ORIGINAL ARTICLE

Reference range of testosterone and dehydroepiandrosterone sulfate levels in women during reproductive age in the Iranian population

Rokhsareh Meamar[1,2], Awat Feizi[3], Ashraf Aminorroaya[1], Masoud Amini[1], Bijan Iraj[1], Maryam Heidarpour[1]

[1]Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Isfahan Clinical Toxicology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Department of Biostatistics and Epidemiology, School of Public Health, Isfahan University of Medical Sciences, Isfahan, Iran

Backgrounds: To determine the average cutoff values of serum-free and total testosterone (FT, TT) and dehydroepiandrosterone sulfate (DHEAS) among healthy premenopausal women. **Materials and Methods:** Participants were women aged 18–55 years without signs and symptoms of hyperandrogenism (n = 489). Participants if Ferriman–Gallwey (FG) scores between 6 and 8 were considered a group located in the upper spectrum related to the normal hirsutism score (n = 30). DHEAS, TT, and FT levels were compared between different populations. Upper limits of 97.5 and 95 and lower limits of 5 and 2.5 percentiles were calculated to provide the reference intervals for DHEA, TT, and FT in the total sample and in the population with FG 6–8. **Results:** In the total population, the mean ± standard deviation (SD) serum FT, TT, and DHEAS levels were 1.40 ± 0.63 pg/mL, 0.42 ± 0.17 ng/mL, and 1.5 ± 0.97 µg/ml, respectively. The cutoff values of FT at 1.35 and TT at 0.49 were obtained for differentiating the patients with FG 6–8 scores from the normal population, with the corresponding specificity of 0.60, the sensitivity of 0.67, and area under the ROC curve (AUC) (confidence interval 95%) of 0.63 (0.52–0.73), P = 0.01 and 0.68 (0.58–0.78) P = 0.001, respectively. **Conclusions:** In our study, the mean ± SD serum FT level was 1.40 ± 0.63 pg/mL, the TT level was 0.42 ± 0.17 ng/mL, and the DHEAS level was 1.5 ± 0.97 µg/ml, in premenopausal women between 18 and 49 years of age. Furthermore, in a population with FG 6–8 score, a cutoff value of FT at 1.35 and TT at 0.49 was obtained. Although the irregular menstrual cycle did not change the reference range when compared with the normal group.

Key words: androgen, reference range, reproductive, testosterone

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INTRODUCTION

Although androgens were produced in small amounts in women have substantial impacts on many aspects of female physiology,[1-3] they are also precursors of all types of estrogens.[4] Testosterone is the main androgenic compound, even in women. In the testosterone biosynthesis pathway, dehydroepiandrosterone sulfate (DHEAS) is one of the most important precursors.[5] DHEAS is the most abundant circulating adrenal steroid in adult life, and its levels are generally 15%–20% higher in men than women.[6]

Several reports have suggested that hyperandrogenism is one of the most common endocrinopathies (5%–10%) in reproductive age women.[7] The measurement of androgens in the blood is essential in the diagnosis of both gonadal and adrenal functional disturbances, as well as for monitoring treatments. Clinical hyperandrogenism has been associated with many pathologic signs and symptoms, the most prevalent being acne, hirsutism, and irregular menstrual cycle (IMC).[7] Previously has

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Address for correspondence: Dr. Maryam Heidarpour, Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: heidarpourmaryam110@gmail.com
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been reported that circulating levels of free testosterone (FT) to total testosterone (TT) in hirsute women are double those of nonhirsute women.[8]

In addition, there are a number of reference ranges published for serum testosterone concentrations in premenopausal women.[9-12] Most of the available ranges are derived from a relatively small number of subjects, as required by the National Committee for Clinical Laboratory Standards.[13,14] On the other hand, these ranges may not have been controlled for factors known to influence testosterone concentrations. Indeed, the amount of these hormones examined is low; it may be influenced by numerous factors.[15,16]

