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The effect of induction of ovulation on glucose tolerance test in women with polycystic ovaries

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ABSTRACT

Design. This is a case controlled study
Site. The outpatient clinic of Suez Canal University Hospital in Ismailia Egypt.
Duration of the study from February 1999 to September 2000.
Objective: This investigation was conducted to study the effect of ovulation induction on the glucose tolerance test in women with polycystic ovarian syndrome.
Materials and Methods: This study included 42 cases suffering from polycystic ovarian syndrome divided into two groups. The first group of 21 cases was scheduled for ovulation induction using clomiphene citrate 100 mg daily for five days starting from the third menstrual day for five days. The induction of ovulation was repeated for three cycles. Glucose tolerance test was carried out before induction of ovulation and after three months of successful induction of ovulation. The second group of 21 cases of matched age and obesity and hormonal profile were scheduled for oral glucose tolerance test. The glucose tolerance test was performed using 75 gm glucose according to the recommendations of the World Health Organizations (WHO).
Results : The results of this study illustrated that cases with impaired glucose tolerance test showed improvement in 50% of them after successful induction of ovulation. Cases with frank diabetes mellitus did not show any improvement after ovulation induction.
Conclusion. Polycystic ovarian syndrome is a disease, which includes a metabolic abnormality in carbohydrate metabolism. The induction of ovulation per se will improve this abnormality besides its reported beneficial effect on the possibility of treatment of infertility.

Abbreviations. Polycystic ovarian syndrome (PCOS), Body mass index (BMI), Follicle stimulating hormone (FSH), Lutinizing hormone (LH), Oral glucose tolerance test (GTT), Ultrasound (US)

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common, but perhaps least understood endocrine disorder of women. Over the >60 years since PCOS was first recognized as a common entity, clinicians have entertained the notion that PCOS is a genetic disease. However, the exploration of the genetics of PCOS has been hampered by several factors. First, PCOS is associated with infertility and low fecundity. Thus, it is rare to find large pedigrees with multiple affected women with whom to perform linkage analysis. Second, assigning phenotypes to premenarchal girls and postmenopausal women is not straightforward, a problem that also limits the use of pedigrees. Third, there has been an ongoing debate over disease phenotypes. The larger the number of distinct phenotypes within the affected category, the more complex the genetic analysis and the greater the

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likelihood that investigators using different diagnostic criteria will arrive at different conclusions. Fourth, although a male phenotype has been postulated, there are no rigorously established clinical or biochemical features that can be used to identify PCOS males. This makes formal segregation analysis as well as genetic linkage studies more difficult. Fifth, the lack of animals that spontaneously develop a PCOS-like phenotype, especially mice, precludes the use of powerful tools of genetic mapping.

The diagnosis of PCOS has traditionally been based on the historical variables of oligomenorrhea and hirusutism; biochemical markers such as circulating total or bioavailable androgens and gonadotropin levels; or the ultrasound image of the ovaries. The criteria that emerged from the 1990 National Institutes of Health-National Institute of Child Health and Human Development (NICHD) conference identified PCOS as unexplained hyperandrogenic chronic anovulation, making it in essence a diagnosis of exclusion[1]. The "consensus" definition did not include the polycystic ovary morphology found on ultrasound of multiple 2– to 8–mm subcapsular preantral follicles[2]. The rationale for not incorporating ovarian morphology in the diagnostic criteria is that polycystic ovaries are distinct from PCOS; ≤30% of an unsolicited population may have polycystic ovaries on ultrasound examination, and many of these women have normal androgen levels and regular menstrual cycles[2,3]. The consensus definition also did not include insulin resistance, a common but not invariable finding in PCOS. However, insulin resistance may be a key factor in the pathophysiology of PCOS, exacerbating an underlying metabolic abnormality. The associated compensatory hyperinsulinemia can affect hypothalamic control of gonadotropin secretion and appetite, stimulate adrenal and ovarian androgen secretion, and suppress circulating levels of sex hormone-binding globulin, thus increasing the pool of bioavailable androgens.

The biochemical evidence for involvement of multiple organ systems in PCOS including hyperandrogenemia of ovarian origin, elevated adrenal androgen production, insulin resistance, and abnormal pancreatic β-cell function raises several important questions: is PCOS many diseases, or do the factors that influence reproductive function also impact different cell types simultaneously, resulting in the multisystem PCOS phenotype? Are the metabolic abnormalities detected in different cells the result of a shared intrinsic defect, or are they the consequence of exposure to an altered endocrine state (i.e., increased androgen or increased insulin)? The answers to these questions have profound significance when one contemplates the role of genetics in PCOS.

