

The comparison of free androgen index and serum free testosterone levels in women with hirsutism or polycystic ovary syndrome

Hirsutizmli veya polikistik over sendromlu kadın hastalarda serbest androjen indeksi ile serum serbest testosteron düzeylerinin karşılaştırılması

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ABSTRACT

Objectives: In many laboratories free testosterone can not be measured, so that free androgen index is suggested instead. The aim of this study was to compare free androgen index and serum free testosterone levels measured by radioimmunoassay in women with hirsutism or polycystic ovary syndrome.

Materials and methods: Totally 94 women referred to the polyclinics of Ankara Numune Hospital were retrospectively included. Three patient groups were composed; 55 of hirsutism, 20 of polycystic ovary syndrome and 19 of both hirsutism and polycystic ovary syndrome. Total testosterone and sex hormone binding globuline levels were measured by chemiluminescence method and free testosterone levels were measured by radioimmunoassay. Free androgen index was calculated from total testosterone and sex hormone binding globuline.

Results: There was a significant positive correlation between free testosterone and free androgen index in patients with hirsutism, in patients with polycystic ovary syndrome, in patients with hirsutism and polycystic ovary syndrome, and in total patient group [r(hirsutism)=0,597, r(PCOS)=0,617, r(hirsutism and PCOS)=0,779, r(total patient group)=0,649, P<0,01].

Receiver operating characteristics curves were drawn to assess the diagnostic power of parameters for all patient groups [For hirsutism (n=55) auROC (FT)=0,431 auROC (FAI)=0,485] [For PCOS (n=20) auROC (FT)=0,431 auROC (FAI)=0,359] [For hirsutism and PCOS (n=19) auROC (FT)=0,676 auROC (FAI)=0,669]. In our study, free testosterone and free androgen index were found useful to diagnose 'hirsutism and polycystic ovary syndrome' but not others.

Conclusion: Free androgen index can be used instead of free testosterone in hirsutism and polycystic ovary syndrome for diagnosis. *J Clin Exp Invest* 2011;2(2):152-6

Key words: Free androgen index, free testosterone, hirsutism, polycystic ovary syndrome.

ÖZET

Amaç: Birçok laboratuvarında serbest testosteron ölçümü yapılamadığı için yerine serbest androjen indeksi önerilmiştir. Bu çalışmanın amacı hirsutizmli veya polikistik over sendromlu kadınlarda, serbest androjen indeksi ile radyoimmunoassay yöntemle ölçülen serbest testosteron düzeylerini karşılaştırmaktır.

Gereç ve yöntem: Retrospektif olarak yapılan çalışmaya, Ankara Numune Eğitim ve Araştırma Hastanesi polikliniklerine başvuran toplam 94 kadın hasta dahil edildi. 55 hirsutizmli, 20 polikistik over sendromlu, 19 hirsutizm ve polikistik over sendromlu üç hasta grubu oluşturuldu. Total testosteron ve seks hormon bağlayıcı globulin düzeyleri kemilüminesans yöntemle, serbest testosteron düzeyleri radyoimmunoassay yöntemle çalışıldı. Serbest androjen indeksleri total testosteron ve seks hormon bağlayıcı globulin kullanılarak hesaplandı.

Bulgular: 'Hirsutizmli hastalarda', 'polikistik over sendromlu hastalarda', 'hirsutizm ve polikistik over sendromlu hastalarda' ve 'toplam hasta grubunda'; serbest testosteron ile serbest androjen indeksi arasında pozitif yönde anlamlı bir ilişki saptandı [r(hirsutizm)=0,597, r(PCOS)=0,617, r(hirsutizm ve PCOS)=0,779, r(toplam hasta grubu)=0,649, P<0,01].

