Biochemical Evidence of Overweight, Androgen Excess and Hyperinsulinaemia in Women with Polycystic Ovarian Syndrome in Nauth, Nnewi, Nigeria

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is the most frequent androgen disorder of ovarian function. This was a cross-sectional study designed to evaluate the levels of sex hormone-binding globulin (SHBG), fasting insulin and some select androgens in women with polycystic ovarian syndrome (PCOS) in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria.

Materials and Methods: Seventy four (74) women aged between 18-40 years were conveniently selected. 37 of them attending the gynecological clinic were diagnosed with PCOS based on the Rotterdam criteria. The remaining 37 participants were apparently healthy hospital staff recruited as control. Blood sample was collected from all the participants for determination of SHBG, fasting insulin, testosterone, dehydroepiandrosterone sulfate (DHEA-S) and estradiol (E2) levels using standard laboratory methods.

Results: Fasting insulin, testosterone, DHEA-S in participants with PCOS were significantly higher compared with their corresponding control participants ($p = 0.005, 0.001, 0.001$ respectively), while SHBG in participants with PCOS was significantly lower compared with the control participants ($p = 0.002$). Body mass index (BMI) and free androgen index ratio (FAI) were significantly higher in participants with PCOS compared with their corresponding controls ($p = 0.043, 0.001$). Waste/hip ratio showed a strong negative correlation with levels of SHBG and E2 in participants with PCOS ($p = 0.025, 0.015$, $r = -0.368, 0.397$ respectively), while BMI showed a strong negative correlation with E2 in participants with PCOS ($r = -0.374$, $p = 0.023$).

Conclusion: Significantly higher BMI value shows evidence of obesity and or overweight in female participants with PCOS. Significantly higher levels of testosterone, DHEA-S, fasting insulin, FAI, with lower SHBG suggests androgen excess and hyperinsulinemia which may subsequently predispose the women to type II diabetes.

Keywords: *Overweight, androgen excess, hyperinsulinaemia, women, polycystic ovary*

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Introduction

Polycystic ovarian syndrome (PCOS) is the most frequent androgen disorder of ovarian function. It is the most commonly encountered endocrinopathy in women of reproductive age. It has significant reproductive and nonreproductive consequences [1]. Diagnostic criteria for

PCOS mostly use the revised Rotterdam 2003 criteria [2]. However, the etiology and pathophysiology of PCOS remain unclear, and the multiple risk factors such as genetics, environment, nutrition, lifestyle, and much more are still under investigation. There is heterogeneity of symptoms and in severity of disease but most have central obesity or android fat deposition (fat at abdominal wall and viscera). Android fat deposition is relatively resistant to insulin hormone [3]. According to [4]central obesity is diagnosed when waist circumference is more than 80 centimeters for women. However, women of any ethnic background can present with PCOS. Both of central obesity and androgen excess in PCOS accelerates insulin resistance which promotes incidence of diabetes mellitus [5]. Hyperinsulinemia with insulin resistance is believed to be highly associated the symptoms of PCOS including anovulatory, infertility and hyperandrogenism and these symptoms can be reversed by reducing the hyperinsulinaemia [6]. This study therefore, was designed to investigate some biochemical evidence of overweight, androgen excess and hyperinsulinaemia in women with PCOS in Nnewi, Nigeria.

Materials and Methods

This was a cross sectional study, a total of seventy four (74) women within the ages of 18 and 40 years were recruited at the Gynecology Clinic, Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria, using convenient sampling technique. They include thirty seven (37) women with mean age (29.00±6.0) attending the Gynecology Clinic at the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria in whom ultrasound and clinical diagnosis of PCOS have been established and based on Rotterdam criteria. This criterion states that for PCOS to be established, two out of the following three criteria must be present: (i) Oligo- and/ or anovulation, (ii) hyperandrogenism (clinical and/or biochemical) and (iii) polycystic ovaries. Thirty seven (37) apparently healthy women recruited among the staff served as control. A well structured questionnaire was used to ascertain the reproductive history and biodata of the participants. All the participants were all in Nnewi metropolis.