Sex and age appear to be the major determinants of serum levels of DHEAS. In fact, it is about twice in men than in women while showing a progressive reduction from the end of puberty to the elderly age in both sexes.[17] The age-related decrease in serum DHEAS levels has been recently shown in obese women[18] and normal ones. In addition, several studies have shown that serum testosterone concentrations decrease gradually with age.[11,19,20]

In general, it seems that accurately determining the age-specific validated normal ranges of testosterone and DHEAS in women of differing ages can help to reduce diagnostic ambiguities in those who have androgen levels in the lower or upper limits of the normal range. On the other hand, other factors, such as hormonal drugs, including oral contraceptives, can influence sex hormone-binding globulin (SHBG) concentrations and influence total, free, and bioavailable testosterone concentrations.[14,21,22]

This population-based study aimed to determine the reference ranges for serum testosterone (i.e. free and total) and DHEAS in healthy premenopausal women and, for the first time, the determination cutoff point in a subgroup that is nearly located in the upper spectrum related to the normal hirsutism score. In addition, we have also explored the effects of natural and demographic factors on these androgens levels.

MATERIALS AND METHODS

Study population

Participants in the cross-sectional study were women aged 18–55 year (during reproductive age), recruited from April 2018 to August 2019. All participants (n = 489) provided written informed consent. All participants completed a demographic questionnaire, including age, education level, and medical history at baseline. Currently, pregnant or lactating patients, recently postpartum patients (within the past 6 months), or patients with a history of hyperandrogenism, acne, hirsutism, adrenal disorder, or treatment with glucocorticoids or oral contraceptives (within the previous 6 months), psychiatric illness, renal failure, liver dysfunction, cardiovascular disease or any other acute major illness, gynecological surgery, or active malignancy or cancer treatment were excluded from the study. This study was conducted under the supervision of the Isfahan Endocrine and Metabolism Research Center (code: 95013). Ferriman–Gallwey (FG) scoring system was used to determine the hirsutism score of nine body areas. Participants included if an FG score was below 8 and an FG score between 6 and 8 considered as a group that had been located in the upper spectrum related to hirsutism score (n = 30).[23] On the other hand, a regular menstrual cycle was defined, ranging between 25 and 35 days, with self-reported normal menstrual cycles. However, individuals generally are located two-sided of the normal spectrum ± 2 days (23–25 and 35–37 days) as defined as mild IMC (n = 54).[24] Therefore, in this study, we considered a normal population (n = 413) without having any history of hyperandrogenism against a mildly abnormal population has been defined as a sum of participants with mild hirsutism and mild IMC (n = 79).

Anthropometric and clinical measurements, including body mass index (BMI) (dividing weight [kg] by the square of height [m^2]), waist circumference, hip circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP), were recorded.

Laboratory measurement

Blood was drawn on days 3–7 of the follicular phase of the menstrual cycle and after an 8-h fast. The serum was frozen at − 80°C. TT was assessed with a solid-phase 125I radioimmunoassay based on a testosterone-specific antibody immobilized to the wall of a polypropylene tube. The assay precision was ± 15.5 percent. The normal range of free testosterone (FT) concentration was 0.04–4.2 pg/mL. FT intra- and interassay CV were 5.2% and 3.9%, respectively. The normal range of TT concentration was 0.14–0.76 ng/mL. The normal range of DHEAS concentration was 0.03–5.88 μg/ml.

Fasting blood sugar (FBS) (mg/dL) was measured by Pars Azmon kit, Lot number: 94011 (photometric method), and cholesterol, low-density cholesterol (LDL), high-density cholesterol, and triglyceride (TG) were also measured.

