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Research Article

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Analysis and Comparison of Total, Bio-available and Free Testosterone Levels in Various Age Groups of Men

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Abstract In recent years several studies recommended the estimation of total as well as bio-available and free testosterone levels to assess the variations provided by the measurements and thus developing the foundation for interpreting hormone status in all groups of men. Therefore present study documents the current testosterone status i.e. total, free and bio-available, including sex hormone binding globulin (SHBG) in variable age groups of men aged 16-24 yrs "young" (n = 26), 25-35 yrs "adult" (n = 22), 36-50 yrs "middle aged" (n = 33) and 51-70 yrs "older" (n = 21). Serum total testosterone and SHBG were measured by Electro Chemiluminescence's (ECL) technology whereas bio-available and free testosterones were calculated from pre-described calculation methods. According to aged-groups, total testosterone level is 16-24 yrs = 16.95 ± 3.55; 25-35 yrs =15.10 ± 4.50 nmol/L; 36-50 yrs = 13.99 ± 2.90 nmol/L; 51-70 yrs = 7.01 ± 2.35 nmol/L. Estimated values of SHBG are 16-24 yrs = 48.01 ± 9.05 nmol/L; 25-35 yrs = 38.95 ± 8.15 nmol/L; 36-50 yrs = 39.05 ± 8.96 nmol/L; 51-70 yrs = 70.10 ± 8.40 nmol/L. Total testosterone levels are comparable to each other in adult and middle age groups, however significantly differ ($P < 0.001$) among older and younger group. Significant difference in free testosterone values were obtained for younger men in comparison with middle age group ($P< 0.001$) and moderate level of significance were noted when same was compared with adult and middle aged groups ($P < 0.05$). In conclusion, the levels of total, free, bio-available testosterone and SHBG were compared with their normal ranges and noted to be match-able with their respective age groups accordingly.

Keywords Total testosterone, free testosterone, bio-available, sex hormone binding globulin (SHBG)

Introduction

It is reported that the concentrations of total, free and bio-available testosterone decline as men ages. Moreover, it is a clinically accepted norm that the majority of elderly men have testosterone levels in the young adult range [1]. In this regard, testosterone concentrations in men are now measured as a routine test for initial indexing of androgen status [1]. Most clinical laboratories all around the world and especially at tertiary care centers, uses automated analyzers for total testosterone estimation, results of which are available within hours. Nonetheless, it is recommended that when concentrations of plasma testosterone are at the lower limit of normal (9.0 nmol/l), levels of bioactive testosterone group should also be analyzed for assessing clinical status of men. This could be the free form (non-protein-bound) or bio-available form (free plus albumin-bound) of bio-active testosterone. Methods available for measurement of both are either analytical or calculation by using any one of a variety of available mathematical formula [1]. Currently, researchers, clinicians and endocrinologists are recommending the estimation of testosterone

as well as that of bio-available and free testosterone levels. This will help in controlling the gaps in variations occurred during measurements and developing the foundation for interpreting hormone status in all clinical groups [2].

Measuring all sets of androgens in individuals and groups also seems to be more relevant nowadays when several studies pointed out increasing risk of mortality in people with low free and bio-available testosterone [3]. Furthermore, another recent study showed that men with type 2 diabetes taking statins as lipid lowering medications exhibited low total testosterone levels. Moreover, it is well documented that there are a further 20–25% with levels in the low normal range that may also be compatible with a diagnosis of hypogonadism, depending on clinical symptoms [4].

Current study documented and described the measurements of testosterone status i.e. total, free and bio-available, including sex hormone binding globulin (SHBG) in variable age groups of men, between 16 yrs to 70 years.

Materials and Methods

Research Design: Males, aged between 16 to 70 years were recruited from outpatients' facility at Department of Biochemistry Laboratory services and Chemical Pathology, Liaquat National Hospital Karachi, during Jan 2015 to Dec 2017. Subjects were given verbal information regarding the study, and seventy eight gave their informed consent to take part. The study population comprised patients' routinely visiting medicine, endocrine and related specialty clinics. Demography, medical history, and drug histories were collected using a questionnaire. Clinical and biochemical assessments of androgens status were made inclusive of other measurements such as routine blood pressure, non-fasting lipid levels, height, weight, and waist circumference. Any additional information was obtained from our patients' database.

Analytical Procedures

Patients were visiting usually in the morning to noon timings between 8:00 and 1:00 pm. No symptoms of hypogonadism were reported in the selected group and thus included in the study. Venous blood was taken; serum samples were produced by centrifugation and stored at -20°C for future analysis. Serum total testosterone and SHBG were measured by Electro ChemiLuminescence (ECL) technology on Roche Cobas e411 (Roche Diagnostics, Basil) immunoassay analyzer according to manufacturer's directions. Bio-available and free testosterone were calculated from total testosterone, SHBG and albumin concentration by a previously described calculation method [5, 6]. These methods of assessing bio-available and free testosterone have been used in previous studies [1, 4, 6] and have determined to be reliable for the assessment of androgen status in men [7, 10]. As recommended earlier [7, 10] weight and height were recorded and used to derive BMI and waist circumference was measured midway between the lower costal margins and the iliac crests. Blood pressure was recorded using a manual sphygmomanometer. Serum albumin was analyzed by Cobas c501 chemistry analyzer (Roche Diagnostics, Basil) using standard method.

