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Serum total testosterone in eumenorrhoeic young Bangladeshi women

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Abstract

Background: Population and an assay-specific cut-off value of total testosterone (TT) is required to diagnose different conditions with mild hyperandrogenemia in females of reproductive age.

Objective: To determine the serum TT cut-off limits for eumenorrhoeic women and to assess whether testosterone levels alter as individuals age and BMI change.

Methods: This cross-sectional study initially included 251 healthy eumenorrhoeic women between the ages of 16 and 35 who gave written, informed consent. Pregnancy, breastfeeding, history of smoking or alcohol intake, history of oral contraceptives within three months of enrollment (n=2), and having significant hirsutism (modified Ferriman-Gallwey score ≥8) or acne (n=5) were excluded. Venous blood was collected to measure alanine aminotransferase (ALT), creatinine, thyroid stimulating hormone, prolactin, and total testosterone. All the hormones were measured by the chemiluminescent microparticle immunoassay. Four participants were excluded for impaired renal and liver function. Any participant having impaired thyroid function (n= 4), hyperprolactinemia (n=4), and very high TT value (n=2) were also excluded. A total of 230 healthy participants were included in the final analysis. The average TT level of the healthy population was calculated by mean±2×SEM. The values between 25th and 75th were considered as reference ranges for healthy women.

Results: The mean±SD of age and BMI of the study population were 24.63±4.17 years and 22.44±3.69 kg/m^2 respectively. The mean serum total testosterone was 25.50±1.36 (23.82 – 27.18) ng/dL. The 25th percentile and 75th percentile of TT were 17.50 ng/dL and 33.72 ng/dL respectively. Serum TT had inverse but insignificant associations with age and BMI.

Conclusions: The reference range of TT in eumenorrhoeic young Bangladeshi women may be considered between 18.0 ng/dL and 34.0 ng/dL. The study findings might provide clinicians with baseline data for detecting hyperandrogenic conditions in young reproductive-age women. *[J Assoc Clin Endocrinol Diabetol Bangladesh, July 2022; 1 (2): 44-49]*

Keywords: Total testosterone, Young women, Eumenorrhea

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Introduction

Testosterone is the predominant male sex hormone that aids in building muscle, voice deepening, and sperm production. Females have much lower amounts of testosterone than males whom it regulates some bodily functions and contributes to libido. Serum androgen is emerging as an important factor in women's health. Sex-specific response to testosterone levels to different disease conditions also varies between sex.[1] Moreover, hyperandrogenemia is a well-known diagnostic criterion of polycystic ovary syndrome (PCOS) where women do not have the clinical features of androgen excess. The prevalence of PCOS is rising globally and for proper diagnosis of PCOS, serum testosterone cut-off needs to be settled.[2] Extraction methods are the gold standard but are expensive, time-consuming, and not available in our country. Similarly, measurement of sex hormone binding globulin (SHBG) is required to calculate the free androgen index, again not widely available in our country. So, clinicians have to rely on only the total testosterone (TT) levels to determine biochemical hyperandrogenemia many times.

However, we need a method-specific and population-specific reference range, which is not currently available in our country. The majority of commonly employed testosterone immunoassays have been developed for the analysis of samples from males and do not offer the precision and accuracy needed for the analysis of samples from women.[3] Previous studies have also shown that testosterone level decreases with age from early reproductive years and the normal cut-off may also vary in different ethnicity.[4-6] In regularly cycling women of reproductive age, testosterone levels have been observed to rise by 20-30% during the mid-cycle period, coinciding with the luteinizing hormone (LH) spike.[7] Furthermore, serum testosterone concentrations vary throughout the day, with maximal concentrations observed in early morning samples.[3]

The use of testosterone treatment in women is becoming more acceptable. A certain group of women may benefit from testosterone therapy although there is little information on its safety and efficacy.[8] Blood testosterone levels need to monitor regularly if testosterone therapy is started for a valid indication.[9] In Bangladesh we do not have any recognized cut-off value which can be used for diagnosing mild hyperandrogenemia in women though testosterone-related disorders seem increasing. The purpose of the current study was to determine the serum testosterone cut-off limits for eumenorrhoeic women and to assess whether testosterone levels alter as individuals age and BMI changes.

