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Original Research Article

"A Correlative Study of C3 in Women with Polycystic Ovarian Syndrome"

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is a metabolic disorder with an impact on the reproductive, metabolic, and cardiovascular health disease of women. It affects approximately 5%-10% of women in the reproductive age groups. The disorder can be morphological polycystic ovaries or predominantly biochemical hyperandrogenism, a clinical hallmark of PCOS, which can cause inhibition of follicular development, micro cysts in the ovaries, anovulation, and menstrual changes. ***Aims and Objective:*** A Correlative Study of C3 in Women with Polycystic Ovarian Syndrome**.** ***Materials and Methods:*** This was hospital based cross-sectional study carried out among PCOS was conducted in the Department of Obstetrics & Gynecology, Index Medical College Hospital, and Indore. This study was conducted from 1st January 2018 to 31st December 2019. A total of 260 subjects with age group between 15 to 45 years were divided into two group; cases (130) and controls (130). ***Results:*** Among a total of 260 cases, based on clinical and different biochemical parameters, 130 were diagnosis with PCOS and 130 were healthy women. The mean ±SD of various parameters among PCOS cases were; body mass index (BMI) 32.97±8.466, total cholesterol (TC) 188.42±31.126, triglyceride (TG) 134.43±50.01, high density lipoprotein (HDL) 36.29±9.55 TC/HDL ratio, 5.54±1.865 serum C3, 160.66±29.155 versus BMI 22.87±2.470, TC 155.42±26.333, TG 110.00±42.19, HDL 41.22±10.912, TC/HDL ratio 4.08±1.39, serum C3 127.48±35.60in healthy control. ***Conclusion:*** In this study, the role of inflammation and different biochemical markers were studied among PCOS cases. It was found that a majority of the PCOS patients were obese having insulin resistance. The levels of C3 as a marker of chronic low grade inflammation were higher in newly diagnosed PCOS as compared to the controls. The C3 values correlated well with various physiological and biochemical parameter.

Keywords: Polycystic ovary syndrome, PCOS, C3, BMI, total cholesterol, triglyceride.

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a metabolic disorder with an impact on the reproductive, metabolic, and cardiovascular health disease of women. It affects approximately 5%-10% of women in the reproductive age groups [1, 2]. And the most common cause of PCOS is anovulatory infertility. PCOS is a complex biochemical disorder that is associated with conditions such as obesity, insulin resistance, hyperinsulinemia, cardiovascular risk factors, and sleep apnea. It presents with clinical features of irregular menstrual cycles, hirsutism, and anovulatory infertility.

The disorder can be morphological polycystic ovaries or predominantly biochemical hyperandrogenism, a clinical hallmark of PCOS, which can cause inhibition of follicular development, micro cysts in the ovaries, anovulation, and menstrual changes [3]. Typical clinical features include hirsutism, irregular menses, chronic anovulation, and infertility. The persistent hyperandrogenism is associated with impaired hypothalamic-pituitary feedback, LH hyper secretion, premature granulose cell luteinization, aberrant oocyte maturation, and premature arrest of activated primary follicles [4].

Like other acute-phase proteins, C3 is synthesized not only by the liver but also by activated macrophages and adipocytes.Its hepatic production is induced by cytokines like interleukin-1 and tumor necrosis factor, alpha, which may interfere with insulin receptor functioning and cause insulin resistance [5]. Although serum C3 is associated with the main endogenous cardiovascular risk factors, it has also been strongly predictive of myocardial infarction independently of them. Evidently, and differently from

CRP, the association of C3 with insulin resistance is not mainly mediated by adipose tissue [6].

2. MATERIALS AND METHODS

It was a hospital-based cross-sectional study carried out among PCOS subjects conducted in the Department of Obstetrics & Gynaecology, Index Medical College Hospital and Research Centre, Indore from the 1st January 2018 to 31st December 2019. A total of 260 subjects aged between 15 to 45 years (reproductive age group) were divided into two groups as cases and control. About 130 patients were diagnosis of women with PCOS, and 130 healthy women were enrolled.

2.1. Inclusion criteria

1. History of oligomenorrhea and/or anovulation,
2. Clinical (hirsutism, FerrimanGallweyscore≥8) and /or biochemical signs of hyperandrogenism Serum testosterone ≥ 2.5nmol/L or DHEAS ≥ 8.5µmol/L.
3. Ultrasonographic evidence of polycystic ovaries and exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome)[7].

