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RESEARCH ARTICLE

An observational analysis of blood and urine testosterone in diagnosis of polycystic ovarian syndrome

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Abstract

Background: An important diagnostic tool for the diagnosis of Polycystic ovarian syndrome (PCOS) in women is elevated blood testosterone levels. The presence of testosterone in the urine is very likely to support the PCOS diagnosis; however, more investigation is required to determine whether blood testosterone levels are associated with PCOS. **Objective:** A total of 30 PCOS-positive women participated in this observational study using a diagnostic test approach. **Method:** The ELISA technique was used to measure the amounts of testosterone in the woman's urine and serum. **Result:** The levels of testosterone in the urine of 30 PCOS women were lower than those in the serum, with values of 2.688 nmol/L and 8.067 nmol/L, respectively. The Spearman correlation test findings revealed a value of 0.39 at a significance level of 0.05, with a cut-off value of 2.6010 nmol/L at sensitivity and specificity of 0.625 and 0.571, showing the importance of urine testosterone levels in detecting hyperandrogenic PCOS patients. **Conclusion:** In women with PCOS, serum and urine testosterone levels are correlated.

Introduction

Polycystic ovary syndrome (PCOS) is an ovarian dysfunction syndrome that often conflicts with systemic insulin resistance, characterised by polycystic ovarian morphology. European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) in 2003 oligo/anovulation; and clinical or biochemical evidence of hyperandrogenism (Fauser, 2004). This classification has now been used for more than ten years. Although the principle is still valid, each of the three criteria must be updated (Dewailly, 2016). Therefore, it is necessary to evaluate the diagnostic criteria for PCOS women.

Hyperandrogenism is an important feature and one of the three main criteria for diagnosing PCOS. Clinical hyperandrogenism is usually defined as androgenic hirsutism and alopecia. Increased total testosterone, androstenedione, free testosterone, and free androgen index (FAI) are generally used to identify hyperandrogenaemia (Zhou *et al.,* 2017). The ratio of total testosterone to dihydrotestosterone correlates with a worse metabolic profile in PCOS patients and healthy subjects without hyperandrogenism (Ambroziak *et al.,* 2017). In comparative studies of clinical and hormonal characteristics in the phenotype of PCOS patients, it is also known that patients who suffer from PCOS have the highest testosterone levels compared to other phenotypes, while the patients that only have oligo-/anovulation and polycystic ovary morphology criteria without the presence of hyperandrogenic testosterone levels are relatively the same as testosterone levels in regular patients (Jamil *et al.,* 2016). Based on these facts, determining testosterone levels in the blood is essential in supporting the diagnosis of PCOS patients.

About 66% of blood circulating testosterone is bound to sex hormone-binding globulin (SHBG). With a lower affinity bound to albumin by 33%, so serum testosterone levels are highly dependent on the number of bonds with SHBG and albumin. The combination of free testosterone and albumin-bound testosterone is a bioavailable form of testosterone. In women, this bioavailable testosterone is found in nanomolar to micromolar concentrations (Korkidakis & Reid, 2017).

Determination of testosterone levels for PCOS diagnosis continues to be done to find the most rapid, easy, accurate, and unnecessary method to hurt patients. So far, the blood specimen is the *best* diagnostic criterion for providing an accurate picture of the patient's condition (Sheehan 2014). However, taking a patient's blood is considered to be hurting the patient and providing a feeling of discomfort. For this reason, researchers are looking for alternative samples that adequately represent the picture of the patient's condition without hurting or making the patient feel uncomfortable, for example, using urine samples.

Detection of hyperandrogenemia biomarkers in the form of total testosterone in urine is very likely to support the diagnosis of PCOS (Dadachanji, Shaikh & Mukherjee). However, there is a need to prove its correlation to testosterone levels in the blood. For this reason, further research in order to determine the role of testosterone levels in urine as a diagnostic support for women with PCOS is paramount. In this study, one of the methods that will be used to determine the role of measurement of testosterone levels in urine is to look for a cut-off value of urine testosterone levels in distinguishing hyperandrogenic and non-hyperandrogenic PCOS patients based on gold standard testosterone levels in the blood.