The complexity of this syndrome stems from its typical heterogeneity outlined in Table 1. It is clear however, that more than one gene (possibly several) contribute to the heterogeneous phenotype[9] and the clinical and biochemical presentation is undoubtedly influenced by additional environmental factors such as diet and exercise[10]. Nevertheless, results from recent studies using animal models together with supporting clinical evidence indicates that the development of PCOS is a linear process with an origin before adolescence (the contemporary clinical perception of age of onset of PCOS). Superimposed on this developmental process are interacting genetic and environmental factors that may alter phenotypic expression of PCOS during adult life, particularly the susceptibility to anovulation[11,12].

Women with PCOS are profoundly insulin resistant, and the resulting hyperinsulinemia plays a role in the pathogenesis of the reproductive disturbances[4–6]. Abnormalities in insulin action are poorly detected by a single determination of either glucose or insulin levels[7,8]. This diagnosis requires iv administration of glucose, insulin, and/or other substances in a research setting. Such tests are time, labour, and, above all, cost-intensive and are not feasible for large scale screening of populations or routine interval assessment individuals at risk.Based on these considerations the concept has evolved by which PCOS is a self-perpetuating disease in which the hypothalamus, pituitary, ovaries and adrenal all contribute to an endocrine that is usually associated with oligo-ovulation, hirsutism and infertility[13].

Almost the previous two decades of research have greatly increased the knowledge in the complex field of metabolic aberrations in PCOS, but still many problems remain unsolved. It is realized since early eighties that mild hyperinsulinaemia and insulin resistance are common findings in PCOS[14,15] and that a derangement of insulin secretion may represent a main component of the pathogenesis and the clinical expression of the syndrome[16,17]. It is still unknown if hyperinsulinaemia and insulin resistance are primary causes of PCOS or the hyperandrogenism associated with PCOS initiates metabolic changes that finally result in hyperinsulinaemia, insulin resistance, glucose intolerance and type II diabetes mellitus[18].

The objectives of this investigation are to study the possible effects of induction of ovulation on glucose tolerance test in women with PCOS by evaluation of

1. The glucose tolerance in women with PCOS.

2. The changes in glucose tolerance test after effective induction of ovulation in women with PCOS, and

3. The effect of obesity on the changes of glucose tolerance test after effective induction of ovulation in such cases.

MATERIALS AND METHODS

The study design is a case control study comprising 42 cases (21 cases in each group) who were selected from the out patient clinic of the Department of Obstetrics and Gynecology Faculty of Medicine Suez Canal University in Ismailia during the period from February 1999 to September 2000.

The inclusion criteria were

1. Age reproductive age.

2. Infertility

3. Obesity with BMI of 26 or more.

4. The diagnostic criteria of PCOS in this study are laboratory criteria and ultrasound (US). The US criteria are the increase ovarian volume; the presence of a thick capsule,hyperechogenic stroma and the presence of multiple subcortical follicles which do not exceed 10 mm in diameters. The laboratory tests include the determination of FSH and LH. Elevated LH compared to FSH levels and LH/FSH ratio >3 are suggestive of PCOS.

Exclusion criteria

This includes pregnancy and endocrine disorders that mimic PCOS as Cushing's syndrome, ovarian tumors, adrenal tumors, thyroid diseases and hypogonadotropic hypogonadism.

Control group

Control cases were selected from PCOS cases on the condition of being of matched age and BMI. The control cases were not subjected to any form of ovulation induction.

METHODS

Patients were subjected to

1. History taking

2. Complete physical examination.

3. Calculation of the BMI.

4. Ultrasound examinations of the ovaries using transvaginal probe 5 and 7.5 MHz and folliculometry was done regularly on days 8, 10, 12 and 14 of each menstrual cycle using the Pie Medical 8 C 240 Ultrasound machine. A follicular diameter >18 mm indicates successful induction of ovulation.