Statistical analysis

The normality of continuous data was evaluated using Kolmogorov–Smirnov test and Q-Q plot and reported as mean ± standard deviation (SD) (median (interquartile range). Categorical data were reported as frequency (percentage). DHEAS, TT, and FT levels were compared between different populations, using

independent samples *t*-test or Mann–Whitney *U* test, while between different groups analysis used variance (ANOVA) and nonparametric Kruskal–Wallis test. Upper limits of 97.5 and 95 and lower limits of 5 and 2.5 percentiles were calculated to provide the reference intervals for DHEA, TT, and FT in the total sample and in the population with FG 6–8 and mild IMC. The association between DHEAS, TT, and FT levels with demographic and laboratory data was evaluated using Pearson's and Spearman's correlation coefficients in different populations. Using the receiver operating characteristic (ROC), the area under the curve (AUC) and 95% confidence interval (CI) for AUC were calculated in crude and adjusted models. All statistical analyses were conducted using SPSS software version 15. (SPSS Inc. Chicago, IL, USA).

RESULTS

Data on mean ± SD of serum TT, FT, and DHEAS levels and other characteristics of the subjects in the entire study population (n = 498) and mild hirsutism (n = 30), mild IMC (n = 54) participants, and finally population with the sum of FG 6-8 and mild IMC (n = 79) and normal population (n = 413) in reproductive age women are presented in Table 1. In the total population, the mean ± SD serum FT level was 1.40 ± 0.63 pg/mL, the TT level was 0.42 ± 0.17 ng/mL, and the DHEAS level was 1.5 ± 0.97 µg/ml. A significant difference in TT level was observed, so the mean ± SD of TT levels in abnormal women was higher (0.45 ± 0.18) than in normal women (0.41 ± 0.16). In FG 6–8 subjects, the mean ± SD of TT, FT, and DHEAS were higher than the control ($P < 0.05$). Whereas, in subjects with mild IMC, in androgens level, no significant difference was observed. In different variables, bleeding days, BMI, BP, and TG levels in the mild IMC were higher than in the normal population. However, in FG 6–8 subjects, this difference was observed only on the bleeding day, BP, and in IMC participants, BMI showed a higher amount than the normal population.

The distributions of DHEAS, FT, and TT concentrations in different percentiles were presented in Table 2. The 5th–95th (%95 CI) and 2.5th–97.5th (%95 CI) of serum DHEAS were 0.54–3.38 (1.41–1.59) and 0.3–3.71 (1.41–1.59), respectively, in the normal population. However, the 5th–95th (%95 CI) and 2.5th–97.5th (%95 CI) of serum FT were 0.60–2.37 (1.33–1.46) and 0.5–2.6 (1.32–1.45), respectively. In addition, the 5th–95th (%95) and 2.5th–97.5th (%95 CI) of serum TT was 0.20–0.70 (0.39–0.42) and 0.1–0.76 (0.39–0.42), respectively.

FT levels in all populations in this study [Table 3] were negatively correlated with age. In subjects with mild IMC, all androgens levels (DHEAS, FT, and TT) were negatively correlated with age and LDL levels. Simultaneously, this negative correlation was observed between only DHEAS and TT levels with cholesterol levels. Besides, the FT level has only a positive correlation with the TG level. A significant negative correlation between DHEAS and age, cholesterol, and LDL was detected in the subnormal population with FG 6–8 and mild IMC. However, in the normal population, such a negative correlation between DHEAS and age, BMI, cholesterol, and TG levels was observed.