Inclusion and Exclusion criteria

Women with evidence of anovulation, hyperandrogenism and polycystic ovaries who are between the ages of 18 and 40 years were included in the study. Women outside the age bracket of 18 and 40 years were excluded; women with metabolic and cardiovascular disorders, Pregnant and lactating mothers and women on contraceptive pills were also excluded from the study. Data was collected through the use of questionnaires, anthropometric measurements and biochemical analysis of blood samples. Ultrasound was also done to obtain ovarian morphology.

Anthropometric measurements

The Physical measurements (body weight and height) was taken using a standard beam balance scale and a stadiometer respectively, participants were advised to wear light clothing with no shoes for an accurate measurement, values obtained was used to calculate BMI (kg/m^2) [weight (kg)/(height)^2 (m^2)]. Blood pressure (systolic and diastolic) was also measured using standard clinical mercury sphygmomanometer to rule out any cardiovascular disorder.

Collection of blood samples

About 5ml of fasting venous blood was collected aseptically from each participant by venopuncture technique from the cubital fossa and dispensed into properly labeled plain containers. The samples were allowed to clot and centrifuged at 4,000 rpm for 10 minutes. The serum was extracted and transferred into properly labeled tubes and stored at -20^{o}c.

Laboratory methods

The reagents were commercially obtained and the standard operating procedures were strictly adhered to base on manufacturer's instructions. All the parameters were determined using Enzyme-linked immunosorbent assay (ELISA) methods.

Sex hormone-binding globulin (SHBG) was done as described by Ly and Handelsman [7]. Testosterone was done as described by Ganie *et al,* [8]. Dehydroepiandrosterone-sulfate (DHEA-S) was done as described by Elmlinger *et al* [9] and Estradiol as described by Liu *et al* [10]. Determination of Fasting insulin level was done as described by Jolanta *et al* [11], while FAI was calculated by the use of the equation, 100 × T/SHBG.

Statistical analysis

The collected data was analyzed using the Student's t-test and mann-Whitney test. Values were deemed statistically significant if p value ≤ 0.05, Pearson correlation coefficient was used for correlation of the parameters.

Results

Levels of some anthropometric parameters in participants with PCOS and control group

The mean values of age (years), waist circumference (cm), hip circumference (cm), waist/hip ratio (cm), systolic blood pressure (mmmHg), diastolic blood pressure (mmHg) and BMI (kg/m^2) in participants with PCOS were (29.00±6.00, 84.27±15.74, 100.03±17.08, 0.84±0.06, 110.27±9.35, 74.32±7.65, 26.70±4.61) compared with control participants (28.43±4.20, 80.65±9.49, 98.35±8.32, 0.82±0.07, 108.11±8.45, 72.84±8.21, 24.77±3.35) (*P = 0.100, 0.234, 0.593, 0.120, 0.300, 0.423, 0.043*) respectively, only BMI was significant higher in PCOS women compared with controls (Table 1).

Levels of steroid hormones in participants with PCOS and control group

The mean serum SHBG (ng/ml) level in participants with PCOS (1.69±4.29) was significantly lower compared with control participants (4.54±3.08) (*P = 0.002*). On the contrary, the mean serum testosterone (ng/dl), DHEA-S (µg/dl), levels in participants with PCOS (107.43±28.08, 243.97±96.04) were significantly higher compared with control participants (35.46±17.69, 127.03±47.54) *(P = 0.001, 0.001* respectively). However, the mean E2 (mlU/ml) level was not significantly different between PCOS and control participants (107.08±98.26) (*P = 0.507*) (Table 2).

Correlation of waist/hip ratio, BMI, SHBG and Estradiol in participants with PCOS

Waist hip ratio was negatively correlated with SHBG and estradiol in participants with PCOS (r = -0.368, -0.397) (*P = 0.025*, 0*.015*) respectively. Similarly, there was a strong negative correlation between BMI and estradiol in participants with PCOS (r = -.374, *P = 0.023*) (Table 3).

