***J Geriatr Cardiol*** 2011; 8: 35-43.
8. Tsujimura A, Matsumiya K, Miyagawa Y, Takao T, Fujita K, Takada S, et al. Comparative study on evaluation methods for serum testosterone level for PADAM diagnosis. ***Int J Impot Res*** 2005; 17: 259-163.
9. Demers LM. The adrenal cortex. In: Burtis CA, Ashwood ER, Burns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostic. Philadelphia (PA): Elsevier Saunders; 2006. p. 2003-52.
10. Clapauch R, De Castro Braga DJ, Marinheiro LP, Buksman S, Schrank Y. Risk of late-onset hypogonadism (andropause) in Brazilian men over 50 years of age with osteoporosis: usefulness of screening questionnaires. ***Arq Braz Endocrinol Metab*** 2008; 52: 1439-1447.
11. Tenover JS. Declining testicular function in aging men. ***Int J Impot Res*** 2003;15 Suppl 4: S3-S8.
12. Li JY, Li XY, Li M, Zhang GK, Ma FL, Liu ZM, et al. Decline of serum levels of free testosterone in aging healthy Chinese men. ***The Aging Male*** 2005; 8: 203-206.
13. Hwang TIS, Juang GD, Yeh CH, Chang YH, Chou KY, Chen HE. Hormone levels in middle-aged and elderly men with and without erectile dysfunction in Taiwan. ***Internati J Impotence Resear*** 2006; 18: 160-163.
14. Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts Male Aging Study. ***J Clin Endocrinol Metab*** 2002; 87: 589-598.