Table 1: Demographic and laboratory findings of the study populations

Variables (mean±SD)	Total population (*n*=498)	Normal population (*n*=413)	Population with FG 6–8 and mild irregular menstrual cycle (*n*=79)	*P*	Population with FG 6–8 (*n*=30)	*P*	Mild irregular menstrual cycle population (*n*=54)	*P*
DHEAS (µg/ml)	1.5±0.97	1.5±0.88	1.58±1.31	0.48	1.91±1.83	0.02	1.42±0.84	0.57
FT (pg/ml)	1.40±0.63	1.39±0.63	1.44±0.67	0.54	1.70±0.74	0.01	1.34±0.63	0.54
TT (ng/ml)	0.42±0.17	0.41±0.16	0.45±0.18	0.034	0.53±0.19	0.001	0.42±0.17	0.76
Age (year)	34.54±8.4	34.49±8.44	34.78±8.28	0.78	35.5±7.80	0.52	34.5±8.3	0.99
Age of menstruation (year)	13.39±1.52	13.39±1.52	13.41±1.52	0.88	13.53±1.24	0.6	13.35±1.62	0.86
Bleeding day	4.40±2.25	4.29±2.15	4.93±2.64	0.02	5.13±2.39	0.04	4.71±2.71	0.18
Weight (kg)	66.01±11.68	65.43±11.64	68.8±11.36	0.016	69.01±13.26	0.1	68.07±10.09	0.09
Waist circumference (cm)	83.63±39.47	83.41±43.19	84.64±10.29	0.79	86.03±11.16	0.73	83.71±9.65	0.95
BMI (kg/m^2)	25.71±4.35	25.46±4.35	26.92±4.13	0.005	26.86±4.57	0.08	26.72±3.85	0.03
BP-maximum (mmHg)	95.96±14.88	95.04±14.58	100.42±15.60	0.003	101.5±16.97	0.02	100.3±15.31	0.01
BP-minimum (mmHg)	63.83±11.15	63.22±11.02	66.83±11.41	0.009	71.29±10.52	0.001	65.5±12.05	0.14
FBS (mg/dL)	91.44±11.83	91.6±12.42	90.58±8.24	0.48	91.6±9.29	0.99	90.48±7.82	0.51
Cholesterol (mg/dL)	184.18±34.85	184.13±33.79	184.41±40.05	0.94	190±37.19	0.37	183.1±41.5	0.84
HDL-c (mg/dL)	44.53±8.23	44.59±7.95	44.24±9.57	0.73	46.17±9.67	0.31	43.24±9.66	0.25
LDL-c (mg/dL)	91.7±20.21	91.76±19.73	91.63±22.62	0.95	93.17±21.76	0.71	91.1±22.69	0.83
TG (mg/dL)	119.32±82.93	116.57±78.86	133.22±100.4	0.01	130.28±96.13	0.38	143.42±108.87	0.02

FG=Ferriman–Gallwey; DHEAS=Dehydroepiandrosterone sulfate; FT=Free testosterone; TT=Total testosterone; BMI=Body mass index; BP=Blood pressure; FBS=Fasting blood sugar; FBG=Fasting blood glucose; HDL-c=High-density lipoprotein cholesterol; LDL-c=Low-density lipoprotein cholesterol; TG=Triglyceride; SD=Standard deviation

A cutoff value of FT at 1.35 and TT at 0.49 [Table 4] was obtained for differentiating the population with FG 6–8 score from the normal population, with the corresponding specificity of 0.60, the sensitivity of 0.67, and area under the ROC curve (AUC) (CI 95%) of 0.63 (0.52–0.73), $P = 0.01$ (adjusted AUC 0.61 [0.5–0.71; $P < 0.05$ after adjustment for age and BMI]) and 0.68 (0.58–0.78) adjusted 0.67 (0.56–0.79, after adjustment for age and BMI) and 0.68 (0.58–0.78); $P = 0.001$, respectively. The crude area under the ROC curve of TT and FT for the differentiation population with FG 6–8 score is shown in Figure 1. The adjusted AUC for DHEA was obtained (0.58 [0.45–0.68]

Table 2: The distribution of dehydroepiandrosterone sulfate, free testosterone, and total testosterone concentrations in different percentiles