Methods

Design

This study is observational with a diagnostic test approach. The consent has been obtained by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga. Every patient who reads the sample criteria and is willing to participate in this study has been given enough information and has signed an informed consent agreement as a respondent. In this study, the research subjects to be observed were 30 patients who were diagnosed with PCOS based on a pre-determined inclusion criterion These criteria include any two of the following criteria: polycystic ovaries oligo/anovulation, clinical manifestation and biochemical evidence of hyperandrogenism. Polycystic ovaries on ultrasonography show 12 or more follicles measuring 2-9mm and ovarian volume > 10 cm^3. Menstrual cycle disorder can also be termed as the absence of regular flow (oligomenorrhea, known as more than six cycles per year with a length of more than 36 days) when the patient does not experience menstrual bleeding for three consecutive months, for one year, obesity with a BMI value >25kg/m^2 (Connor, 2022). Typical signs of hyperandrogenism include hirsutism, alopecia, acne, and acanthosis nigricans.

Assessment

Determination of testosterone levels in blood and urine at the Institute of Tropical Disease Universitas Airlangga. The research sample collection was conducted in Surabaya. A total of 30 PCOS patients were obtained by non-probability purposive sampling, where each sample was determined based on pre-determined inclusion criteria. Testosterone levels in this study were determined using the human testosterone ELISA kit bioassay technology laboratory, with the iMark Microplate absorbance reader, Bio-RAD Instrument, equipped with microplate manager® 6 Software. Samples were taken in the form of blood and urine. Each piece was prepared according to the procedure in the ELISA Kit. All standards, samples, and controls were carried out simultaneously so that all test conditions were the same.

Results

The results of showing the characteristics of the research subjects can be seen in Table I. The 30 respondents were successfully collected with features of mean age of 29.57 ± 4.15 years, and body mass index (BMI) 30.22 ± 3.39 kg / m^2. On ultrasonography, 30 individuals exhibited polycystic ovary morphology, out of whom 21 had anovulation disorders, and eight individuals showed signs of hyperandrogenism. Using the Rotterdam criteria, PCOS patients were divided into four phenotypes, namely phenotype A, which had every PCOS criteria; phenotype B had the criteria for menstrual disorders in the form of oligo-/ anovulation and clinical signs of hyperandrogenism. Phenotype C had clinical evidence of hyperandrogenic and polycystic ovary morphology; as well as phenotype D which had oligo-/anovulation and ovarian morphology (Dewailly, 2016). Based on the division of the phenotype group, in this study, there were six patients with phenotype A; none of the respondents had a type B phenotype; Nine patients with phenotype C where

seven of them had a high BMI though the signs of hyperandrogenism are not very clear; and 15 patients with phenotype D. It was shown that the 15 patients with clinical signs of hyperandrogenism were phenotypes A and C, and 15 others did not have any clinical sign of hyperandrogenism. From these data, The normality test using the Shapiro-Wilk test indicates that the blood testosterone level data is not normally distributed, with a *p*-value = 0.000. urinary testosterone levels were usually distributed with $p = 0.429 > 0.05$. So based on the results of the normality test above, the correlation test uses a non-parametric correlation coefficient that is Spearman's rho.

Table I: Summary of the clinical data of research subjects

Parameter	Total
Age (year)	29.57±4.15
BMI (kg/m2)	30.22±3.39
Polycystic ovary	30
Anovulatory disorder	21
Signs of hyperandrogen	8

The clinical study will be carried out in this research to find the value of cutting data on urine testosterone levels in PCOS patients who add hyperandrogenism or not based on the value of testosterone levels in the blood. Blood testosterone levels are considered the gold standard or reference for distinguishing female patients with PCOS from those with hyperandrogenism but not using PCOS. In various studies, the cutoff values for blood testosterone levels were reported as 2.37 nmol/L (Hahn et al., 2007) and 2.39 nmol/L (Zhao et al., 2018). These values are used to differentiate between the two conditions and aid in accurate diagnosis and treatment decisions. The cut-off value to be used is the lowest, which is expected to provide a high sensitivity and specificity value.

Testosterone levels can be used to determine whether or not the subject has PCOS with hyperandrogenism. The test results showed that the positive subjects were hyperandrogenic PCOS. Therefore, the ROC (receiver operating characteristics) analysis was performed on urine testosterone levels. The level of urine testosterone is determined based on the highest sensitivity and specificity values from the ROC curve results (Figure 1). The analysis shows that the cut-off value of urine testosterone levels of 2.6010nmol/L has the highest sensitivity and specificity values of 0.625 and 0.571.