Methods

This cross-sectional study included participants retrospectively from the studies done as a comparator healthy group of PCOS patients in the department of Endocrinology of Bangabandhu Sheikh Mujib Medical University (BSMMU). Using the following formula [$n= (Z^2 \times 4 \times SD^2) \div d^2$], at 95% confidence interval (CI) (Z=1.96), taking SD from a previous study (SD=15.5), and desired width of CI of 2.5 ng/dL on either side of the mean (d=5), the minimum sample size was 148.[7,10]

We initially included 251 healthy eumenorrhoeic women of 16 - 35 years consecutively by convenient sampling. Informed written consent was taken from each participant. None of the participants had a history of pregnancy, lactation, and any smoking or intake of

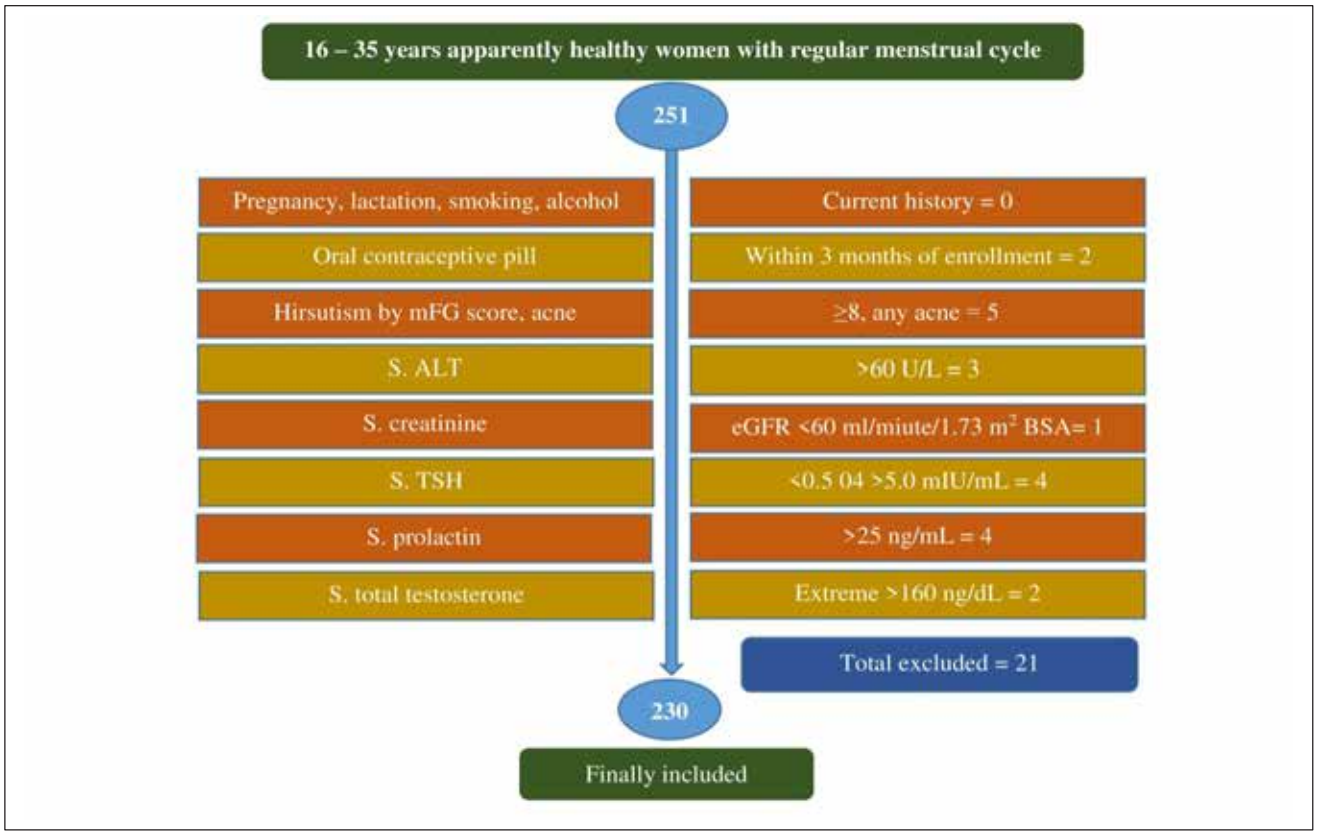

Figure-1: The study flow chart

alcohol. Women with a history of intake of oral contraceptives within three months of enrollment were excluded (n=2). Five participants had either significant hirsutism (modified Ferriman-Gallwey score ≥8) or acne were also excluded. Venous blood was collected from each participant to measure alanine aminotransferase (ALT), creatinine, thyroid stimulating hormone (TSH), prolactin, and total testosterone. All the hormones were measured by the chemiluminescent microparticle immunoassay (CMIA). ALT ≥60 U/L (n=3), estimated glomerular filtration rate (eGFR) by CKD-EPI formula <60 ml/minute/1.73 m^2 body surface area (n=1) were excluded. Any participants having TSH <0.5 mIU/ml or >5.0 mIU/ml (n=4) and prolactin >25 ng/ml (n= 4) were also excluded. Finally, two participants' TT values were extreme and excluded from the final analysis. A total of 230 healthy participants were included in the final analysis (Figure-1).