2.2. Exclusion criteria

1. History of connective tissue diseases, immunological diseases, myocardial disease,
2. Malignancy, familial hyperlipidemia, chronic liver and kidney diseases women on chemotherapy.
3. On medicine like oral contraceptive pills and corticosteroids. Women with a history of hyperprolactinemia, type 2 diabetes mellitus, type 1 diabetes mellitus, late-onset congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, or patients taking medications that could alter the hormonal or biochemical profiles [7].

2.3 Ethical Consideration

The study process was conducted only after obtaining ethical approval from the institutional ethical committee. All the information about the participants was kept confidential.

2.4 Blood Samples

Approximately 5ml overnight fasting blood sample was collected from each participant in plain vial for estimation of serum biochemical parameters, citrated vial for C3 assessment.

2.5 Methods of Estimation

The estimation of various parameters was done as per the following.

2.6 Estimation of C3

It was analyzed on an auto analyzer Roche/Hitachi COBAS c-501.

2.7 Estimation of Blood Glucose

It was done by using standard glucose oxidase-peroxidase method (Erba EM 200).

2.8 Estimation of Lipid Profile

It was assayed by using the fasting blood sample based upon the spectrophotometric principle and with the help of auto analyser machine (Erba EM 200).

2.9 Statistical Analysis

For statistical analysis, SPSS v20 (IBM©, Chicago, IL, USA) software was used. Quantitative results have been provided as a mean and a standard deviation (SD) and measured, if appropriate, by the ANOVA (F) method. Different demographic and test parameters were calculated and tabulated in the master chart and analyzed. The qualitative result is provided as numbers and percentages and contrasted, if possible, with the Chi-square (X2) method. The value of P was considered statistically significant when it came <0.05.

4. RESULT

The test parameters were analysed and tabulated as per the master chart. The results were expressed in terms of mean ± SD. The mean±SD of age was 25.19 ± 3.54 in cases and 27.49±5.158 in controls as shown in Table-1. It also shows that maximum number of patients (46.1%) was in the age grouping 26–30 years.

Table-1: Age distribution in the study subject with PCOS

Age groups	Number of Cases	Number of Control	Total	$\chi^2_{(p\text{-value})}$
15-20	12(9.2%)	11 (8.5%)	23	
21-25	53(40.8%)	38 (29.2%)	91	
26-30	60(46.1%)	46(35.4%)	106	27.12
31-35	4(3.1%)	22 (16.9%)	26	(P< 0.001)
>35	1(0.8%)	13(10%)	14	
Total	130(100%)	130 (100.0%)	260	
Mean±SD	**25.19±3.54**	**27.49±5.158**		

Blood pressure, blood glucose, body mass index was compared between the cases and controls was shown in table 2. The Mean ± SD of systolic and diastolic blood pressure was 119.05±8.426, 113.57±8.857 and 78.64±6.406, 77.03±5.670 between cases and controls respectively and this value was statistically significant. The Mean ± SD for the fasting and post prandial blood sugar between cases and

controls was 93.37±11.623, 85.09±8.454 and 99.82±13.25, 99.90±11.36.and The Mean ± SD of body mass index below 20 kg/m^{2}was 33.98±10.71 and 22.17±1.87 and above 20kg/m^2 was 32.95±8.3 and 22.98±2.51 in case and control respectively and this was statistically significant (P <0.001).

Table-2: Physiological parameter in the study subjects:

Parameter	Cases	Controls	P value
SBP (mm Hg)	119.05±8.426	113.57±8.857	0.000
DBP (mm Hg)	78.64±6.406	77.03±5.670	0.033
FBS	93.37±11.623	85.09±8.454	0.000
PPBS	99.82±13.25	99.90±11.36	0.959
BMI <20kg/m^2	33.98±10.71	22.17±1.87	0.0001
BMI >20kg/m^2	32.95±8.3	22.98±2.51	0.0001

The Mean ± SD of Serum complement component 3, an inflammatory marker, was compared in the table 3 between cases and controls. The Mean ± SD of C3 between cases and controls was160.66±29.155 and 127.48±35.60 respectively. The level of C3 shows statistically significant difference between the cases and controls. The cases show an elevated level of C3 in the blood.

Table-3: Serum complement component 3 of the study subjects

Serum complement component 3	Cases	Controls	P value
Serum complement component 3	160.66±29.155	127.48±35.60	<0.001

Comparisons between various parameters of lipid profile among the study subjects table-4 represents statistically significant difference (P<0.001) in the serum levels of total cholesterol, triglyceride, high density lipoprotein (HDL) and total cholesterol/ high density lipoprotein ratio between cases and controls. The mean ± SD of cases and controls for total cholesterol, triglycerides, is 188.42±31.126 and 155.42±26.333, 134.43±50.01 and 110.00±42.19 respectively.

