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Original Research Article

Evaluation of the impacts of insulin on ovulation in Nigerian women with polycystic ovarian syndrome: a cross-sectional sonographic study

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ABSTRACT

Background: Insulin resistance (IR) is a metabolic state characterized by a decrease in cellular ability to respond to insulin signaling, which contribute to pathophysiological mechanism in the development of all metabolic complication of polycystic ovary syndrome (PCOS). The aim of the study was to match categorized values of patient's biochemical predisposing factors for polycystic ovaries such as insulin with change in follicular sizes as determined by sonography following CLOMID inducement therapy.
Methods: This experimental study was carried out in Anambra State, Nigeria from June 2018 to May 2021. Those included in the study were women of child bearing age (18 to 45 years) for both groups. The ultrasound examinations and insulin levels measurements were performed on each subject and data such as follicular sizes, insulin levels before and after treatment were recorded. Obtained data were analyzed using both descriptive and inferential statistical tools.
Results: There were no statistically significant mean differences in the insulin levels ($t=1.16$, $p=0.81$) and maximal follicular size ($t=0.39$, $p=0.70$) of women with and without polycystic ovary who had successful and failed ovulation before the clomid treatment. Both the insulin level ($t=2.85$, $p<0.01$) and follicular size ($t=4.88$, $p<0.01$) showed statistically significant mean differences. There was significant difference in insulin ($F=7.55$, $p<0.01$), with the control having the lowest insulin concentration.
Conclusions: There were statistically significant mean differences in the insulin level and follicular size in women with polycystic ovary after clomid treatment. Therefore, clomiphene citrate inducement triggers increase in serum concentration of insulin.

Keywords: Follicular, Insulin, Polycystic ovaries

INTRODUCTION

Polycystic ovarian syndrome (PCOS) accounts for more than 80% of ovarian dysfunction-related infertility.[1,2] It is associated with an increased risk of diabetes and cardiovascular events in adulthood and is the most commonly diagnosed ovarian dysfunction.[2] The reported prevalence of PCOS ranges from 2.2% to 26% in various countries, depending on the recruitment process of the study population, the criteria used for its definition, and the method used to define each criterion.[3] However two studies in Nigeria reported the prevalence of PCOS as 18.1% and 12.2% respectively, but these studies were based on the Rotterdam criteria.[3]

The pathophysiology of PCOS has remained controversial and the role of BMI, androgens and insulin in PCOS development have not been fully understood.[4] Insulin is the most important factor in the regulation of plasma glucose homeostasis, as it counteracts glucagen and other hormones- epinephrine, glucorticoid, and growth hormones.[5] Low insulin level (Diabetes type I) causes

elevated sugar level. Insulin resistance (IR) is a metabolic state characterized by a decrease in cellular ability to respond to insulin signaling, and seem to be an essential pathophysiological mechanism in the development of all metabolic complication of PCOS.[6] Insulin stimulates ovarian theca cells androgen production and secretion, and suppresses the hepatic production of sex hormone binding globulin (SHBG). Hyperinsulinemia may also directly cause premature follicular atresia and antral follicle arrest. The resulting anovulation also leads to unopposed estrogen production and endometrial proliferation in women with PCOS, leading to an increased risk of endometrial hyperplasia. Outstanding proportion of women with PCOS are also diagnosed with diabetes mellitus 2 (DM2) or metabolic syndrome (MS), as well as isolated criteria from the latter.[7] By the foregoing, hyperinsulinemia results in increased ovarian androgen biosynthesis and decreased sex-hormone-binding globulin synthesis from the liver, leading to increased bioavailability of free androgen which in turn promotes PCOS.

Elevated body mass index (BMI), increased concentration of serum androgens and insulin are the major features of PCOS. They cause ovulation failure by suppressing folliculogenesses. Sonographic assessment of PCOS subjects undergoing treatment with clomifen citrate can be more accurate if rate of follicular growth after CLOMID inducement is matched with individual patient's major predisposing factors for PCOS such as insulin. The objective of this study was to match categorized values of patient's biochemical predisposing factors for polycystic ovaries such as insulin with change in follicular sizes as determined by sonography following CLOMID inducement therapy.

METHODS

This was an experimental study, which involved 100 subjects made up of all fertility challenged women due to PCOS who were undergoing treatment and women with no history of reproductive or thyroid hormone insufficiency at the aforementioned selected hospitals during the period of this study. This study was conducted at the obstetrics and gynecology department of general hospital, Onitsha, and other selected hospitals in Anambra State, Nigeria from June 2018 to May 2021. The sample size of 100 was determined using the formula for a finite population described by Yemani as cited by Ukaji et al and Ogolodom et al was used as shown below.[8,9]

$$n = \frac{N}{1} + N\,x(e)^2$$

Where n=Minimum sample size, N=Population under study, e=Error margin, which may have values (010, 0.05, or 0.01), Whereas N= 2000 (The population for the two years under consideration), Using error margin, e=0.10, and n=93.5 and this was increased to 100 in order to increase the validity of the study.

