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Diabetic and cardio-damaging aspects of the polycystic ovary syndrome among Indian women

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Abstract

Insulin resistance is a common feature of PCOS and is more marked in obese women, suggesting that PCOS and obesity have a synergistic effect on the magnitude of the insulin disorder. It leads to increased insulin secretion by beta-cells and compensatory hyperinsulinemia. Hyperinsulinemia associated with insulin resistance has been causally linked to all features of the syndrome, such as hyperandrogenism, reproductive disorders, acne, hirsutism and metabolic disturbances. If beta-cell compensatory response declines, relative or absolute insulin insufficiency develops which may lead to glucose intolerance and type 2 diabetes. The key characteristics in the studied group [20 women] include irregular menstruation, obesity, infertility, acne and hair growth on the face, chest, and back (hirsutism) and ovarian cysts. Also as Insulin resistance precipitates dyslipidemias, thus the PCOs affected victims are more prone for developing CVDs. We performed this study to determine the prevalence of glucose intolerance and parameters associated with risk for CVDs in PCOS women. PCOS women, aged 20-48 years, were prospectively evaluated in Bilaspur city. The PCOS women were compared to 20 control women of similar age and demographical profile. Their biochemical profile including serum levels of estrogen, progesterone, Testosterone, C-peptide level, C-reactive protein, total lipid profile, BRI, waist-hip ratio, Glucosylated Hemoglobin were assessed and compared with the biochemical values in the control group. The values in the PCOs victim group in terms of Serum Insulin level, Serum Testosterone level Hb1Ac, obesity, serum Cholesterol, Triglyceride, LDL were significantly higher than the values estimated in controls, but lower in terms of serum estrogen and progesterone levels. Also the serum value of creatine Kinase was higher in the subjects, a clear indication of cardio-damaging effect of PCOs driven dyslipidemia. We concluded that - PCOS women are at significantly increased risk for type 2 diabetes mellitus and CVDs, suggesting that PCOS may be a more important risk factor than assumed previously for developing Insulin resistant Diabetes and CVDs.

Keywords: Polycystic Ovary Syndrome, Hyperinsulinemia, Dyslipidemia, Creatine Kinase, estrogen, progesterone.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common disorders affecting women of reproductive age. As a syndrome, it has multiple components, including reproductive, metabolic (diabetic & hypothyroidism related effect) and cardiovascular, with long-term health concerns that cross the life span [1, 2]. Although still not well understood, insulin resistance seems to underlie many of the clinical manifestations of PCOS. Insulin resistance also appears to increase the risk of glucose intolerance, type 2 diabetes, and lipid abnormalities [2, 3]. Treatment of this disorder should focus on reduction of androgen-associated symptoms, the protection of the endometrium and reduction of the long-term risks of diabetes and cardiovascular complications [4]. Polycystic ovarian disease is a lifestyle disorder that has no specific etiology and manifests as a group of symptoms making its diagnosis difficult. It affects about 30-40% of young girls in their reproductive age in India. Polycystic ovary syndrome or PCOS is quite a common hormonal disorder in women and is characterized by ovarian cysts [4-6]. The magnitude of the problem of PCOs is very vast- also we Indian are already prone for Diabetes and Cardio vascular diseases, the incidences of hypothyroidism is still steady increasing among Indian females, this significant health problem of Indian ladies should be given more attention, also as the traditional treatment of PCOs is found not only very effective, but discontinuation of therapy results in reoccurrence of all symptoms and complications in a more aggressive way [7-10].

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Epidemiology

- Very prevalent disease affecting between 6.5 and 9.8 percent of women overall.
- Indian adolescent girls have prevalence of PCOs is 13.22%.
- Prevalence much higher in obese women (28% versus 5.5%)
- Most common cause of infertility in Indian women.
- Non-alcoholic steato-hepatitis and higher levels of C-reactive protein, higher body mass index (BMI)
- Disturbance in hypothalamic- pituitary –ovarian axis.
- Aromatization of C-14 Steroids-Progesterone & Estrogens, resulted in hyper-androgenic profile.
- The body may have a problem using insulin, called insulin resistance when the body doesn't use insulin well, blood sugar levels go up. Over time, this increases chance of getting diabetes.
- PCOs causes dislipidemias and hyperlipidemias, thus precipitates cardiac problems in later course of the disease [11, 12].