	Total population	Normal population	Population with FG 6–8	MPopulation with mild irregular menstrual cycle
DHEAS (µg/mL)				
5th–95th (CI %95)	0.54–3.21 (1.41–1.58)	0.54–3.38 (1.41–1.59)	0.62–2.96 (1.33–1.87)	0.23–2.84 (1.19–1.67)
2.5th–97.5th (CI %95)	0.29–3.71 (1.43–1.6)	0.3–3.71 (1.41–1.59)	0.54–…	0.2–4.02
FT (pg/mL)				
5th–95th (CI %95)	0.60–2.40 (1.34–1.47)	0.60–2.37 (1.33–1.46)	0.67–3.40 (1.42–2.0)	0.53–2.84 (1.17–1.52)
2.5th–97.5th (CI %95)	0.50–2.92 (1.34–1.46)	0.5–2.6 (1.32–1.45)	0.6–	0.33–3.23
TT (ng/mL)				
5th–95th (CI %95)	0.20–0.70 (0.40–0.43)	0.20–0.70 (0.39–0.42)	0.25–1.0 (0.45–0.61)	0.20–0.76 (0.37–0.46)
2.5th–97.5th (CI %95)	0.1–0.78 (0.40–0.43)	0.1–0.76 (0.39–0.42)	0.2–	0.1–0.86

FG=Ferriman–Gallwey; DHEAS=Dehydroepiandrosterone sulfate; FT=Free testosterone; TT=Total testosterone; CI=Confidence interval

Table 3: Correlation between androgenic hormones and demographic-clinical finding in reproductive age women

	Normal population			Population with FG 6–8 and mild irregular menstrual cycle			Population with FG 6–8			Population with mild irregular menstrual cycle		
	DHEAS	FT	TT	DHEA	FT	TT	DHEA	FT	TT	DHEA	FT	TT
Age (year)	−0.438**	−0.27**	−0.27**	−0.267*	−0.284*	−0.185	−0.210	−0.365*	−0.159	−0.342*	−0.295*	−0.291*
Age of menstruation (year)	−0.019	−0.072	−0.034	0.116	0.214	0.059	0.050	0.188	0.143	0.168	0.233	0.012
Bleeding days	0.079	−0.009	−0.010	−0.120	−0.095	0.068	−0.211	−0.085	−0.083	−0.070	−0.108	0.131
Weight (kg)	−0.150**	0.036	−0.05	−0.021	0.208	0.050	−0.027	0.211	−0.019	−0.033	0.196	0.041
Waist circumference (cm)	−0.260**	−0.049	−0.102*	−0.040	0.120	−0.026	0.034	0.076	−0.143	−0.134	0.081	−0.065
BMI (kg/m^2)	−0.206**	0.003	−0.079	−0.107	0.125	−0.068	0.083	0.150	−0.132	−0.241	0.091	−0.100
BP-maximum (mmHg)	−0.081	−0.043	0.034	−0.179	−0.082	0.133	0.003	0.347	0.111	−0.169	−0.287*	0.052
BP-minimum (mmHg)	−0.075	0.018	0.078	−0.073	0.021	0.235*	0.099	0.235	0.265	−0.115	−0.108	0.118
FBS (mg/dL)	−0.050	0.102*	0.066	−0.055	0.174	−0.018	0.118	0.118	−0.106	−0.155	0.156	0.007
Cholesterol (mg/dL)	−0.156**	0.002	0.010	−0.349**	−0.201	−0.181	−0.336	−0.286	−0.110	−0.450**	−0.261	−0.392**
HDL-c (mg/dL)	0.059	0.108*	0.103*	−0.161	−0.181	−.088	−0.320	−0.360	−0.181	−0.205	−0.219	−0.233
LDL-c (mg/dL)	−0.100	−0.004	−0.007	−0.315**	−0.216	−0.210	−0.287	−0.248	−0.050	−0.409**	−0.273*	−0.413**
TG (mg/dL)	−0.144**	−0.034	−0.026	−0.022	0.185	−0.024	0.061	0.218	0.031	0.020	0.272*	0.029