Table 1. Levels of some anthropometric parameters in participants with PCOS and control group

Parameters	PCOS	Control group	T-test	P-value
Age	29.00±6.00	28.00±4.20	1.800	0.100
Waist circumference (cm)	84.27±15.74	28.00±4.20	1.199	0.234
Hip circumference (cm)	100.03±17.08	98.35±8.32	0.537	0.593
WHR	0.84±0.06	0.82±0.07	1.574	0.120
BMI (kg/m2)	26.70±4.61	24.77±3.35	2.059	0.043*
SBP (mmHg)	110.27±9.35	108.11±8.45	1.044	0.300
DBP (mmHg)	74.32±7.65	72.84±8.21	0.806	0.423

Key: WHR- Waist/Hip Ratio, DBP- Diastolic Blood Pressure, BMI- Body Mass Index PCOS- Polycystic Ovarian Syndrome, SBP- Systolic Blood Pressure

Table 2. Levels of steroid hormones in participants with PCOS and control group

Parameters	PCOS	control group	T-test	p-value
SHBG (ng/ml)	1.69±4.29	4.54±3.08	-3.283	.002*
Testosterone (ng/dl)	107.43±28.08	35.46±17.69	13.193	0.001*
DHEAS (µg/dl)	243.97±96.04	127.03±47.54	6.638	0.001*
E2 (mlU/ml)	91.52±102.37	107.08±98.26	-0.667	0. .507
Insulin (mlU/l)	6.31±3.51	4.51±1.01	3.001	0.005*
FAI	153.10±85.19	18.91±26.53	9.148	0.001*

Key: SHBG - Sex Hormone-Binding Globulin, DHEA-S- Dehydroepiandrosterone Sulphate E2-Estradiol

Table 3. Correlation of waist/hip ratio, BMI, SHBG and E2 in participants with PCOS (n=37).

Parameter	r	p-value
WHR vs SHBG	-0.368	0.025*
WHR vs E2	-0.397	0.015*
BMI vs E2	-0.374	0.023*

Key: WHR- Waist/hip ratio, BMI-Body mass index, SHBG- Sex Hormone-Binding Globulin E2- Estradiol, r- Pearson Correlation Co-efficient.

Discussion

Polycystic ovary syndrome (PCOS) is a common cause of ovarian dysfunction in women of childbearing age. Among the most frequent symptoms of PCOS are anovulation, infertility and hyperandrogenamia which is produced by the presence of Hyperinsulinemia with constant occurrence of insulin resistance in the affected individuals [6]. This study was carried out on women with PCOS who are at their peak reproductive age of 18 - 40 years. In this study, majority of the women who had PCOS were within the age group of 20-31 years, with mean age of 29.00±6.00 years. This is in line with the work done by Igwegbe *et al,* [12]

The higher incidence of overweight observed in this study may be attributed to the food habits adopted and lack of exercise among the participants. This is consistent with previous studies [13]. Markopoulos reported that approximately half of women with PCOS are obese or overweight and obesity plays an important role in the development of hyperandrogenic state in PCOS individuals. Lack of exercise and inadequate fiber intake has been highly implicated in the onset and development overweight in young females in Nigeria [13], and this could subsequently result to variation in steroid hormones hence, PCOS [14]. Several women with PCOS become overweight just before or during puberty and this

could lead to onset of obesity in this period of life which may further develop to PCOS [15]. It has been shown that more than 5-10% weight loss improves fertility and menstrual cycles in women with PCOS [16]. Adam and Edmonds, [17] also reported that improvement in lifestyle with a combination of exercise and diet to achieve weight reduction is important to enhance the prospects of both spontaneous and drug induced ovulation.