The statistical analysis was done by SPSS software version 22.0. From the normal distribution curve the mean, standard deviation (SD), and standard error of the mean (SEM) along with 5th and 95th percentile values of TT were determined. The average TT levels of the healthy population were calculated by mean±2×SEM. From a violin plot with a median (interquartile range), the values between the 25th and 75th were considered reference ranges for healthy women. TT level was compared among different subgroups of age, BMI, WC, and BP by Kruskal Wallis one-way ANOVA or Mann-Whitney U test as appropriate. A two-tailed p <0.05 was considered statistically significant.

Results

The baseline characteristics of the study population are shown in Table-I. The mean age of the study

Table-I: Characteristics of the study population (N=230)

Variables	Minimum	Maximum	Mean	Standard deviation
Age, years	16	35	24.63	4.17
BMI, kg/m^2	13.22	36.33	22.44	3.69
WC, cm	56.0	106.0	75.10	8.23
Systolic BP, mm-Hg	80	145	103.37	11.58
Diastolic BP, mm-Hg	50	100	67.65	8.21

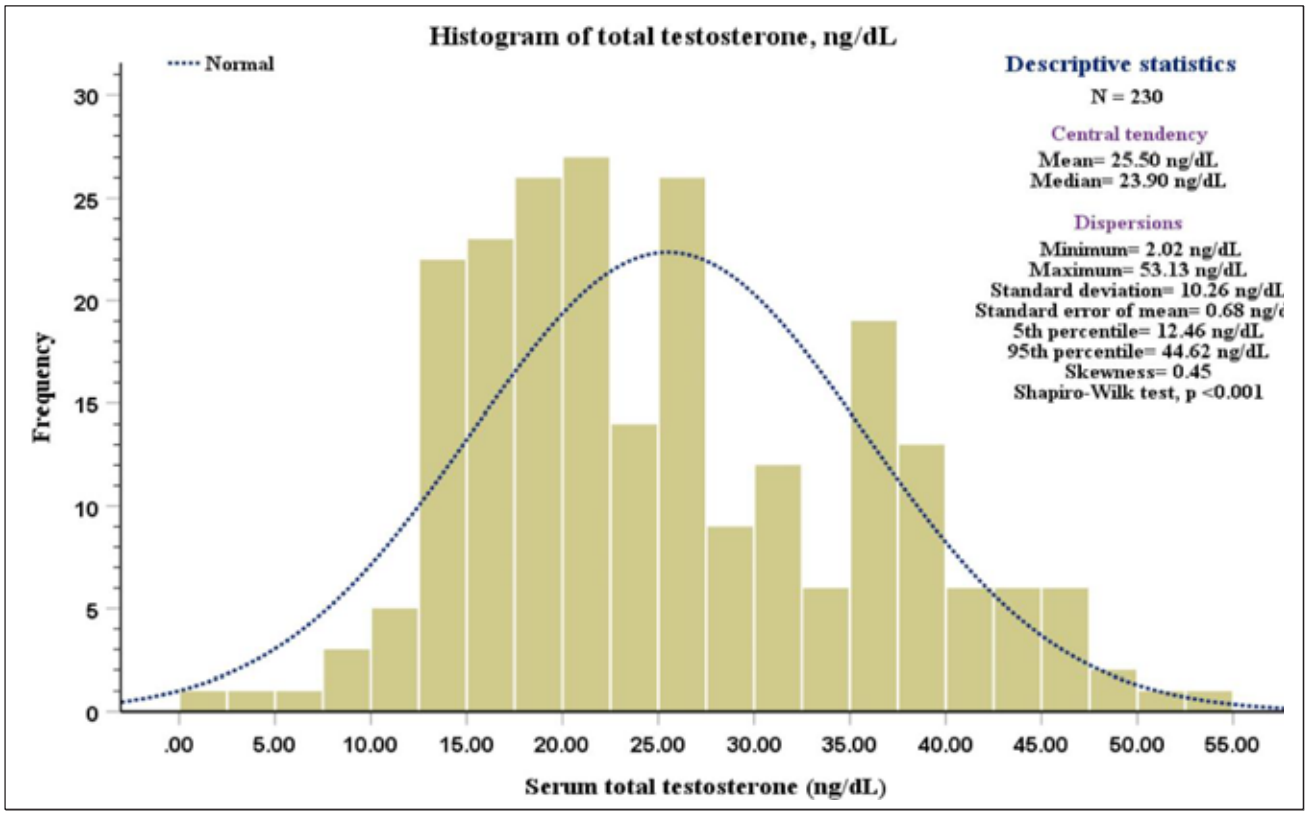

Figure-2: Distribution of serum total testosterone in the study population (N=230)