*P–value <0.05, **P-value < 0.001. FG=Ferriman–Gallwey; DHEA=Dehydroepiandrosterone; DHEAS=DHEA sulfate; FT=Free testosterone; TT=Total testosterone; BMI=Body mass index; BP=Blood pressure; HDL-c=High-density lipoprotein cholesterol; LDL-c=Low-density lipoprotein cholesterol; TG=Triglyceride; FBS=Fasting blood sugar

Table 4: Area under the curve, sensitivity and specificity of androgenic hormones for differentiating the population with FG 6–8 score and population with mild IMC from normal population

	ROC curve				
	AUC (%95 CI)	*P*	Sensitivity (%)	Specificity (%)	Cut point
DHEA					
Population with FG 6–8	0.58 (0.48–0.68)	0.11			
Population with mild irregular menstrual cycle	0.51 (0.43–0.60)	0.66			
FT					
Population with FG 6–8	0.63 (0.52–0.73)	0.017	0.67	0.60	1.35
Population with mild irregular menstrual cycle	0.53 (0.45––0.61)	0.36			
TT					
Population with FG 6–8	0.68 (0.58–0.78)	0.001	0.67	0.60	0.49
Population with mild irregular menstrual cycle	0.5 (0.42–0.58)	0.41			

DHEA=Dehydroepiandrosterone; FT=Free testosterone; TT=Total testosterone; ROC=Receiver operating characteristic; AUC=Area under the ROC curve; CI=Confidence interval

and 0.44 [0.36-0.54]) for differentiating the population with FG 6–8 score and population with mild IMC from normal population which was not statistically significant ($P > 0.05$). Also the AUC (0.47 (0.37–0.56) for TT and AUC (0.41 (0.32–0.50) for FT was not statistically significant (both $P > 0.1$) that indicate insignifical differential rols of TT and FT between normal population and women with mild IMC.

DISCUSSION

Diagnosis of hyperandrogenemia and appropriate monitoring of its therapeutic management cannot be accomplished without laboratory studies' availability. Therefore, access to a laboratory capable of providing valid, precise, and temporally repeatable results is essential. Furthermore, detailed information about the normal or reference ranges and a precise description of the source population used to define the reference range are critical. The study reported herein addresses one of these major issues of the normal or reference range and avenues used to obtain it.[7]

Analysis of publications for the past four decades dealing with serum testosterone levels in normal control subjects and clinically hyperandrogenic women showed a clear separation between normal and hyperandrogenic subjects based on the blood testosterone levels.[25]

In our total population, the mean ± SD serum FT level was 1.40 ± 0.63 pg/mL, the TT level was 0.42 ± 0.17 ng/mL, and the DHEAS level was 1.5 ± 0.97 μg/ml. The lowest testosterone levels (0.41 ± 0.16 ng/ml) were found in the normal group. Therefore, this euandrogenic group was considered the normal study reference population. In contrast, the highest testosterone levels were noted in the subjects with FG 6–8 score of 0.53 ± 0.19 ng/ml. Thus, we reported a significant elevation of three androgenic hormones in the population with an FG 6–8 score. The result only in TT level was observed in FG 6–8 plus mild IMC group compared with the normal group. Indeed, we reported mildly hyperandrogenic women in this research. Because selecting a population devoid of any abnormality would be extremely difficult, we include some women near both sides of the normal range for mild IMC. In subjects with FG 6–8 scores, all androgenic hormones were higher than the normal population whole; this did not occur for the mild IMC. Therefore, it would be explained that mild hirsutism is categorized in the FG scoring system. However, individuals who are generally located two-sided of the normal spectrum ± 2 days are classified as a normal group. This finding is also compatible with Ayala *et al*.[7] It showed that in women with menstrual dysfunction but no acne or hirsutism, the mean testosterone level was significantly higher, with mild hirsutism, it further increased; and with moderate to severe hirsutism, it was still higher. Serum DHEAS levels showed similar patterns.[7] Several studies showed that half of the women with hirsutism have high levels of androgen hormones, and one-third of the women with levels of androgen hormones have hirsutism.[26]