An ethical approval for this study was obtained from the human research and ethical committees of Anambra State ministry of health, Awka, Nigeria (MH/AWK/M/321/390), a convenient sampling technique was adopted to select the subjects based on the inclusion criteria, which were women of child bearing age (18 to 45 years) for both groups. The experimental group include only subjects with PCOS while the as the control group include subjects with no history of reproductive and thyroid hormone abnormality. Subjects with reproductive hormone abnormality and history of hysterectomy/ oophorectomy were excluded from this study. Confirmation of patients with PCOS was ascertained with the presence of polycystic ovaries in ultrasound.[2]

Blood Sample collection was done in two phases for the subjects in the experimental group. The first was a clomid-free sample collection, during the menstrual cycle at which the investigation began. The second phase was during the next menstrual cycle following the first collection. Clomid was administered this time. Five ml of blood was drawn from each participant by venipuncture in each phase. The sample was allowed to clot and centrifugation performed at 5000 RPM for 5 minutes. Serum was extracted and stored at -10^0 C before analysis. Blood sample collection in the control group was done only once, and that was at the menstrual cycle of the onset of the investigation.

The serum insulin level was determined according to the method described by Eastham.[10] It is an enzyme linked immunosorbent assay (ELISA) method. Six hours fasting blood sample were drawn from each subject.[10] Utilizing the direct sandwich technique a merocodia insulin ELISA (A two-site enzyme immunoassay) was made. The specimen, control, or standard were pipetted into the sample well. This was followed by the addition of peroxidase-conjugated anti-insulin antibodies. Insulin present in the sample was to bind to anti-insulin antibodies bound to the sample well.[11] While the peroxidase-conjugated anti-insulin antibodies also bound to the insulin at the same time. After washing to remove unbound enzyme-labelled antibodies, TMB-labelled substrate was added to bind to the conjugated antibodies. Hydrochloric acid was added to the sample well to stop the reaction, and the colorimetric endpoint was read on a microplate spectrophotometer set to the appropriate wavelength. The essential computerization was performed in the data system management result entry to generate the output values.

The ultrasound examinations were performed by the researchers using standard scanning methods described by Sanders on Mindray-DCN3 (Mindray electronic instrument company limited, Jiangsu, China, 2014) ultrasound scanner with adjustable transducer's frequency between 6-10 MHz.[12] Measurement of follicular size was made by taking two orthogonal diameters (d1 and d2) at the largest follicle plane on real-time 2D image of the follicle that will be obtained. This was determined by placing calipers at the inner follicle borders. Each 2D

follicular diameter was examined two times consecutively during the examination and the mean follicular diameter obtained. Mean follicular diameter was obtained using the formula, d1+d2/2 and the average value from three measurements for each follicle was obtained and used for statistical analysis as previously documented by Ping et al.[13]

Statistical analysis was carried out using the statistical package for social sciences version 22.0 (SPSS Inc., Chicago Illionis). Mean distribution for the insulin levels and follicular sizes of the test and control groups were presented in tables. Independent t-test was used to compare the insulin level and maximal follicular size between women with polycystic ovary before clomid treatment and healthy control. Chi-square test was used to determining the differences in distribution of women with polycystic ovary before clomid treatment and healthy control across different categories of insulin levels, and maximal follicular size. The level of statistical significance was set at p value less than 0.05.

RESULTS

The mean value of insulin among the experimental and control group before and after clomid administration were (experimental group: 14.90±25.10 and 21.76±25.41) and 10.33±6.62 for the control group (Table 1).

Table 1: Mean value of insulin among women with polycystic ovary and healthy control.

Variables	Mean ± SD		
	Before clomid	After clomid	Control
Insulin	14.90±25.10	21.76±25.41	10.33±6.62

There were no statistically significant mean differences in the insulin levels (t=1.16, p=0.81) and maximal follicular size (t=0.39, p=0.70) of women with and without polycystic ovary who had successful and failed ovulation before the clomid treatment while there were statistically significant mean differences in the insulin level (t=6.92, p<0.01) and follicular size (t=2.04, p=0.04) in women with polycystic ovary after clomid treatment. Both the insulin level (t=2.85, p<0.01) and follicular size (t=4.88, p<0.01) showed statistically significant mean differences (Table 2). There was significant difference in insulin (F=7.55, p<0.01), with the control having the lowest insulin concentration. Post Hoc analysis revealed that the difference in insulin is between the test after treatment group and the control group (p<0.05) (Table 3). There was no statistically significant relationship between the ovulation success/failure rates among test group (before treatment) with different concentration categories of insulin (χ^2=1.45, p=0.49) (Table 4).

