Research Article
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Levels of insulin and testosterone in women with polycystic ovarian syndrome (PCOS) in ekiti sate, nigeria

Abstract

Polycystic Ovarian Syndrome (PCOS) is a hormonal disorder that involves multiple organ systems within the body characterized by irregular menstrual cycle, infertility (inability to get pregnant), development of cysts (small fluid-filled sacs) in the ovaries. This study was carried out to determine the levels of insulin and testosterone in women with PCOS in Ekiti State, Nigeria. Freshly diagnosed PCOS subjects (n=100) and healthy control subjects (n=50) from Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti and Federal Medical Centre, Ido-Ekiti, Ekiti State, Nigeria were used for the study. Insulin and testosterone are assessed in the subjects' blood sample using enzyme-linked immunosorbent assay (ELISA). Menarche age and body mass index (BMI) of the PCOS patients were also determined. Results were subjected to statistical analysis ($p<0.05$). Insulin and testosterone levels increased significantly inclusive of Body mass index (BMI) but Menarche age of PCOS subjects ($p<0.05$) decreased significantly. This study revealed that increase in BMI, testosterone and insulin are part of the major parameters to be tested in the diagnosis of PCOS while menarche age is equally implicated.

Keywords: polycystic ovarian syndrome, polycystic ovary, body mass index, testosterone, insulin and menarche age

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Introduction

Polycystic ovarian syndrome (PCOS) is the most common female endocrine disorder, affecting approximately 5%-10% of all females and 4-6% of adolescent girls and young women. PCOS is a hormonal disorder that involves multiple organ systems within the body and is believed to be fundamentally caused by insensitivity to insulin.[1] It can be diagnosed in all phases of life - in girls as young as 8-9 years of age through post-menopausal females. Although PCOS is one of the leading causes of infertility, the reproductive aspects of the disorder are secondary. PCOS is not limited to women of reproductive age or potential.[2] Polycystic ovary syndrome (PCOS) is a set of symptoms due to elevated androgens (male hormones) in women.[3] Many components are involved in the pathophysiology of the syndrome, especially markers of insulin resistance and hyperandrogenism. Although adult diagnostic criteria have become more refined; adolescent diagnosis remains obscure regardless of BMI.[4] signs and symptoms of PCOS include irregular or no menstrual periods, heavy periods, excess body and facial hair, acne, pelvic pain, difficulty getting pregnant, and patches of thick, darker, velvety skin. Polycystic ovary syndrome causes irregular menstrual cycles, excessive body or facial hair and polycystic ovaries as its main symptoms. Polycystic means "many cysts," and PCOS often causes clusters of small, pearl-sized cysts in the ovaries. The cysts are fluid-filled and contain immature eggs. Women with PCOS produce slightly higher amounts of male hormones known as androgens, which contribute to some of the symptoms of the condition.

This topic has had extensive research over the past five years because of the growing incidence among adolescent females, as well as the systemic repercussions that are being discovered with older women who were not managed optimally due to lack of research available to guide treatment. Although there is still much to learn about the topic, treatment guidelines and diagnostic criteria have been developed that allow healthcare providers to confidently manage and treat women with symptoms suggesting PCOS. When glucose and insulin levels are tested and insulin resistance is revealed, discovering insulin resistance in obese adolescent plants the idea of PCOS being a differential diagnosis. Upon further clinical evaluation, excess distribution of body hair (hirsutism), acne, or menstrual abnormalities are noticed. The difficulty of diagnosing PCOS in adolescence is the mere awkwardness of the adolescent stage of development. There are many hormonal transitions taking place that could also manifest such as increased acne due to skin oil composition changes or irregular menses for the first year of menarche as the body's hormones are adjusting during puberty. Pediatric healthcare providers know when to screen for PCOS in adolescent females who are normal weight. Emans described PCOS as a diagnosis that is correlated to insulin resistance, not being overweight. There are many normal weight female adolescents and adults who have insulin resistance.[1] Polycystic ovary syndrome treatment starts with a proper diagnosis. The primary treatments for PCOS include: lifestyle changes, medications and surgery.[5] There is no cure yet, but there are many ways one can decrease or eliminate PCOS symptoms and feel better. Losing as little as 5% excess weight can help women ovulate more regularly and lessen other PCOS symptoms. The ideal way to do this is through nutrition and exercise. When females have a higher baseline level of testosterone, they have higher increases in sexual arousal levels but smaller increases in testosterone, indicating a ceiling effect on