In our normal population, the 5–95th percentile and 2.5–97.5th percentile values of serum TT were 0.20–0.70 ng/ml (0.39–0.42) and 0.1–0.76 ng/ml (0.39–0.42), respectively. Hashemi *et al*. in a study on Iranian women of reproductive age reported the serum level of TT and DHEA as 0.63 ± 0.6 ng/ml, and 164.3 ± 105.1 μg/ml, respectively.[27] We reported for the first time the reference range of androgenic hormones for FG 6–8 and the mild IMC group. While these percentiles were increased only for the population with FG 6–8 score, TT level was 0.25–1.0 (0.45–0.61) and 0.2. Although the IMC did not change the reference range when compared with the normal group.

Previously, a study reported an estimated 5th and 95th percentiles for women of reproductive age, TT, 15–46 ng/dL, and FT, 1.2–6.4 pg/mL.[14] The levels of total and free testosterone and DHEAS reported in our study are comparable with those reported by others, some of whom used well-validated testosterone assays and controlled for some of these factors.[12,19]

In our study, the mean BMI and TG levels in the mild IMC group were higher than the control group when combined with hirsutism. High BMI increases the risk of ovulatory dysfunction and may cause heavy menstrual blood loss.[28] In line with our findings, Van Anders and Watson showed that even in healthy women, menstrual irregularities are associated with elevated androgen levels.[29] In one study, BMI was not correlated with menstrual cycle length and menses but was positively associated with menstrual blood loss.[28] Spencer *et al*. noted that BMI had relatively little effect on SHBG, TT, or FT in their regression models.[11]

Our present findings in a large cohort of women showed a negative association between DHEAS levels and BMI in the normal population. On the other hand, we did not report any relationship between BMI and DHEAS levels in another studied group. In accordance with our data, Mazza *et al*. showed that increased BMI and insulin secretion were not associated with DHEAS reduction in women.[17] Therefore far, data in the literature concerning the relationship between BMI and DHEAS are contradictory. In fact, DHEAS levels were found not associated with BMI to be either in pre-[30] or postmenopausal[31] women by some authors. However, DHEAS reduction in obesity has been reported by others.[32]

Although weight and BMI are known to be inversely correlated with SHBG, we do not estimate that correcting

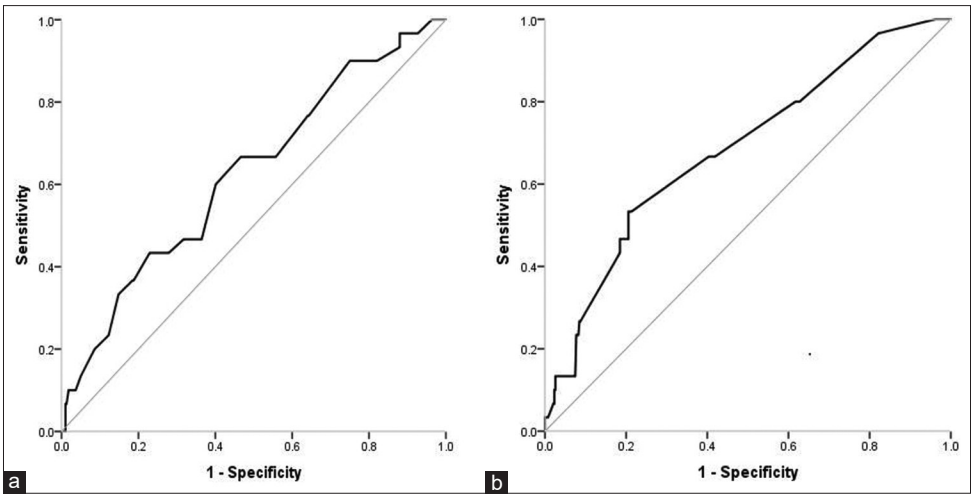

Figure 1: Receiver operating characteristic curves for free testosterone (a), and total testosterone (b) for differentiating the patients with FG 6–8 scores from the normal population

for weight or BMI would exert a considerable influence or yield a reference range that would be more useful in clinical practice.[14]