Table 2: Insulins levels and maximal follicular size of women with and without polycystic ovary who had successful and failed ovulation.

Variables	Mean±SD		T	P
	Failure	Success		
Women with polycystic ovary before clomid treatment				
Insulin	15.16±26.82	13.79±2066	1.16	0.81
Max. follicular size	23.38±10.26	22.59±3.52	0.39	0.70
Women with polycystic ovary after clomid treatment				
Insulin	41.55±24.87	11.04±18.69	6.92	<0.01*
Max. follicular size	27.91±5.10	25.86±4.59	2.04	0.04*
Control group				
Insulin	16.11±15.45	9.75±4.85	2.85	<0.01*
Max. follicular size	30.56±23.74	23.74±3.65	4.88	<0.01*

Table 3: Analysis of variance and Bonferroni Post Hoc analysis comparing insulin levels among test (before and after treatment) and control group.

Groups	Follicular size, (Mean±SD)			F	P
	Test (Before)	Test (After)	Control		
Insulin	14.79±25.21	21.72±25.53	10.23±6.58	7.55	<0.01

Table 4: Chi-square test comparing ovulation success/failure rates among test group (before treatment) with different concentration categories of insulin.

Variables	Class	Frequency (percentage)		X^2	P
		Failed	Successful		
Insulin	Low	22 (31.0)	10 (38.5)	1.45	0.49
	Normal	37 (52.1)	0 (38.5)		
	High	12 (16.9)	6 (23.1)		

DISCUSSION

PCOS is the most common endocrine disorder responsible for subfertility among the young adult.[14] The prevalence of PCOS is increasing and as high as 15-20%.[15] Safe and effective ovulation induction is important for women with WHO group II anovulation.[16] Clomiphene citrate has been used for ovulation induction since 1960s. It is still considered first line drug for anovulatory PCOS woman.[16] Clomiphene resistance occurs in 15% to 20% of patients.[16] Also, PCOS makes it more difficult for the body to use the hormone insulin which is responsible for converting sugars and starches from food into energy, this condition called insulin resistance can cause insulin and sugar glucose to build up in the bloodstream hence body fat. Insulin resistance is a common finding in PCOS that is independent of obesity. Insulin mediated glucose disposal, reflecting mainly insulin action on skeletal muscle is decreased by 35-40% in women with PCOS compared to weight comparable reproductively normal women.[17] This defect is independent of but substantially worsened by obesity. In contrast, hepatic insulin resistance, characterized by both increased post-absorptive glucose production and reduced sensitivity to insulin mediated suppression of endogenous glucose production, is present only in obese women with PCOS compared to control women of comparable body weight.[17] This synergistic deleterious effect of obesity and PCOS on endogenous glucose production may be an important factor in the pathogenesis of glucose intolerance.[17]

Fasting insulin levels are increased in PCOS. Nonetheless, there are defects in insulin secretion that are independent of obesity.[17] These abnormalities are more pronounced in women with PCOS who have a first-degree relative with type 2 diabetes. In PCOS, basal insulin secretion is increased, but insulin responses to glucose are inappropriately low.[17] Under normal circumstances, the relation between insulin secretion and sensitivity is constant so that changes in insulin sensitivity are accompanied by reciprocal changes in insulin secretion that maintain normal glucose tolerance; this relationship is known as the 'disposition index'. Both obese and non-obese women with PCOS have lowered a disposition index compared to weight-matched reproductively normal women.[17] Furthermore, disposition index is significantly lowered by PCOS as well as obesity.