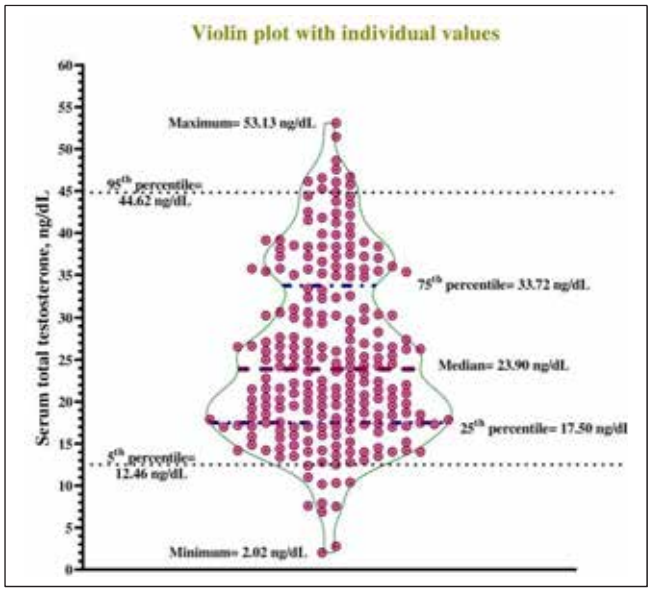

Figure-3: Median (interquartile range) of TT in the study population (N=230)

population was nearly 25 years. The mean BMI, WC, and BP were all within optimum levels.

Figure-2 shows the distribution of serum TT (ng/dL) in the study population. The mean±SD TT level was 25.50±10.26 ng/dl. Considering the 2×standard error of the mean (±0.68), the mean serum TT was 25.50±1.36 (23.82 – 27.18) ng/dL. The 5th and 95th percentiles of serum TT were 12.46 ng/dL and 44.62 ng/dL respectively. The distribution of serum total testosterone was positively skewed (skewness= 0.45, p <0.001).

Figure-3 shows the median values of TT with an interquartile range. The 25th percentile and 75th percentile of TT were 17.50 ng/dL and 33.72 ng/dL respectively.

As shown in Figure-4, serum TT reduced with increasing age groups without any statistical significance with respect to distribution (H=3.87, df=3, p=0.0276) and median values (H=3.58, df=3, p=0.311).

Figure-5 is showing serum TT according to BMI category. There is a trend of gradual decrement of serum TT with increased BMI category without any statistical significance both in distribution (H=6.62, df=3, p=0.085) and median values (H=8.96, df=3, p=0.030; all adjusted p-values after pairwise comparison= NS).

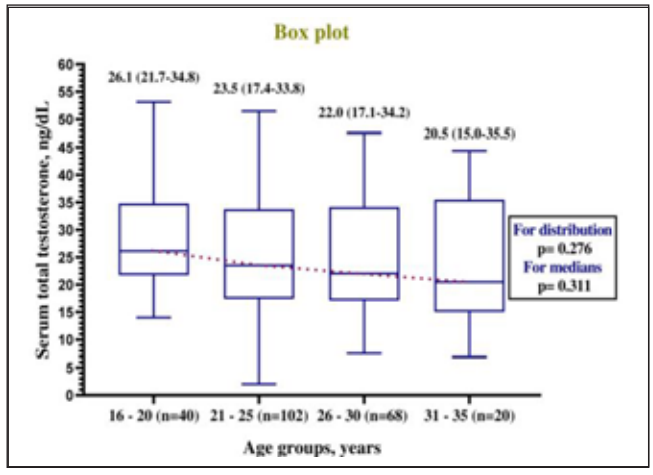

Figure-4: Total testosterone levels according to age groups (N=230)
Kruskal-Wallis one-way ANOVA test was done