According to this point, hyperandrogenism and hyperinsulinemic insulin resistance contributed to hypertriglyceridemia in the IMC.[28] This condition could explain the TG's high level in the mild IMC group and a positive correlation between TG and FT levels in our mild IMC group.

Testosterone levels have also been shown in several,[9,19] but not all studies[12,20] decline with age in women, with a steeper decline observed in the early childbearing age. Moreover, previously, premenopausal women between the ages of 18 and 49 years presented such results;[14] besides, our data supported this hypothesis.

FT levels in all populations in this study [Table 2] were negatively correlated with age. A negative correlation was observed in normal populations with DHEAS and age. All androgens levels (DHEAS, FT, and TT) were negatively correlated with age in subjects with mild IMC. The negative influence of age on DHEAS synthesis and secretion is widely accepted.[17] The mechanisms underlying the negative effect of age in women probably include an age-dependent decrease in enzyme activity.[33] In recent years, DHEAS has received great attention. In animals and men, reduced DHEAS levels may be present in patients with different age-related illnesses, including brain aging, some forms of cancer, and disorders associated with hyperinsulinemia and insulin resistance, particularly ischemic heart disease.[17,34]

The relationships of both androgens and estrogens with individual characteristics of the metabolic syndrome (MetS) such as HTN, insulin resistance, and dyslipidemia have been reported in pre-and postmenopausal women; however, few studies have evaluated the relationship between endogenous sex hormone levels and MetS.[35] However, in our population study, we did not report any meaningful difference in FBS between the studied groups.

In our population, the mean BP was higher in hirsutism alone and was significantly higher than normal when joined to the IMC group. In this latter group, a positive correlation between TT and mean BP was observed. Recently, a meta-analysis showed that reproductive age women with polycystic ovary syndrome (PCOS) are at higher risk for HTN.[36] Testosterone levels in women with PCOS increased the risk of elevated SBP and DBP.[37] On the other hand, there is growing evidence suggesting that menstrual cycle dysfunction is linked to breast cancer, cardiovascular disease, and endometrial malignancy.[38,39]

All the above documented against the correlation of hirsutism and IMC, caused we planned to determine the reference range for two groups with the mild disturbance that will be ignored most of the time.

However, for only FG 6–8 population, a cutoff value of FT at 1.35 and TT at 0.49 was obtained to differentiate the patients with FG 6–8 from the normal population. In the previous study, a cutoff for TT was reported as 2 (nmol/L) with a sensitivity of 59.1% and specificity of 74.3% for differentiation of PCO from non-PCO patients.[40] Indeed, we reported for the first time the cutoff point of TT and FT in a population with FG 6–8 score to differentiate the normal population. Although several assays are available to measure TT, FT, and DHEAS, there are significant methodological biases between different manufacturers.[41] Finally, the sample size of this study was not sufficiently large to obtain reliable estimates of the 2.5 and 97.5 percentiles.

CONCLUSIONS AND IMPLICATIONS

In our study, the mean ± SD serum FT level was 1.40 ± 0.63 pg/mL, the TT level was 0.42 ± 0.17 ng/mL, and the DHEAS level was 1.5 ± 0.97 μg/ml, in premenopausal women between 18 and 49 years of age. Also, in a population with FG 6–8 score a cutoff value of FT at 1.35 and TT at 0.49 was obtained. Therefore, FT and TT only significant predictors of hirsutism and none of three indices have significant predictor role for differentiating population with FG 6-8 score and population with mild IMC from normal population.

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Conflicts of interest
There are no conflicts of interest.

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