Symptoms

- Acne
- Weight gain and trouble in losing weight.
- Extra hair on the face and body. Often women get thicker and darker facial hair and more hair on the chest, belly, and back. (Hirsutism)
- Thinning hair on the scalp.(Alopecia)
- Irregular periods. Often women with PCOS have fewer than nine periods a year. Some women have no periods. Others have very heavy bleeding (Dysmenorrhoea & Oligo-menorrhoea)
- Fertility problems. Many women who have PCOS have trouble getting pregnant (infertility).
- Depression.

PCOs as an etiological cause of Diabetes

Insulin resistance/Type II diabetes is most common complication. Many previous studies concluded that women with PCOS have an elevated prevalence of insulin resistance and type II diabetes, even when controlling for body mass index (BMI). PCOS also makes a woman, particularly if obese, prone to gestational diabetes. First and foremost, it is known that insulin resistance is a major component of PCOS, and 50-70% of those suffering from it have high insulin levels or impaired blood sugar regulation. It's important to note that insulin resistance develops many years before diabetes, so the most common tests for diabetes don't often pick it up. The resultant obesity and hyper androgenic profile (more testosterone) are the precipitants of this very condition. Insulin is a hormone that signals muscle and fatty tissue to take up glucose from the bloodstream and to store it as fat or energy. When the body tissues are "resistant" to insulin, the pancreas simply makes more insulin to compensate and to keep the blood sugar levels controlled. As such, a woman with PCOS will often have much higher insulin levels in her blood than normal. With PCOS, even though other tissues in the body are resistant to insulin, for some reason the ovaries and pituitary gland remain very sensitive to it. High insulin levels cause the pituitary gland to make too much luteinizing hormone (LH), and too much LH causes the overproduction of testosterone, thus hindering ovulation [15]. As part of this vicious cycle, the high testosterone in PCOS sparks even more insulin resistance. One can get a general idea of how high levels of insulin contribute to the overall picture of PCOS as a result:

the higher the insulin, the more severe hormonal deregulations occurs. Women with type 1 diabetes are at increased risk for PCOS, further suggesting that insulin may be a key player. A possible link between PCOS and type 1 diabetes may be that the large swings in insulin levels that accompany insulin injections may place extra stress on the ovaries. Likewise for people with type 2, who tend to have high levels of insulin in the early stages of diabetes because their bodies whip it out to fight their insulin resistance. Researchers suggest that raised insulin has a direct effect on ovaries, enhancing testosterone levels, which may be behind PCOS [16]. The mechanism by which hyperinsulinemia is implicated in the development of PCOS includes the co-gonadotrophic effects of insulin (augmentation of gonadotrophin action by insulin) on ovarian and adrenal steroidogenesis, reflected by an association between levels of insulin and testosterone. Other mechanisms include the inhibitory effects of insulin on ovarian folliculogenesis and on the synthesis of sex hormone-binding globulin within the liver. The development of PCOS and Type 2 Diabetes share many common features, coupled with subsequent weight gain and associated insulin resistance. The pathogenic overlap between these two conditions explains the pathogenesis of Type 2 Diabetes includes a combination of insulin resistance and β -cell dysfunction. Insulin resistance is also a feature of many women with PCOS, particularly those with obesity. As outlined previously, there is some controversy regarding insulin resistance in lean women with PCOS, with some data to support a lack of insulin resistance in this subgroup. However, the majority of obese women with PCOS appear to manifest insulin resistance. One of the cardinal features of T2D is β -cell dysfunction and consequent insulin deficiency. By contrast, hyperinsulinemia (secondary to insulin resistance) is likely to play a key role in the development of PCOS. Therefore, a key difference between Type 2 Diabetes and PCOS would appear to be related to β -cell function. In women with PCOS, insulin stimulates ovarian theca cells, enhancing biosynthesis of ovarian androgens such as testosterone and causing arrest of ovarian follicle development. Hyperinsulinemia is also likely to have adverse effects on other tissues including the liver (i.e., suppression of sex hormone-binding globulin production), adrenal and pituitary gland. The steroidogenic effects of insulin in the context of insulin resistance can be explained through impairment of the PI3K-mediated insulin signal transduction pathway, with preservation of signalling through the alternative MAPK pathway (which typically mediates the effects of insulin on cell growth). These post-insulin receptor effects are likely to pertain to both Type 2 Diabetes and PCOS. Evidence from an effect in PCOS comes from the observation of reduced abundance of GLUT4 from adipocytes in women with PCOS (compared with adipocytes from weight-matched control women) despite there being no abnormalities in insulin receptor number or affinity. As India is the global diabetic capital, thus identification and treatment of all possible etiological factors like PCOs are of prime importance now.