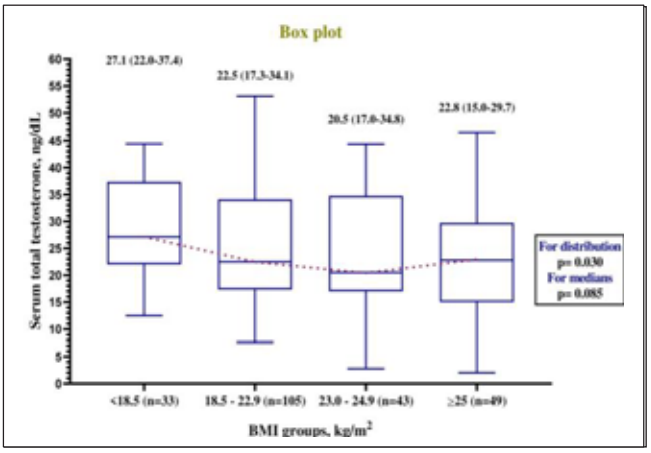

Figure-5: Serum total testosterone levels according to BMI categories (N=230)
Kruskal-Wallis one-way ANOVA test was done

There were also no significant differences in serum TT (ng/dL) with WC and BP categories [WC category: centrally obese (WC≥80 cm, n= 53) vs. non-obese (WC <80 cm, n= 177): 22.42 (15.03-32.94) vs. 24.23 (18.30-34.87), p=0.222; BP category: hypertensive (≥ 140/90 mm-Hg, n= 4) vs. normotensive (<140 & 90 mm-Hg, n= 226): 33.65 (24.41-43.84) vs. 23.50 (17.47-33.22), p=0.108, median (IQR)].

Discussion

This cross-sectional study included 230 healthy young women to find out an acceptable reference range of TT at the population level. The median value of TT was 23.90 ng/dL. The 25th percentile and 75th percentile of TT were approximately 18.0 ng/dL and 34.0 ng/dL respectively. Braunstein et al. (2011) showed 46.0 ng/dL as the upper limit of normal TT level (95th percentile value) - a similar finding to us.[7] However,

TT levels in our study were skewed. So we took the 75th percentile value of TT (34.0 ng/dL) as the upper limit of the normal TT level. Whereas an earlier study conducted in Australia revealed a considerably higher maximum level with a similar minimum level, and their sample size was larger than the USA-based study.[11] Both the studies employed the radioimmunoassay (RIA) method after extraction and chromatography, however, the current study used the direct chemiluminescent immunoassay method, where reference intervals are not well established and are relatively inaccurate compared to other methods.[3]

Several studies have found a declining tendency in serum testosterone with the increasing age of women, which is consistent with our findings.[4-7] Interestingly, the greatest decline in testosterone levels occurred during the early reproductive years, with little to no further decline in mid- and later life.[11] As we included only the reproductive age group commenting about the later age group is beyond our capacity.

It is a well-known fact that a higher BMI is associated with hyperandrogenemia in women, but in the present study, we found a decreasing trend of testosterone with a higher BMI. Though no statistical difference was observed among different BMI categories. The mean TT was comparable to those with normal BMI even in obese participants (>25 kg/m^2). This reinforces the idea that obesity is not the major cause of hyperandrogenemia; rather, insulin resistance, genetic, and environmental factors may all play a role.[12]

The results presented here have some limitations. We did not assess sex hormone binding globulin, which might alter TT findings, and we also did not use the gold standard dialysis equilibrium method for TT analysis. The different menstrual phases may also influence the TT level. Massafra et al. suggested that if a larger number of samples are taken during the menstrual cycle, greater differences between the early follicular phase and ovulatory levels of testosterone can be seen.[13] We collected the serum sample during the follicular phase, but we were unable to detect a change since we did not measure the level during the mid-luteal phase. Besides, we could not do ultrasonography of ovaries to exclude normal variant of polycystic ovarian morphology.

Conclusion

The mean serum TT levels of young reproductive-aged females were between 24.0 ng/dL and 27.0 ng/dL; the reference ranges between 18.0 ng/dL and 34.0 ng/dL using a CMIA method. The study finding might provide baseline data to clinicians for diagnosing hyperandrogenic disorders in young reproductive-aged women. Future studies should be done by measurement of SHBG or free testosterone by ultrasensitive assay to find out the actual cut-off for hyperandrogenemia among the Bangladeshi population.

Acknowledgement

We are grateful to the participants of the study for giving the consent.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Financial Disclosure

The author(s) received no specific funding for this work.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Written informed consent was taken from the participants. The study was approved by Institutional Review Board (IRB) of the institute.

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