Statistical Analysis

Data were analyzed using the SPSS package ver 17 (SPSS, Chicago, IL). Testosterone and SHBG were assessed by using Student's *t* test for comparison of group means. Within smaller sub-groups, the normal distribution was assessed using Pearson's correlation testing. The two-way ANOVA was also used to compare groups when data did not fulfill the normal distribution. Results were considered statistically significant at $P < 0.05$.

Results

Total, free and bio-available testosterone levels in 102 patients from boys aged 16 to older men ages 70 were assessed in the present study. The patients were divided into four groups to evaluate a generalized androgen status of selected male population. The data of each group was assessed according to their age group and compared with other groups as well. Four groups includes male aged 16-24 yrs "young" (n = 26), 25-35 yrs "adult" (n = 22), 36-50 yrs "middle aged" (n = 33) and 51-70 yrs "older" (n = 21). Except for slightly higher weight index in middle age and older group of men as compared to younger ones, all other parameters are in comparable range with each other.

The androgen hormone levels of the participants in the study span broad ranges of values (Table 1). According to aged-groups, total testosterone level is 16-24 yrs = 16.95 ± 3.55; 25-35 yrs =15.10 ± 4.50 nmol/L; 36-50 yrs = 13.99 ± 2.90 nmol/L; 51-70 yrs = 7.01 ± 2.35 nmol/L. Estimated values of SHBG are 16-24 yrs = 48.01 ± 9.05 nmol/L; 25-35 yrs = 38.95 ± 8.15 nmol/L; 36-50 yrs = 39.05 ± 8.96 nmol/L; 51-70 yrs = 70.10 ± 8.40 nmol/L. It should be noted that lower levels for total testosterone and free testosterone may indicate various factors related to progressing or older age. Another possibility of obtaining lower levels of total testosterone in older group is their self reported health status and medications. Therefore, some subjects may be taking unreported medications or had unreported conditions thus affecting hormone levels and thus this fact cannot be ruled out. However, the majority of values are within normal ranges. The assessment of data was not very different for bio-available testosterone as well; accept when older group was compared with middle aged men, it was found to be non-significant. All measured levels of total, free, bioavailable testosterone and SHBG were compared with their normal ranges and noted to be match-able with their respective age groups accordingly. Calculated values for Free Testosterone as per age-groups are 16-24 yrs = 0.67 ± 0.15 nmol/L; 25-35 yrs = 0.61 ± 0.15 nmol/L; 36-50 yrs = 0.55 ± 0.09 nmol/L; 51-70 yrs = 0.15 ± 0.08 nmol/L whereas for Bio-available testosterone 12.90 ± 3.40 nmol/L, 11.80 ± 3.05 nmol/L, 10.85 ± 2.15 nmol/L and 2.88 ± 1.90 nmol/L.

Table 1: Determination of total, free and bio-available testosterone and sex hormone binding globulin in various age groups of men

Men Age groups	T. Testosterone nmol/L	SHBG nmol/L	Free-Testo nmol/L	Bio-Testo nmol/L
16-24 yrs	16.95 ± 3.55[a]	48.01 ± 9.05[b]	0.67 ± 0.15[a]	12.90 ± 3.40[a]
25-35 yrs	15.10 ± 4.50[b]	38.95 ± 8.15[a]	0.61 ± 0.15[b]	11.80 ± 3.05[c]
36-50 yrs	13.99 ± 2.90[b]	39.05 ± 8.96[d]	0.55 ± 0.09[b]	10.85 ± 2.15[d]
51-70 yrs	7.01 ± 2.35[a,b]	70.10 ± 8.40[a,b]	0.15 ± 0.08[a,b]	2.88 ± 1.90[a,c,d]

Normal ranges: Total Testosterone (nmol/L) = Children = < 0.69, Males = 9.9-52.3; SHBG (Sex hormone binding globulin-nmol/L) = Children = 10-80, Males = 14-48; Free Testosterone (Free-T-nmol/L) = Males = 0.31-1.041; Bio-available testosterone (Bio-Testo-nmol/L) = Young Males = 2.88-8.90, Adult Males = 2.49-8.15, middle-aged males = 2.11-6.59, old aged-males = 1.388-5.82).

Results are expressed as mean ± SD; [a]Significant difference < 0.001, [b]Significant difference < 0.01, [c]Significant difference < 0.05, [d]non-significant.