In summary, PCOS is associated with defects in insulin sensitivity and secretion that are further exacerbated by obesity i.e., obesity is strongly associated with PCOS and may be present in up to 50% of cases, as seen in this study where majority of those with PCOS are overweight with a mean BMI of 25.69±5.99 kg/m^2.[18-22] Obese women with PCOS are more likely than thin women with PCOS to suffer from anovulation.[18] This effect on ovulation may be secondary to insulin resistance, which in turn results in hyperinsulinemia and stimulation of excess androgen production from the ovaries. Intra-ovarian hyper-androgenism in turn inhibits follicular maturation.[22]

Insulin resistance might be considered as a potent contributor to follicular atresia in polycystic ovaries. Impairment of normal follicular development leads to anovulation and subsequent oligo/amenorrhea in that patient.[23] After clomiphene citrate was administered, the frequency of ovulation failure dropped from 73% to 35% there by indicating a high improvement in successful ovulation following treatment using clomiphene citrate. In other words, there was a 65% ovulation success rate after clomiphene citrate was administered. This is slightly higher than that of a study by Kar, where clomiphene citrate administration brought about an ovulation success rate of 60.78% but lower than those of previous studies where ovulation success rate was 70.9%, 74.7% and 72% respectively after administering clomiphene citrate.[24-26] This efficient ability of clomiphene citrate in successful ovulation was in agreement with another study by Hughes et al who concluded that clomiphene citrate (at doses between 50 to 250 mg per day), appears to be an effective method of inducing ovulation and improving fertility in oligoovulatory women.

This study also showed that Insulin level was higher in women with PCOS than the control group, this further explain findings of lowered SHBG among women with PCOS which is related to insulin resistance that is a common occurrence in PCOS, insulin resistance will lead to hyperinsulinemia hence the increased level of insulin found in this study among women with PCOS as compared to the control group. Furthermore, the hyperandrogenic state in PCOS also seems to be linked with the action of insulin. The increased insulin secretion possibly mimics the tropic action of luteinizing hormone on ovarian theca cells, which further causes an increase in androgens.[27] This is further validated by the fact that the improvement of insulin resistance in PCOS women decreases the level of hyperandrogenism.[28]

Findings from this study showed no significant difference in Insulin level and max. follicular size between test group with failed ovulation and those with successful ovulation before clomid treatment whereas after clomid treatment, there was significant difference in the Insuline level and follicular size between those with failed ovulation and those with successful ovulation. Those with successful ovulation had significantly lower insulin level and follicular. This is also similar with that of the control group in this study as a significant lower insulin level and follicular size was observed among those with successful ovulation in the control group. The reduced insulin in women with successful ovulation after clomiphene citrate treatment and those in the control group in this present study also supports the conclusion drawn in an article that the effect on ovulation may be secondary to insulin resistance, which in turn results in hyperinsulinemia and stimulation of excess androgen production from the ovaries. Intra-ovarian hyperandrogenism in turn inhibits follicular maturation.[22] Also the increased SHBG in women with successful ovulation after clomiphene citrate treatment also concur with Zhu et al who stated that "low serum SHBG levels are considered a biomarker of

abnormal metabolism and are related to insulin resistance, compensatory hyperinsulinemia and abnormalities in glucose and lipid metabolism in PCOS patients and this reduces chances of successful ovulation.[29,30]

There was an increased level of insulin after clomiphene citrate as compared to before clomiphene citrate for women with PCOS but this difference was not statistically significant (14.90 and 21.76; p=0.06). This high level of insulin after clomiphene is in contrast to a previous study where both insulin and testosterone level dropped.[30] This difference could be due to the previous study using clomiphene together with herbal mixture whereas this study only used clomiphene. This increased level of insulin could be due to clomiphene resistance as reported by a previous study where obesity and hyper-insulinaemia are well correlated with clomiphene citrate resistance.[31] Also, this study showed that PCOS women with successful ovulation had lower insulin level than those with failed ovulation and it was observed that after clomiphene citrate, those PCOS women with failed ovulation had increased insulin whereas those with successful ovulation had a drop in insulin after clomiphene citrate. This entails successful ovulation could be dependent on low Insulin. Insulin resistance with resultant hyper-insulinaemia is a prominent feature of PCOS, and it is seen both in obese and normal weight women.[30]

There was no significant distribution of successful and failed ovulation of women with PCOS before clomiphene citrate was administered across different categories of insulin level. Although before clomiphene citrate was administered, the women with PCOS who had failed ovulation showed varying degrees of insulin level majorly normal (52.1%) while those with successful ovulation had successful had varying degrees of insulin level but this time majorly low insulin level further indicating that the lower the insulin level the higher the chances of ovulation though in this case, the pattern of distribution wasn't significant. This is further supported by a previous study by Parsernezhad et al who found out that clomiphene citrate responders had higher insulin levels while non-responders were hyper-insulinemic.[32]

CONCLUSION

There were no statistically significant mean differences in the insulin levels and maximal follicular size of women with and without polycystic ovary who had successful and failed ovulation before the clomid treatment while there were statistically significant mean differences in the insulin level and follicular size in women with polycystic ovary after clomid treatment. Therefore, clomiphene citrate inducement triggers increase in serum concentration of insulin.

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