Her hastalık grubu için parametrelerin tanılabilirliğini değerlendirmek amacıyla receiver operating characteristics eğrileri çizildi [Hirsutizm için (n=55) auROC (FT)=0,431 auROC (FAI)=0,485] [PCOS için (n=20) auROC (FT)=0,431 auROC (FAI)=0,359] [Hirsutizm ve PCOS için (n=19) auROC (FT)=0,676 auROC (FAI)=0,669]. Çalışmamızda serbest testosteron ve serbest androjen indeksi, 'hirsutizm ve polikistik over sendromu grubunda' tanılabilir olarak yararlı bulunmuştur ancak bu grup dışında tanılabilir bilgi sağlamamaktadır.

Sonuç: Hirsutizm ve polikistik over sendromu birlikteliğinde tanı için serbest androjen indeksi serbest testosteron yerine kullanılabilir. *Klin Deney Ar Derg* 2011;2(2):152-6

Anahtar kelimeler: Serbest androjen indeksi, serbest testosteron, hirsutizm, polikistik over sendromu.

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INTRODUCTION

In hypogonadal disorders of men and in hirsutism and virilism of women to measure serum testosterone levels may be very useful. In men, testosterone is mainly synthesized in Leydig cells of testis; in women it is produced in ovaries, in adrenal glands and by conversion of periferal prior (precursor) hormone. While testosterone is responsible for development of primary and secondary sex characteristics in men, excess amounts cause clinical problems in women.¹ The testosterone production in women is 5-10% of men,¹ approximately one third of it comes from ovaries and rest of it from metabolism of adrenal androgens.²

After testosterone is released, almost all of it is bound to carrier proteins. Small amount of it remains unbound. As in most biochemical parameters, the biological active form of testosterone is free form. One of testosterone binding proteins is sex hormone binding globuline (SHBG); a glycoprotein which is tightly bound to testosterone and synthesized in liver, and the other one is albumin which is bound weakly to testosterone. Free androgen index (FAI) or free testosterone index is the ratio of total testosterone (TT) and sex hormone binding globuline and a marker of anormal androgen condition. Free androgen index increase in severe acne, men type baldness, hirsutism. However increased free androgen index in early follicullary phase may be a sensitive and specific marker of polycystic ovary syndrome (PCOS) in oligomenorrhoea without obesity or hirsutism.¹⁰

The total of weakly bound testosterone and free testosterone (FT) is called biological available testosterone (bioavailable testosterone). Bioavailable testosterone is measured by selective precipitation of SHBG with ammonium sulfate. Testosterone bound SHBG is precipitated by 50% ammonium sulfate.¹ In some conditions like hirsutism to determine bioavailable testosterone levels is an important diagnostical test.

Equilibrium dialysis is regarded as reference method for free testosterone measurement and selective precipitation is most reliable method for bioavailable testosterone.¹

Free testosterone levels measured by equilibrium dialysis/ultrafiltration, radioimmunoassay (RIA) methods and calculated free androgen index values

were compared for estimation of free testosterone. In these comparisons free androgen index was found remarkably convenient in clinical assessment. In laboratories that cannot measure free testosterone, free androgen index may be used in clinical diagnose and assessment of prognosis of disease.

In this study, we aimed to ascertain whether FAI may be used instead of free testosterone in hirsutism and PCOS diagnose. The values of free testosterone levels measured with radioimmunoassay method and the values of free androgen index were compared and the relationship with hirsutism and PCOS was evaluated.

MATERIALS AND METHODS

Study Group

Totally 94 women patients referred polyclinics of Ankara Numune Hospital, 55 of them with hirsutism, 20 with PCOS and 19 with both hirsutism and PCOS were included in the study retrospectively (Table 1).

Materials

The total testosterone and SHBG levels measured by chemiluminescence immunoassay method with DXI 800 (Beckman-Coulter) hormone analyser with original reagents and free testosterone levels measured with free testosterone kit (RADIM) by radioimmunoassay were retrospectively seeked. Free androgen index was calculated by formula [FAI = (TT / SHBG) x 100]. Total testosterone and SHBG units were nmol/L in formula. However both of two parameters were measured in ng/mL, so they were converted from ng/mL to nmol/L using conversional factors. For total testosterone every results were multiplied by conversional factor 3,47 and results in nmol/L unit were obtained. For SHBG every results were divided by conversional factor 95 and results in nmol/L unit were obtained. Therefore, FAI (%) was calculated for every patient using the obtained results and formula. Calculated FAI and free testosterone levels measured by RIA were compared.