5. Estimations of serum FSH, LH and Testosterone were performed using radioimmunoassay methods.

6. Oral glucose tolerance test before and after induction of ovulation in the study cases and in the control cases at the beginning of the study and after three months. The cases in this study were subjected to 75grams oral glucose after an overnight fast with fasting, 30 minutes, 60 minutes, 90 minutes, and 120 minutes blood samples for estimation of the glucose level. The test was carried out on all subjects before and after ovarian stimulation and the occurrence of successful ovulation repeatedly for three months. Effective induction of ovulation was emphasized by folliculometry to be sure that induction resulted in one or more follicles. The test was repeated again after three successive ovulatory cycles. Diabetes was diagnosed when the fasting blood sugar exceeds 7.8 mmol (140 mg/dl) and the value after two hours to be more than 11.1 mmol. (200mg/dl). Impaired glucose tolerance was diagnosed when the two-hour glucose value was 7.8-11.1 mmol. (140-200 mg/dl).

7. Enzyme immunoassays using commercially available kits were used for the quantitative determination of LH, FSH and testosterone in the female serum.

8. Induction of ovulation was carried out using clomiphene citrate 100 mg daily for 5 days starting from the third day of the start cycle. This was only applied to the study group.Successful induction was monitored by frequent US folliculometry on days 8,10,12 and 14 from the start of the menstrual cycle and the finding of a follicle that is more than 18 mm in diameter in any ovary.

RESULTS

The study group included 42 women of whom 21 were diagnosed as PCOS. As presented in Table 1, 11/21 women with PCOS were obese (26.2%), 9 had acne (21.4%) 13 with hirsutism (31%) while 4 had amenorrhoea (9.5%) and 12 presented with oligomenorrhoea (28.6%). The control group of non-diabetic women was evenly matched for all these clinical features.

Of the PCOS group, 10 women were not obese

(23.8%) and of the remaining 11 women, 7 had mild, 3 had severe and 1 with morbid obesity (Table 2). No significant differences were noted in the control group of women with respect to obesity criteria included in this study (P>0.05).

The mean ±SD levels of testosterone, LH and FSH between the PCOS groups vs control women were also not significantly different in the 2 groups (Table 3), while the corresponding values of LH/FSH ratio was 4.88 in women with PCOS (Table 4). The results of glucose tolerance test in both groups of women is illustrated in Table 5. The blood glucose levels before and after induction of ovulation were significantly decreased after ovulation induction particularly after LH of GTT in women with PCOS (Table 6).

TABLE 1
Prevalence of the major clinical features of PCOS

	Obesity		Acne		Hirsutism		amenorrhea		Oligomenorrhea	
Groups	No.	%	No.	%	No.	%	No.	%	No.	%
Cases	11	26.2	9	21.4	13	31	4	9.5	12	28.6
Control	10	23.8	8	19.0	14	33.3	4	9.5	9	21.4
Total	21	50	17	40.5	27	64.3	8	19.0	21	50.0
P value	0.76		0.756		0.750		1.00		0.222	

No significant difference between both groups P>0.05

TABLE 2
Classification of obesity in both groups

	Not obese		Obese							
			Mild		Severe		Morbid		Total	
Groups	No.	%	No.	%	No.	%	No.	%	No.	%
Cases	10	23.8	7	16.7	3	7.1	1	2.4	11	26.2
Control	11	26.2	8	19.0	2	4.8	0	0	10	23.8
Total	21	50.0	15	35.7	5	11.9	1	2.4	21	50.0

P value>0.05 = no statistically significant difference

TABLE 3
Serum testosterone,LH and FSH levels in both group

	Testosterone			LH			FSH		
Groups	Mean	S.D	Range	Mean	S.D.	Range	Mean	S.D.	Range
Cases	1.04	1.02	0.25-4	92.5	38.5	41-180	18.86	2.37	15-22
Control	1.05	0.99	0.3-4.2	97.33	42.77	39-184	18.67	2.13	14-21
Total	1.04	0.99	0.3-4.2	94.9	40.27	39-184	18.76	2.23	14-22

No statistically significant difference between both groups (P>0.05)

TABLE 4
LH/FSH ratios in both groups of this study

Groups	Mean LH/FSH	S.D.	Range
Cases	4.88	1.97	2.2-9.25
Control	5.24	2.3	2.05-10.82
Total	5.06	2.11	2.05-10.82

No statistically significant difference between both groups (P>0.05)

TABLE 5
Glucose tolerance test in both groups before and after induction of ovulation

GTT	Study cases				Control cases			
	Before induction		After induction		Before induction		After Induction	
	No.	%	No.	%	No.	%	No.	%
Normal	9	42.9	14	66.7	9	42.9	8	38.1
IGT	10	47.6	5	23.8	10	47.6	11	52.4
DM	2	9.5	2	9.5	2	9.5	2	9.5
Total	21	100	21	100	21	100	21	100
P value	0.025 NS				0.157 NS			

IGT= impaired glucose tolerance test.
DM= diabetes mellitus
N.S.= not significant.