testosterone levels in females. Sexual thoughts also change the level of testosterone but not level of cortisol in the female body, and hormonal contraceptives may affect the variation in testosterone response to sexual thoughts.[6] Testosterone may prove to be an effective treatment in female sexual arousal disorders, and is available as a dermal patch. Testosterone may be a treatment for postmenopausal women as long as they are effectively estrogenized.[7] However, this research work is set to estimate the levels of Insulin and Testosterone hormones in women with PCOS and to detect the implication of its different concentrations in the diagnosis and treatment of the syndrome.

Materials and methods

Subjects

The specimens used for the analysis were blood samples collected from female subjects within the age of 20-45 years at Ekiti State University Teaching Hospital (EKSUTH) Ado-Ekiti, and also at Federal Medical Centre (FMC) Ido-Ekiti, Ekiti State, Nigeria. Freshly diagnosed women with PCOS (n=100) and healthy control subjects (n=50) were used for the study. Questionnaire was used to obtain information and anthropometry data from the participants. The usual precautions as regards the collection of venipuncture samples were strictly observed, as they were collected by a trained professional. The blood samples were collected intravenously using standard venipuncture technique in the entire subject with the use of 5 ml needle and syringe into plain tube. The collected blood samples were centrifuged at 3000 rpm for 10 min using table top centrifuge (model 0508-1) to obtain the serum. The serum was carefully separated with the use of micro pipette. The samples were stored at temperatures of -20ºC.

Methods

Insulin procedure: Appropriate calibrators of 0.050 ml (50 µl), controls and samples was pipetted into the assigned wells, after which 0.100 ml (100 µl) of the insulin enzyme reagent was added to each well. The microplate was swirled gently for 20 sec. to mix well while covered with a plastic wrap. It was thereafter incubated for 120 min at room temperature (20-27ºC). The contents of the microplate were discarded by decantation after which the plate was tapped and blotted dry with absorbent paper. Wash buffer (350 µl) was added and the content of the plate was again discarded by decantation after which the plate was also tapped and blotted dry with absorbent paper. This process was repeated 2 additional times making a total of 3 washes. In all the processes, air bubbles were avoided in the bottle. Working substrate solution 0.100 ml (100 µl) was added to all the wells. The plate was not shake after the working substrate solution was added to all the wells. The sample was incubated at room temperature for 15 min after which 0.050 ml (50 µl) of stop solution was added to each well and mixed gently for 15 sec. The reagents were added in the same order to minimize reaction time differences between wells. The absorbance in each well was read at 450 nm (a reference wavelength of 620-630 nm was used to minimize well imperfections) in a microplate reader. The results were read within 30 min of adding the stop solution.

Calculation of creatinine concentration in serum: The absorbance obtained from the microplate reader was recorded after which a graph was plotted for the absorbance for each duplicate serum reference versus the corresponding insulin concentration in mmol/L on linear graph paper. To determine the concentration of insulin for the unknown, the average absorbance of the duplicates for each unknown was located on the vertical axis of the graph while the intersecting point on the curve was found and the concentration (in mmol/L) was read from the axis of the graph.

Testosterone procedure: Appropriate calibrators of 0.010 ml (10µl), controls and samples was pipetted into the assigned wells, after which 0.050ml (50µl) of the testosterone enzyme reagent was added to each well. The microplate was swirled gently for 20 sec. to mix well while covered with a plastic wrap. It was thereafter incubated for 60 min at room temperature. The contents of the microplate were discarded by decantation after which the plate was tapped and blotted dry with absorbent paper. Wash buffer of 350 µl was added and the content of the plate was again discarded by decantation after which the plate was also tapped and blotted dry with absorbent paper. This process was repeated 2 additional times making a total of 3 washes. In all the processes, air bubbles were avoided in the bottle. Working substrate solution of 0.100 ml (100 µl) was added to all the wells. It was incubated at room temperature for15 min after which 0.050 ml (50 µl) of stop solution was added to each well and mixed gently for 15 sec. The reagents were added in the same order to minimize reaction time differences between wells. The absorbance in each well was read at 450 nm (a reference wavelength of 620-630 nm was used to minimize well imperfections) in a microplate reader. The results were read within 30 min of adding the stop solution.