Statistical analysis

Data were statistically analyzed by SPSS 13.0 for Windows. Descriptive statistics were showed as mean \pm SD for continuous variables. The values showed normal distribution by Kolmogorov-

Smirnov test. Pearson correlation analysis and regression analysis were made to assess the relationship between parameters. Receiver operating characteristics (ROC) curves were used to determine the diagnostic power of every parameter. Every result were accepted statistically significant for $p < 0.05$.

RESULTS

As a result of correlation analysis between parameters there was a positively significant relationship between free testosterone and free androgen index [$r(\text{total patient group})=0.649$, $r(\text{hirsutism})=0.597$, $r(\text{PCOS})=0.617$, $r(\text{hirsutism and PCOS})=0.779$ $P < 0.01$] (figure 1). The most powerful correlation was found in patients with hirsutism and PCOS [$r(\text{hirsutism and PCOS})=0.779$], the weakest correlation was found in patients with hirsutism [$r(\text{hirsutism})=0.597$].

Receiver operating characteristics (ROC) curves were drawn for every disease group for assessment of the diagnostic power of every parameter. There was no diagnostic power of FT and FAI for hirsutism group and PCOS group [For hirsutism ($n=55$) $\text{auROC}(\text{FT})=0,431$ $\text{auROC}(\text{FAI})=0,485$] [For PCOS ($n=20$) $\text{auROC}(\text{FT})=0,431$ $\text{auROC}(\text{FAI})=0,359$]. The diagnostic power of free testosterone was found greater than the diagnostic power of free androgen index for hirsutism and PCOS group [For hirsutism and PCOS $\text{auROC}(\text{FT}) > \text{auROC}(\text{FAI})$] [For hirsutism and PCOS ($n=19$) $\text{auROC}(\text{FT})=0,676$ $\text{auROC}(\text{FAI})=0,669$] (figure 2). FT and FAI provided no diagnostic information except for 'Hirsutism and PCOS group', while they provided weak diagnostic information even for 'hirsutism and PCOS group'

Table 1. Ages and measured free testosterone (FT) and free androgen index (FAI) values of patients (mean \pm SD)

Diagnosis	n	age (year)	FT (pg/mL)	FAI (%)
Hirsutism	55	24.47 \pm 7.50	2.11 \pm 0.81	5.89 \pm 4.33
PCOS	20	22.65 \pm 4.50	2.07 \pm 0.74	4.35 \pm 3.03
Hirsutism and PCOS	19	23.63 \pm 5.63	2.60 \pm 0.77	7.68 \pm 4.23
Total	94	23.91 \pm 6.60	2.20 \pm 0.80	5.93 \pm 4.17

PCOS: Polycystic ovary syndrome

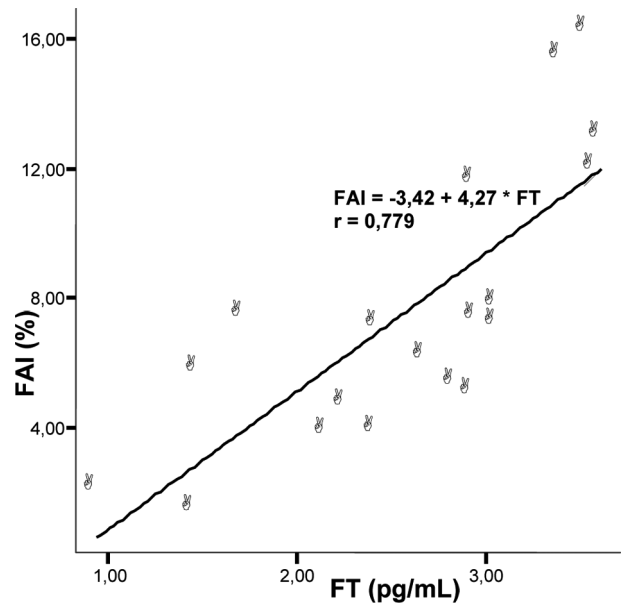


Figure 1. Relation between free testosterone and free androgen index in patients with Hirsutism and PCOS ($n=19$) ($r=0,779$, $P < 0,01$)