TABLE 6
The range, mean and standard deviation of glucose level in women with PCOS before and after induction of ovulation

Timing Of GTT	Minimum		Maximum		Mean		S.D.	
	Before	After	Before	After	Before	After	Before	After
Fasting	76	76	211	196	98.42	92.57	27.39	25.03
30 min.	110	100	263	270	149.42	137.66	31.38	33.67
60 min.	119	108	276	281	161.23	149.71	41.45	43.67
90 min.	121	118	292	292	163.90	147.80	46.54	46.63
120 min.	108	81	293	270	150.42	125.90	45.52	40.26

P value<0.05 statistically significant difference between the values of GTT before and after ovulation induction.

DISCUSSION

The literature lacks a universally accepted definition to PCOS. In this study both the ultrasound criteria and biochemical characteristics of PCOS were used in patient selection[19–22]. This study is an attempt to find out the influence of induction of ovulation on the glucose tolerance test (carbohydrate metabolism). Induction of ovulation when successful will improve the internal hormonal milieu including reduction of serum androgen levels and there may be improvements of other unknown factors that may influence carbohydrate metabolism.

Most previous relevant studies attempted to prove that insulin resistance and hyper-insulinemia are responsible for the development, or play an important role in the pathogenesis of PCOS. Very few studies however tried to prove the reverse that the endocrine and metabolic abnormalities of PCOS may be

responsible for the insulin resistance and glucose intolerance.

Recently Polderman and his colleagues[18], found that androgen administration could induce insulin resistance and glucose intolerance in healthy subjects after 4 months of testosterone administration.

In this study PCOS was found to be a completely heterogenous disorder, all patients had the characteristic US criteria of PCOS but not all the characteristic symptomatology or characteristic endocrinological abnormalities of PCOS. More than two thirds of the cases had abnormal menstrual patterns (Table 4); half of the patients had oligomenorrhoea and one fifth of them had amenorrhoea. These results are consistent with those of Balen and associates[23] but contradicted the findings of Lergo et al[22] who found a higher prevalence of oligomenorrhoea than any other menstrual abnormalities associated with PCOS. Hirsutism was reported in almost two thirds of the study population (Table 4). This was also reported in the work of others[22,23].

Patients in this study were more obese than other reports (Table 4 & 5) in which exactly half of the cases in this study were obese (10 cases of the study group and 11 of the control cases). Mild obesity was reported in 71.4%, severe obesity in 23.8% and 4% of cases had morbid obesity. These results were 10% more than those reported by other workers[23]. This can be explained by the higher prevalence of obesity in Egypt. Ciampelli and Lanzone[24] reported the same incidence of obesity as the current study 50% of cases. The incidence of acne in this study was reported to be higher than previous studies of others[22,23]. This explains the type of presentation of PCOS in Egypt. Also acanthosis nigricans characterized by severe insulin resistance which was reported in only one case in this study was mentioned in 2% of the cases by Balen and his coworkers[23] with nearly the same incidence.

As seen in tables 3 & 4 the serum levels of LH & FSH and LH/FSH ratios were higher than previously reported and coincide with the endocrine abnormality seen in PCOS[25]. As seen in table 5, the study population was divided into normal GTT cases, cases with impaired GTT, and cases with frank diabetes mellitus. In this table almost 50% of cases with impaired GTT improved after three months of successful ovulation induction. Diabetic patients suffered no change at all. This can be explained by the fact that frank diabetes is a long-term chronic illness that has established metabolic abnormality. This condition cannot be reversed by a three-month ovulation induction regimen. Further studies are recommended in this regard.

As seen in table 6 the blood sugar curve and the area under the curve before and after induction of ovulation showed significant reduction at all points of the curve after ovulation induction particularly after 2 hours of GTT. This could be attributed to a decrease in insulin resistance after successful induction or due to something else and further investigations are warranted.

Fasting glucose levels are higher in obese PCOS women secondary to increased hepatic glucose production, which reflects hepatic insulin resistance, but this usually does not achieve statistical significance, as is the case in the present study. As non obese women were not included as an exclusive group, we cannot directly confirm this.

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