Calculation for testosterone

$$\text{Concentration of testosterone in serum (nmol/l)} = \frac{A\ \text{sample} \times \text{Standard concentration (mmol/l)}}{A\ \text{standard}}$$

Where,

A sample=Absorbance of the sample

A standard=Absorbance of the standard

Standard concentration=13.20 mmol/l

Statistical analysis

The results obtained from this study was grouped and expressed as mean±standard error of mean (SEM). Statistical analysis was carried out by one-way analysis of variance and T-test with the Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA). The test for statistical significance was carried out at 95% confidence limit.

Results

Levels of insulin and testosterone in PCOS patient and control subjects

There was significant increase ($P<0.05$) in the concentration of Insulin and Testosterone in the PCOS subjects compared with the control. Also, there was significant decrease ($P<0.05$) in Menarche of the PCOS subjects compared with the control while the age of the PCOS subjects shows no significant difference ($P>0.05$) in comparison with the control subject as shown in Table 1.

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Table 1 Levels of Insulin and Testosterone in patients with PCOS

Group/parameters	Control	PCOS
Insulin (mmol/L)	3.13±0.44[a]	7.89±1.31[b]
Testosterone (nmol/L)	0.42±0.05[a]	0.49±0.05[b]
Menarche age (yrs)	15.00±0.55[a]	13.20±0.34[b]
Age (yrs)	34.82±2.17[a]	33.70±1.47[a]

Values are presented as mean ± SEM. Values with superscript a and b are significantly different at $p < 0.05$ when compared with Non PCOS control group.

PCOS, Polycystic Ovarian Syndrome.

Effect of PCOS on the body mass index (BMI) of participants

There was significant increase ($P<0.05$) in the weight and BMI of PCOS subjects when compared with the control subject while the height of the PCOS subjects showed no significant difference ($P<0.05$) in comparison with the control subject as shown in Table 2**.**

Table 2 Effect of PCOS on the Body Mass Index of Participants

Group/parameters	Control	PCOS
Weight (kg)	62.36±1.35[a]	66.45±2.74[b]
Height (cm)	1.62±0.02a	1.60±0.01[a]
BMI (kg/m^2)	23.79±0.64[a]	25.99±0.92[b]

Values are presented as mean ± SEM. Values with superscript a and b are significantly different at $p<0.05$ when compared with Non PCOS control group.

PCOS, Polycystic Ovarian Syndrome.

Discussion

Insulin is a peptide hormone produced by beta cells of the pancreatic islets. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of, especially, glucose from the blood into fat, liver and skeletal muscle cells.[8] The concentration of insulin in PCOS patients shows that there was significant increase ($P<0.05$) when compared with the control subject (Table 1). When glucose concentration in the blood are high, the pancreatic beta cells secrete insulin into the blood; when glucose levels are low, secretion of insulin is inhibited.[9] This correlates with the research work of Barbieri et al.,[10] who reported that insulin may act at the pituitary level, the ovarian and/or the hepatic level to increase androgen synthesis and/or free testosterone serum concentrations. In addition, an increased ovarian cytochrome P450c17 activity in obese and non-obese hyperinsulinemic women with PCOS which appears to be stimulated by insulin in PCOS.[11] Previous *in-vitro* studies showed that insulin is also able to stimulate androgen secretion from stromal and thecal ovarian cells. Barbieri et al.,[10] However, metformin used in the treatment of hyperglycemia was found to increase clinical pregnancy rates and reduce the risk of ovarian hyperstimulation syndrome (OHSS) in women with PCOS. Farquhar et al.,[12] reported that metformin treatment improves live birth rate in women with PCOS. Testosterone is a steroid from the androstane class containing a keto and hydroxyl groups at the three and seventeen positions respectively. It is biosynthesized in several steps from cholesterol and is converted in the liver to inactive metabolites. It exerts its action through binding to and activation of the androgen receptor.[13]