PCOs as an etiological cause of Cardiac problems-

Cardiac disease is the significant killer for women globally. Adolescents and reproductive age women with PCOS have an increased prevalence of cardiovascular risk factors. These include obesity, impaired glucose tolerance, diabetes, hypertension, increased stress, depression, mood disorders and metabolic syndrome. There is sufficient evidence to confirm the presence of subclinical atherosclerosis in women with

PCOS compared to age matched controls. Hyper androgenic hormone levels precipitates dislipidemias— a major contributory factor for CVD. Women with PCOS often have elevated levels of LDL “bad” cholesterol, low levels of HDL with hypertriglyceridemias with high blood pressure, obesity and of course, Insulin Resistance, which is an underlying cause of CVD. Insulin Resistance can increase a person’s risk of developing Type 2 Diabetes. Previous research has estimated that these factors can cause up to a seven-fold increase in risk for heart attack for women with Polycystic Ovarian Syndrome, compared to those without it. Coronary heart disease (CHD) is a broad term that describes any disorder that can impact the functioning of the heart muscle itself or the cardiovascular system [17]. Conditions that contribute to heart disease include: high blood pressure, Cardiovascular Disease, arteriosclerosis (hardening of the arteries caused by calcium deposits), atherosclerosis and more. Insulin is produced in pancreas and released into the circulatory system where it is the key to the absorption of glucose by cells [18]. If cells resist insulin, both insulin and glucose build up in blood. Excess insulin leads to weight gain and high blood pressure. As insulin comes in contact with the interior wall of the arteries, it damages the tissue, causing the initial injury that produces plaque. Therefore, having Insulin Resistance and PCOS directly cause negative changes in the blood lipids and overall cardiovascular health. The patient of PCOs is many times more prone for Coronary artery disease, Atherosclerosis and Cardiomyopathies [18].

Based on these facts we designed a research work to assess the biochemical parameters related to diabetes and cardiac problems in those women who are having biochemically proved problem of Polycystic Ovary Syndrome.

Study Area- Bilaspur City and nearby villages

Study Time –March 2014 to October 2015.

Duration-About 19 months

Subjects- This is a case-control study. Indian women with age range between 20 to 48 years of age are selected either through visual symptoms and disturbances in menstruation cycle by contacting various gynecologists. (20 women) Later their selection was confirmed by sonographic reports. The visual symptoms of the hormonal imbalances as virilism, hirsutism, infertility, acne, oligomenorrhea/amenorrhea, hypermenorrhea, central obesity, dry skin, alopecia were also considered for drawing any conclusion regarding presence/severity of the disease. Also Biochemically presence of Hyperandrogenemia was taken as criteria for the selection of the subjects. Thus for the selection of subjects we opted the Criteria of National Institutes of Health (NIH) 1990 for PCOs victims.

Subjects -20 subjects with cysts in ovary and frank symptoms of PCOs.

Controls- 20 controls with matched demographic profile with subjects, mostly age matched ladies of the same families from which we picked subjects, so that we got the maximum matched demographic profile of subjects-control.

Methodology

❖ Insulin resistance was measured through Homeostasis Model Assessment (HOMA) and Blood sugar to Insulin ratio (BSIN). HOMA (Katz A, Nambi SS *et al.* 2000) and

BSIN ratio (Legro RS, Finegood D *et al.* 1999) have been shown to be reliable derived indices of insulin status – HOMA be derived by calculating the product of fasting serum insulin- fasting serum glucose, divided by a constant. (Legro RS, Castracane VD *et al.* 2004) HOMA-IR = Glucose x Insulin/405 when glucose in mass units mg/dl.

HOMA-IR = Glucose x Insulin/ 22.5 when glucose in mmol/L

- ❖ Weight was estimated by standard weighing machine. Waist: Hip ratio was also assessed in this connection. B.P. was assessed by standard auscultatory method.
- ❖ Some previous studies showed that androgenic hormonal picture of the affected persons precipitates Higher values of BMI-The BMI of subjects was estimated by weight / height² formula.
- ❖ PCOs precipitates a hormonal imbalance, that precipitates dyslipidemia, the chemicals for the estimation of total serum lipid profile we used were-(A) Cholesterol Estimation Kit (one step method of Wybenga and Pileggi) (Catalog No. – 25924)(B)HDL Estimation Kit (One step method of Wybenga and Pileggi) (Catalog No.– 25924)(C)Triglyceride Estimation Kit (Enzymatic colorimetric method GPO–PAP liquid stable single reagent) (Catalog No. 77034 (6×250 ml)).