Discussion

Normally androgen status, as reported, is essential for male physiological functions, and bio-available androgens, and in this regard, comprised of free and albumin-bound fractions [7, 8, 8-13]. In present study we examined the age-related measurements in serum of total, free and bio-available testosterone levels comparing with each fraction of testosterone and its relationship with respective age group. It is noted that albumin-bound as well as bio-available testosterone levels declined with age, and their decrease was associated their respective age groups as well as with the increase of sex-hormone binding globulin (SHBG) level in ages of fifties and sixties. Similar results were recognized in the level of all three fractions of testosterone, suggesting that SHBG and albumin levels plays an important role for maintaining bio-available sex steroid levels in males aged over sixty. Moreover, our study showed that SHBG levels associated inversely with bio-available sex steroid, in agreement with previous reported studies [1-4, 8]. A study carried out for estrogens also showed similar pattern for albumin and SHBG bound fractions [13]. They postulated that the decrease of bio-available estradiol as well as testosterone is induced by the decrease of albumin-bound fractions in combination with the increase of SHBG-bound fractions in males aged over sixty. In addition their physical characteristics of aging could be induced by the decrease of albumin-bound fractions caused by the decrease of serum albumin regardless of total sex steroid levels [13].

Methodology for the measurements of all forms of testosterone is available at various clinical laboratories all around the world. However several reports suggest that it is essential to understand the differences among various measured analytes [7-12] Thus generally the measurement of serum testosterone or "total" testosterone is usually performed

by ELISA, MEIA and ECL assays and it measures free plus protein bound testosterone. Furthermore, "Free" or dialyzable testosterone measurements are estimates of the fraction of testosterone in blood that is not bound to protein. These assays sometimes require determination of the percentage of unbound testosterone by a dialysis procedure, estimation of total testosterone, and the calculation of free testosterone. Free testosterone can also be calculated if total testosterone, SHBG, and albumin concentrations are known [9]. There is a third analyte of testosterone commonly made of "bio-available" or non-SHBG bound testosterone [11]. This analysis determines the amount of testosterone not bound to SHBG and includes that which is non-protein bound and weakly bound to albumin. This fraction is supposedly readily available to tissues and thus the name "bio-available' [7].

Arguably, as observed in our study, it is reported well that serum testosterone levels begin to decline in normal healthy men, in the mid- to late-thirties and this decline is linear into the nineties, at a rate of 0.4%/year. Men with chronic medical illnesses such as hypertension, heart disease, diabetes when evaluated, showed same age-associated decline in serum testosterone exists, but at a rate of 10–15% below that of healthy age-matched men. Moreover, in addition to this decline with age in total testosterone, there is an inversely proportional increase in sex hormone binding globulin (SHBG) [7, 13-16] as noted in our study. Therefore, undoubtly, as man ages, the total testosterone level decreases, but the serum binding of testosterone increases. This increase in testosterone binding results in a "free" or bio-available testosterone level that decreases to a greater extent than total testosterone, a phenomenon observed in our present study as well. As a result, the availability of the free active form of testosterone in the serum is further reduced compared with total testosterone.

Interestingly, the measurement of testosterone that depends on determination of protein-bound testosterone, may not reflect of a deficiency of it, but may be the result of a change in the binding protein by a problem independent of the androgen state of the man such as seen in type 2 diabetes mellitus patients. As reported earlier, a decrease in SHBG occurs in type II diabetes mellitus as a result of an increase in insulin or insulin-like growth factor I levels [17, 18]. This decline in SHBG level associated with decreased total androgen in diabetic patients, and unrelated to androgen deficiency, may be coincidentally related to impotence. However, this impotency is not because of any alterations in total, bio-available or free testosterone levels but due to the onset of diabetes mellitus and its concomitant physiological disturbances [7].

Several researchers also correlated other biochemical and physiological factors with altered or otherwise levels of androgens. Assessing association of total, free and bio-available testosterone with age groups as well with high or low levels of albumin or SHBG, mostly agreed upon, is another area worth exploring [19-21]. An inverse relationship between serum SHBG concentration and percent of testosterone recovery has been observed and the release of serum testosterone from SHBG via diethyl ether extraction in another bioassay has been shown to increase recovery of serum testosterone [22]. Consistent with the well-established positive relationship between serum SHBG and total testosterone [22-24] the authors found that the median serum total testosterone and bio-available testosterone levels were significantly decreased in serum samples within the lowest quartile of SHBG compared with the highest quartile, well supporting our findings. These findings are highly suggestive of a physiological rather than methodological relationship between the measures [21, 25-27].

Conclusion

In conclusion, total, free and bio-available testosterone levels in 102 patients including boys aged 16 to older men ages 70 divided in four age-matched groups were assessed in the present study. All measured levels of total, free, bio-available testosterone and SHBG were compared with their normal ranges and noted to be match-able with their respective age groups accordingly. It was argued that clinical situations, either of male andropause, impotency or otherwise, points out the difficulty in assessing androgen status when there is no good independent marker of androgen action that can be used *in vivo.* Therefore, assessing total serum testosterone in addition to free and bio-available, as demonstrated in present study, is less expensive and more practical to ascertain the general androgen status in men of varying age groups from young to old.

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