There was significant increase ($p<0.05$) in the testosterone concentration of PCOS group when compared with non-PCOS control group (Table 2). This correlates with the work of Bartolone et al.,[14] who reported an extremely high level of testosterone. When females have a higher baseline level of testosterone, they have higher increases in sexual arousal levels but smaller increases in testosterone, indicating a ceiling effect on testosterone levels in females. Sexual thoughts also change the level of testosterone but not level of cortisol in the female body, and hormonal contraceptives may affect the variation in testosterone response to sexual thoughts.[6] Women with PCOS produce slightly higher amounts of male hormones known as androgens, which contribute to some of the symptoms of the condition. Menarche is the first menstrual cycle, or first menstrual bleeding, in female humans. From both social and medical perspectives, it is often considered the central event of female puberty, as it signals the possibility of fertility. There was significant decrease ($p<0.05$) in the menarche of PCOS subject when compared with the control (Table 1). This correlates with the work of Bartolone et al.,[14] who reported that a low level of menarche in PCOS patients when compared to the control. This shows that menarche age is implicated in PCOS. Also, there was no significant difference ($p<0.05$) in the age of PCOS group when compared with non-PCOS group. This value shows that there is no significant difference in their age range. Therefore, age is not implicated in PCOS.

The weight of PCOS patient is higher than the weight of the control (those without the syndrome). There's a significant different in their weight and this might be due to the high level of blood glucose concentration. This in correlation with the result for insulin which shows high level in the patients confirms the presence of the syndrome. The body mass index (BMI) or Quetelet index is a value derived from the mass (weight) and height of an individual. The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in meters. The BMI of non PCOS patients and Polycystic Ovarian syndrome patients (Table 2) shows a significant increase ($p<0.05$) in the Body Mass Index (BMI) of the PCOS group when compared with Non-PCOS control group which signifies that BMI is implicated in PCOS and thus an important factor to be considered when diagnosing patients for the syndrome. Trent et al.,[15] described the influence that BMI in adolescents with PCOS had on perceived overall health-related quality of life. The adolescent's weight loss strategies need to be investigated while maintaining an enthusiastic mentality for the female during this transition phase. The increase in the BMI of the patients is in correlation with the research work of Trent et al.,[15] who discovered the importance of perceived quality of life related to BMI in adolescent girls diagnosed with PCOS.

Conclusion

In conclusion, PCOS is a common endocrine disorder of female adolescents and adult hood with exact etiology unknown but pathophysiology rooted in insulin resistance, hyperandrogenism, and chronic anovulation. A multitude of clinical factors can be present including hirsutism, menstrual irregularities, metabolic abnormalities, acne, and increased BMI. History, physical exam, and laboratory tests are all components of making a diagnosis as some adolescents do not present with all clinical factors.[16] Treatment options include healthy dietary habits and regular exercise accompanied by additional

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medications, such as metformin or hormone therapy to treat presenting symptoms. This study has proved the fact that Insulin is one of the major determinants of diagnosing patients for PCOS. The increase in the level of insulin present in the patient's blood is as a result of the increase in the number of ovaries which subsequently increases the number of ovarian cells and determines the number of androgen hormones that will be secreted which has an effect on Insulin concentration in the system. This study has been able to prove that increase in BMI, testosterone are part of the major symptoms of Polycystic Ovarian Syndrome, and also showed that menarche age is implicated in PCOS.

Recommendation

Primary care providers should test for the concentration of insulin and testosterone in women as a means of diagnosing PCOS in adolescent females regardless of their weight and menarche age. Consistent medical checkup will facilitate timely recognition, prompt treatment, and potential reduction or elimination of future morbidities until more information is known. Further investigation on some other helpful parameters should be carried out as it will help in achieving the therapeutical target in PCOS treatment research.

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Competing interest

Authors declared that no competing interests exist.

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