Optical Measurements

All routine colorimetric estimations was performed on Spectro-colorimeter 103 and Spectro-photometer 106, and Colorimeter 114, (5-filters) (Systronics, India).Auto analyzer – Model Star 21 was also be used for the analysis of the total lipid profile of the cases and controls.

Calculations-The following formula was used to determine the mg/100 ml value of the following-

(A) Total Cholesterol-

(Normal level -130-250 mg/100 ml in adults)

$$\text{Serum Cholesterol (mg/100 ml)} = \frac{\text{Optical density of Test (Ax)}}{\text{Optical density of Standard (As)}} \times 200$$

(B) High Density Lipo-Protein (HDL)-

(Normal level - 35-75-mg/100 ml for adult female)

$$\text{HDL (mg/100 ml)} = \frac{\text{Optical density of Test}}{\text{Optical density of standard}} \times 50$$

(C) Estimation of Triglyceride (Tg)-

(Normal level = 10-190 mg /100 ml for adult woman)

$$\text{TG (mg/100 ml)} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 200$$

(D) Calculation of Low-Density Lipoprotein (LDL mg /100 ml)

For this the fw formula was adopted =
(Normal Range = 30-60 mg/100 ml)

(A) Triglyceride mg/100 ml = x

(B) X + HDL mg/100 ml = y

(C) Total cholesterol –y = LDL (mg /100 ml)

- ❖ The thyroid status is also affected due to this syndrome. The estimation of T3, T4 and TSH was done by using Mini Vidas-Biomarix Company Model XT-55, by using Kit of Merack Company. Also visual symptoms of hypothyroidism as dry skin, constipation, fatigue, joint stiffness, swelling in face, hair loss was observed for conformation.
- ❖ The presence of Diabetes was assessed by estimating Glucosylated Hemoglobin; (HbA1c) because in comparison to estimation of serum levels of glucose this biochemical parameter is more dependable guide for Diabetes. HbA1 is a series of glycated variants resulted due to attachment of various carbohydrate to N terminal Valine of Hb. Glycation result in increased negative charge and hence runs faster in electrophoresis system. Nycocard (boronate-affinity binding) was used for this estimation.
- ❖ Serum level of fasting and post prandial glucose levels was estimated to assess the condition of diabetes in patients of PCOs by Glucose Oxidize Method by using optical instrument (Auto-analyzer Star 21)
- ❖ Increased rate of inflammation is common feature in PCOs syndrome, this increased incidences of inflammation and infections causes increased serum level of C- reactive protein. C-reactive protein was assessed by using kit of Span Diagnostics, reagent kit, Surat [code 25934] used for in vitro detection of C - reactive protein (CRP) in human sera in auto-analyser by agglutination method. 50 micro ml serum was mixed with 1 ml reagent, clumping was indication of positive test.
- ❖ As Serum Creatine Kinase is bio-indicator of cardiac damage-so quantitative analysis of this enzyme was done to assess the severity of the cardiac damage. For analysis Kit of Tecko Diagnosis was used, for assessment kinetic method was adopted. Sample value of 50 micro ml serum was mixed with reconstituted reagent with buffer, at 37 °C and the absorbance was read at 340nm, the time interval was 30 seconds.
- ❖ The depression and anxiety levels was measured by scale developed by Quarren and Cattle – 8SQ Scale.
- ❖ The insulin level was measured indirectly by measuring c-peptide level by using spectrophotometric method developed by Nicolas.

3. Observations

- 1) The mean serum total estrogen level in the studied group was observed [Follicular Phase-5 days] 92 pmol/L, it is 60% lower than the normal values. 92% ladies of the

experimental group showed trend of lower serum estrogen levels.