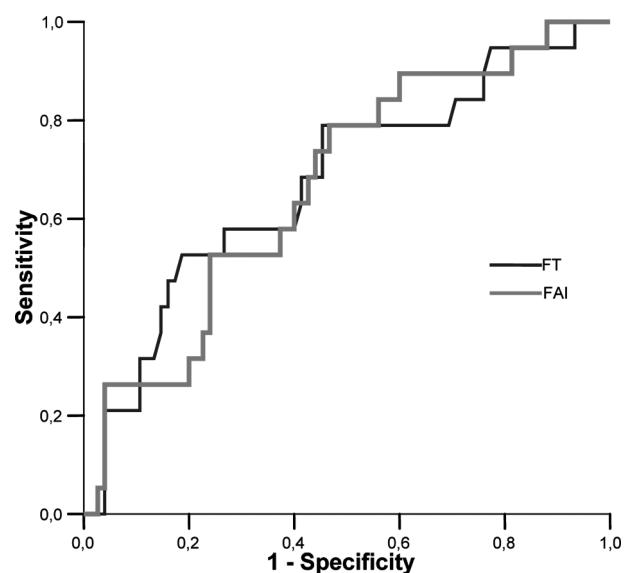


Figure 2. ROC curve for Hirsutism and PCOS ($n=19$). [$\text{auROC}(\text{FT})=0,676$ $\text{auROC}(\text{FAI})=0,669$]

DISCUSSION

In disorders of androgen metabolism the importance of free testosterone comes forward. Because measurement methods of free testosterone are difficult to practice and time-consuming or not enough automatized or not reliable, free androgen index is suggested as a marker to substitute for free testosterone in clinical diagnosis and monitoring.

On the other hand some investigators suggested mathematical models estimating bioavailable testosterone or free testosterone levels. These models used complex calculations based on law of mass action. These calculations were made with formulas using serum total testosterone, SHBG and albumin concentrations. For example Vermeulen and colleagues derived a complex calculation of free testosterone concentration, with measurement of total testosterone and SHBG concentration using also albumin concentration. Although these calculations were complicated, they showed good correlation with equilibrium dialysis and considered to be a reliable indicator of free testosterone.^{1,3} Conditions with abnormal plasma protein concentrations like nephrotic syndrome, cirrhosis and pregnancy required regulations in estimation of albumin concentration. However it was a problem that these calculations were not exactly validated. In their study radioimmunoassay and the values obtained from free androgen index were not found a reliable index of bioavailable testosterone.³

Free androgen index is a simple ratio obtained from total testosterone/sex hormone binding globulin ratio multiplied by 100. TT and SHBG are measured by automatised immunassays. FAI gives an idea about FT levels and its consistence with hirsutism is showed.⁴

Literature present contradictory results about free testosterone estimation methods. Many studies which compared present methods in various patient groups obtained different results.^{3,13} There is no agreement on a parameter of an accepted reliable index of free testosterone estimation.

In this study, in women with hirsutism or PCOS, free androgen index and free testosterone measurement by RIA were compared and the investigation of usability of free androgen index instead of free testosterone measurement by RIA was aimed.

Wilke and Utley⁴ made a study to compare measurement methods of free testosterone. In their study, they found perfect correlation between FAI and calculated free testosterone in hirsute women and showed both of them were providing nearly identical information.

Christ-Crain and colleagues⁵ calculated testosterone in old men with androgen deficiency with various methods and researched their clinical con-

cordance in their study. Calculated free testosterone showed good concordance with free testosterone measured by RIA and FAI. Measurement with RIA, FAI and bioavailable testosterone was not found a reliable index of free testosterone in old men with androgen deficiency.