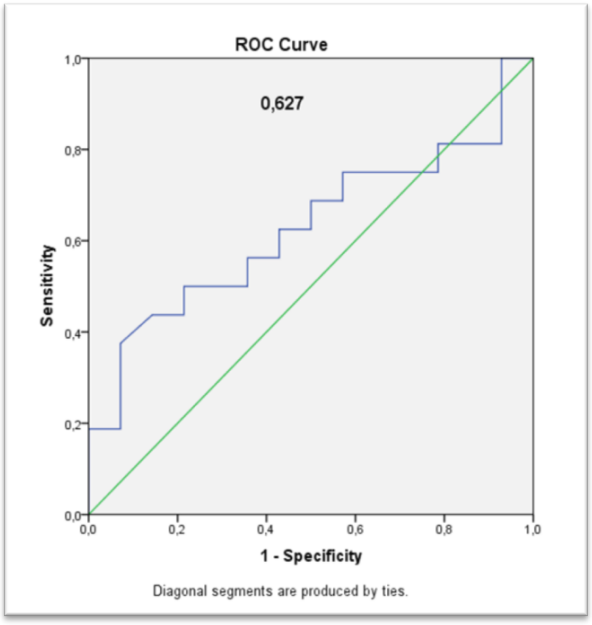

Figure 1: ROC curve of urine testosterone levels with the best sensitivity and specificity values of 0.625 and 0.571

Discussion

This study focuses on the biochemical analysis of hyperandrogenism in determining testosterone levels in blood and urine in women with PCOS. Determining blood and urine levels aims to find alternatives to blood that can be used to represent the patient's clinical picture. This study involved respondents diagnosed with PCOS in the Surabaya region and was by established inclusion criteria. Determination of testosterone levels that have been carried out on 30 blood and urine samples obtained the average value and standard error for blood and urine testosterone levels of 8.067 ± 2.129 nmol/L and 2.688 ± 0.127 nmol/L respectively. According to Hahn and co-authors in 2007, the values obtained from average blood testosterone levels in patients with PCOS was 2.8 nmol/L (Hahn *et al.,* 2007), and in the same year blood testosterone levels in adolescent PCOS women with a value of 0.99mg/mL or equivalent to 3.44 nmol/L (Chen *et al.,* 2007). Although both studies have different results, all researchers have similar results; namely, blood testosterone levels have higher values than ordinary people in the 0.6-2.5nmol/L range for blood testosterone levels (Burger, 2002). In another study, the testosterone level in the urine of PCOS women obtained an average level of 3.06nmol/L, whereas, in intermediate patients, the average level of urine testosterone was 1.50 nmol/L (Wang *et al.,* 2015). Based on the average values obtained from this

study, the study subjects' blood and urine testosterone levels showed more blood and urine testosterone levels than usual.

The main objective of this research is to find a correlation between blood and urine testosterone levels and to analyse the role of measuring testosterone levels in diagnosing patients suspected of having PCOS, especially the measurement of testosterone in urine as an alternative to measuring blood testosterone. Correlation testing between blood and urine testosterone levels was performed using Spearman's rho correlation test (Table II). Correlation test results on the 30 blood and urine samples obtained correlation coefficient values of 0.391 (p = 0.033; $p < 0.05$). Based on the significance value or p-value, it can be stated that there is a correlation between blood testosterone levels and urine testosterone levels.

Table II: Non-parametric correlation analysis results

			Serum	Urine
Spearman's rho	Serum	Correlation coefficient	1	0.391*
		Sig. (2-tailed)	.	0.033
		N	30	30
	Urine	Correlation coefficient	0.391*	1
		Sig. (2-tailed)	0.033	.
		N	30	30

* Correlation is significant at the 0.05 level (2-tailed)

Based on the gold standard of blood testosterone levels that have been determined previously, we obtained two groups: the first group of subjects with blood testosterone values ≥ 2.37nmol/L and subjects with blood testosterone values of less than 2.37 nmol/L. From this disaggregation, 16 PCOS patients had hyperandrogenic conditions, and the rest did not have hyperandrogenic requirements. Based on patient clinical data, six out of eight patients with clinical signs of hyperandrogenism had their blood testosterone values above the limit, while the rest have values below the limit. It shows that the value of testosterone levels in the blood can reflect the clinical condition of hyperandrogenism.

Groups of PCOS patients who had been differentiated based on blood gold standards were then analysed using the ROC method of urine testosterone levels. The ROC analysis results showed inTable II that the cut-off value of urine testosterone levels of 2.6010nmol/L had the highest sensitivity and specificity values, namely 0.625 and 0.571. Despite the AUC value of the ROC curve, urine testosterone levels are in the weak category, with a value of 0.627 or 62.7%. Although included in the weak category, this interrupted value is significant because this value can help diagnose PCOS patients, especially those with hyperandrogenic conditions. Determining the limit value in urine testosterone is expected to assess the patient's condition in hyperandrogenic cases.

Conclusion

There is a correlation between serum and urine testosterone levels in women with PCOS. PCOS patients with hyperandrogenic conditions can be determined by determining their testosterone levels in urine.

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