- 2) The mean serum progesterone of studied group [in pre ovulation phase] was observed-0.73 ng/mL, it is 56% lower than the normal level.
- 3) The mean serum Testosterone level was higher in most of the studied subjects-[74%] –the mean level was 81 ng/dL. (Approx 11% increased)
- 4) The mean C-Peptide level of medicine group was observed significantly high. Approx 49% of these subjects showed higher serum C-peptide levels. [mean 2.3 ng /ml]. This value is approx 109% higher than the normal levels.
- 5) The level of Glucosylated Hb in medicine group was- 8.3% [HbA1c of 6% or less is normal]
- 6) Mean body mass index (BMI) was 27.44 kg/m² in obese subjects from the studied group.
- 7) Mean waist: hip ratio (WHR) was 0.83 in studied PCOs subjects. Seventy percent subjects were overweight, among whom 46.93% had high testosterone levels, 44.2% been hirsute, having significantly high BMI and total testosterone (TT).
- 8) 62.8% fulfilled sonographic criteria for diagnosing PCOS - 43.45% of them bilateral, 12.72% only left-sided and 6.81% only right sided. 59.3% were hirsute and 38.6% hyper insulinemic.
- 9) The 6 subjects from each group had tested for status of thyroid functioning, mean T3 level of experimental group was 0.83 nmol/L, mean T4 level was- 3.34 ug/ dL, and TSH level was 6.12 uIU/ml. This profile indicated marginal hypo-thyroidic status.
- 10) Dis-lipidemias is prominent problem in androgenic hormonal profile, as expected the experimental group had prominent dislipidemia- with hypercholesterolemia, hyper-triglyceridemia, lower HDL serum levels. [mean Cholesterol- 281 mg/dl, Triglyceride-314 mg/dl and HDL – 32 mg/dl., LDL by Fawcett formula- 129 mg/dl, Cholesterol: HDL- 6.1]
- 11) High to marginally high B.P. was observed in the experimental group.
- 12) The 82% experimental subjects have hirsutism and acne, 46% have hypermenorrhoea 23% have severe amenorrhoea, and 29% have oligomenorrhoea, 2% subjects are observed normal in this very aspect. 47% have central obesity.
- 13) The anxiety and depression score of experimental group was 8 on scale. This showed maximum deep depression level.

Statistics of the Study

parameters	(mean ± Sd)		Change in percent-age value
	Experimental Group	Control Group	
Serum Estrogen	92 pmol/L	233 pmol/L	↓ 60.51%
Serum Progesterone	0.73 ng/mL	1.66 ng/mL.	↓ 56.02
Serum testosterone	81 ng/dL	73 ng/dL	↑ 10.95%
Serum Cholesterol	281 mg/dl,	177 mg/dl,	↑ 58.75%
HDL (mg/ml)	32 mg/dl.	41 mg/dl,	↓ 21.95%
Serum Triglyceride	314 mg/dl	183 mg/dl	↑ 71.58%
Cholesterol: HDL	6.1	4.9	↑ 24.48%
LDL (mg/ml)	129 mg/dl,	78 mg/dl,	↑ 65.38%
Waist/ hip ratio	0.83	0.62	↑ 33.87%
BMI	27.44 kg/m ²	21.6 kg/m ²	↑ 27.03%
Glucosylated Hb	8.3	5.2	↑ 59.61%
C-reactive Protein	3.6 mg/L	3.1 mg/L	↑ 16.12%
Creatine Kinase	183 IU/L	71 IU/L	↑ 157.7%
c-peptide	2.3 ng/ mL	1.1 ng/ mL	↑ 109%
Depression Level	8	5.4	↑ 48.15%

Discussion-The biochemical and clinical profile of the PCOs victims significantly showed Hyper-Hyperinsulinemia, (Type 2 Diabetes). The victim group has double amount of c-peptide than the level in controls, this molecule is attached with 2 units of pro-insulin, when c-peptide is detached, 2 molecules of insulin is set free. Significantly high level of c-peptide showed diabetic condition in the PCOs victims. Also the serum value of Glucosylated Hb was 8.3 in experimental group, that showed the condition of frank diabetes in them. The control group has normal values of glucosylated Hb. The experimental group has high BMI and waist-hip ratio, which may be a precipitating etiological factor for Diabetic and abnormal cardiac enzymology of the subjects, which was evidenced by dyslipidemia in victims. The hormonal profile was also showed the declined trend in subjects in terms of Estrogen and Progesterone. The androgenic hormone –Testosterone showed increasing trend in the disease victims. Also the subjects showed MDD (Major Deep Depression) which is a significant symptom of the PCOs victims, level of Depression was significantly higher than the controls. The serum Level of Creatine Kinase was 157% higher in the PCOs victims, it showed an alarming cardiac–pathological condition for them.

Conclusion- We observed a univariate association between PCOs and Type II –Insulin Resistant Diabetes and Cardio damaging effect of the disease. Thus according to HACCP (Hazed Analysis of Critical Control Point System) designed by WHO –we should identify each and every etiological cause of Diabetes and Cardiac Disorders, because we are the biggest victim of both the diseases now. Thus the clinical significance of this study is much to correlate PCOs as significant etiological cause of Type-II Diabetes Mellitus and Cardiac problems.

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