The significantly lower SHBG level in participants with PCOS could be as a result of increase testosterone and or excess body weight. Increased body weight has been significantly implicated in the variation of clinical and hormonal presentation of PCOS [5]. Moran *et al.,* [18] has attributed the cause of PCOS to increased GnRH frequently due to hyperinsulinemia leading to reduction in SHBG level. There are often low levels of sex hormone-binding globulin in particular among obese or overweight women [19]. Martinez-Garcia and colleagues reported reduced serum SHBG level, which regulates the bioavailability of androgens to target tissues resulting in high levels of androgens in PCOS patients [20]. SHBG has been shown to be inversely correlated with body mass index in both premenopausal and post-menopausal obese women [21]. However, it has also been established that other factors such as diet, body fat distribution and insulin could be responsible for metabolic regulation of SHBG thereby; increasing free androgen levels in PCOS patients [22]. Furthermore, the decreased SHBG has been also reported to increase the metabolic clearance rate of circulating SHBG-bound steroids [23].

The mean level of testosterone in participants with PCOS was significantly higher compared with control participants. This could be attributed to inadequate fiber intakes, ovarian or adrenal dysfunction [24]. Earlier study by Teede *et al*., [25] also reported increased serum androgen levels including testosterone and androstenedione in participants with PCOS. Previous study similarly, revealed that the concentration of testosterone are higher in some obese than non-obese women with PCOS [26]. FAI of the ratio of testosterone to SHBG was also significantly high in this study. It has however, documented that about half the total testosterone is tightly bound to SHBG while the other half to albumin, causing reduction in SHBG and increasing the free testosterone level in PCOS patients [27].

The mean DHEA-S level was also significantly higher in participants with PCOS compared with control participants. This could be attributed to increased body weight including ovarian and adrenal dysfunction. The main source of androgen in women with PCOS is the ovary [5]. Another study by Christodoulaki *et al*, [28] also reported a mildly increase in DHEA-S level in women with PCOS. It has been however, documented that peripheral testosterone, androstenedione and DHEAS levels were significantly reduced by weight loss in obese women with PCOS [5]. This therefore, confirms the role of obesity in the development of hyperandrogenism observed in women with PCOS. Similarly, it shows that weight loss increases the SHBG levels, and significantly modified the clearance rate of testosterone and its free fraction in obese PCOS women [23]. This further confirms the effect of weight loss in the reversal of most of the possible risk factors that may predisposes women to androgen excess with subsequently development of polycystic ovary syndrome.

This study also showed that the mean level of insulin in participants with PCOS was significantly higher compared with control participants. This may result from excess body weight as was observed in this study and may predispose the affected individual to type II diabetes. The observation was similar to the earlier study by Richard [29]. The author reported that women with PCOS have increased prevalence of insulin resistance and type II diabetes, even when controlling for body mass index (BMI). Nafiye *et al,* [30] also reported that serum insulin and insulin resistance are higher in participants with PCOS. Insulin has shown to be an important indicator in the regulation of ovarian steroidogenesis [31]. Higher level of serum insulin was reported as a possible cause of increase incidence of hyperandrogenism and reduced SHBG in women with PCOS [23]. Furthermore, increased production of endogenous androgens in women by insulin could be through regulation of SHBG synthesis and metabolism [32]. Insulin has been involved in the inhibition of SHBG thereby, acting as a potent regulator of SHBG concentrations invivo [33]. An inverse correlation between insulin and SHBG concentration has been reported in both pre and postmenopausal women [34]. Waist/hip ratio was also shown to be negatively correlated with SHBG and estradiol in participants with PCOS. This was in line with the previous finding by Sieminska *et al*, [35].

Conclusion

Findings from this study have shown significant evidence of overweight and hyperadrogenaemia (androgen excess) with hyperinsulinamia in women with PCOS. This might subsequently predispose the affected women to type 11 diabetes mellitus and subsequent cardiovascular diseases if not adequately managed. Early identification of patients at risk and prompt initiation of therapies is strictly advocated by including routine analysis of select androgens and fasting insulin in fertility clinics. Proper diet and routine exercise for weight management is also encouraged to ameliorate the metabolic consequences of PCOS in young females.

Ethical consideration and informed consent

Ethical approval for this research work was obtained from the board of ethics committee of Nnamdi Azikiwe University Teaching Hospital, (NAUTHEC), Nnewi, Nigeria. Written Informed consent of the participants was obtained before enrollment into the study. They were assured of confidentiality of the information obtained from them during and after the study.

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Conflict of Interest – NIL

Source of Funding- Self

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