Morris and colleagues⁶ made a cohort study consist of 1072 men. In this study where the measurement of free testosterone cannot be made, the feature of free androgen index to show the free testosterone status was researched. In the study total testosterone and SHBG levels were determined by ELISA, bioavailable testosterone was measured with precipitation of total testosterone with ammonium sulfate. In the study free androgen index, free testosterone with Nanjee-Wheeler method and Vermeulen method were calculated. All methods were found having similar efficiency. FAI was found to be the worst predictor for hypogonadism and for bioavailable testosterone. In the study total testosterone was declared the best marker of hypogonadism and bioavailable testosterone but in borderline it was not found adequate so in these conditions free testosterone measurement by equilibrium dialysis or bioavailable testosterone estimation with ammonium sulfate was suggested.

In their study Morimoto and colleagues⁷ found FAI was parallel to plasma free testosterone and useful to assess androgen status. Qiao and colleagues⁸ in their study at 51 women with hirsutism, suggested that besides testosterone and DHEAS, measurements of SHBG and FAI were necessary in clinical practice.

Brannian and colleagues⁹ found in their study that both in women and in men FAI showed good concordance with FT. They suggested that FAI could be an alternative of FT measurement in the diagnosis of oligomenorrhea and hirsutism in women. Eden and colleagues¹⁰ researched a group of women with oligomenorrhea without obesity and hirsutism with ultrasonography, laparoscopy and biochemical parameters. In these women who had not classical symptoms, PCOS was diagnosed with ultrasound and laparoscopy and it was a common cause of oligomenorrhea and strongly related with elevated FAI. It was stated that to investigate FAI also with standard tests seemed to be reasonable in women with oligomenorrhea without hirsutism.

Urdl and colleagues¹¹ made a study about utility of free androgen index in androgenization in women. In 63 patients with androgenization symptoms, the severity of these symptoms was compared with free androgen index. Also they were compared with control group. This study put forward that there was a significant relationship between severity of these symptoms and free androgen index. At the same time in these individuals free testosterone levels were also found increased.

In their study Hahn and colleagues¹² compared the values of different androgen determinations for PCOS diagnosis in 133 PCOS patients. They compared sensitivities and specificities using ROC curves. Calculated testosterone indexes (bioavailable testosterone, FAI, free testosterone) were found useful parameters in PCOS patients. FAI and bioavailable testosterone were found significantly correlated with TT, androstenedione, LH/FSH ratio and DHEAS. FAI, bioavailable testosterone and FT were found significantly correlated with hirsutism scores, ovarian volume and follicle number.

Vermeulen and Kaufmann¹³ stated that FAI was inadequate to assess hypogonadism in men. In their study they suggested that the best ways to measure FT and bioavailable testosterone values were equilibrium dialysis and precipitation with ammonium sulfate. Because these methods are time-consuming and are not automatized systems, they remarked the difficulty of practice. In their study they presented calculated free testosterone alternatively to methods equilibrium dialysis and precipitation with ammonium sulfate.

Free androgen index is a parameter that is practical, easy to calculate, valuable in assessment of hirsutism and PCOS. But it is not adequate to use in every diagnose group. While it is an appropriate parameter for hirsutism and PCOS it is not found consistent to determine hypogonadism in men.¹³ It is significantly concordant with free testosterone values measured by RIA. But some studies reported that both RIA method and FAI showed weak correlation with equilibrium dialysis which is reference method for free testosterone measurement and selective precipitation which is a reliable estimation method of bioavailable testosterone.^{3,13}

In our study, we compared free androgen index with serum free testosterone values measured by RIA in women patients with hirsutism or polycystic

ovary syndrome. In statistical analysis of the study we saw that there was a significant convenience between free testosterone and free androgen index. We can suggest that free androgen index can be used instead of free testosterone as a practical method where there is no condition of measuring free testosterone.

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