The mean ± SD of HDL is 36.29±9.583 and 41.22±10.912 for all cases and controls. The Mean ± SD of total cholesterol/high density lipoprotein ratio for cases and control is 5.54±1.865and 4.08±1.39 respectively

Table-4: Lipid parameters of study subjects

Lipid parameters	Cases	Controls	P value
Total cholesterol mg/dl	188.42±31.126	155.42±26.333	<0.001
Triglyceride mg/dl	134.43±50.01	110.00±42.19	<0.001
High density lipoprotein mg/dl	36.29±9.55	41.22±10.912	<0.001
Total cholesterol/HDL ratio	5.54±1.865	4.08±1.39	<0.001

C3 shows positive correlation with FBS, total cholesterol, triglyceride, total cholesterol HDL ratio, BMI, and Systolic blood pressure (BP). Diastolic BP is in positive correlation with C3 but the difference was statistically insignificant with p value 0.619 and r value 0.031 as shown in Table-5.

In case of HDL it was negatively correlated with r-value -0.253 but it was statistically significant with C3. In the patient population, when C3 compared with PPBS the r-value was -0.0012 and p-value of 0.850 did not show statistical significance (Table-5 and Image-1&2).

Table-5: Correlations Pearson correlation of serum complement component 3 with clinical variables in cases

Correlation of C3 with clinical variable	r value	P value
Serum complement component 3vsBMI < 20kg/m^2	0.069	0.773
Serum complement component 3vsBMI >20kg/m^2	0.287	<0.001
Serum complement component 3Age <25 Y	-0.115	0.224
Serum complement component 3Age >25 Y	-0.215	0.009
Serum complement component 3vs fasting blood sugar	0.188	0.002
Serum complements component 3vs postprandial blood sugar	-0.012	0.850
Serum complement component 3vs Total Cholesterol	0.238	<0.001
Serum complement component 3vs Triacylglycerol	0.177	0.004
Serum complements component 3vs high density lipoprotein	-0.253	<0.001
Serum complement component 3 vs Cholesterol /high density lipo protein ratio	0.276	<0.001
Serum complement component 3vsSystolic BP	0.267	<0.001
Serum complement component 3vsDiastolic BP	0.031	0.619

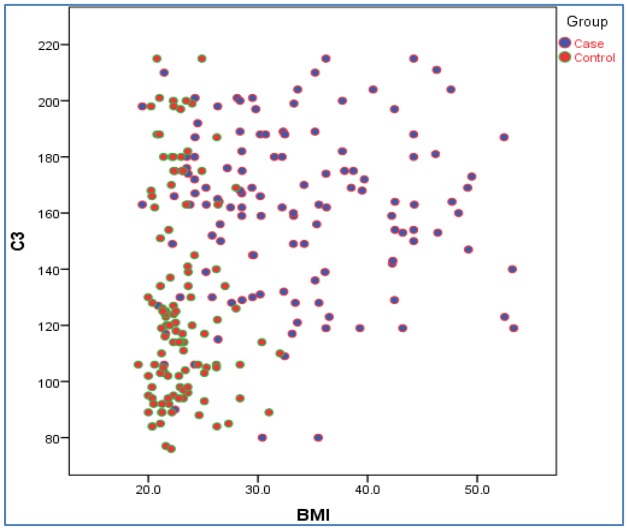

Image-1: The correlation between BMI and C3

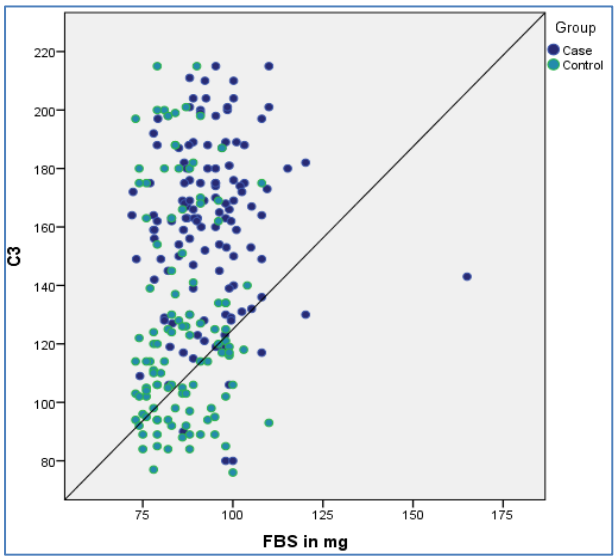

Image-2: The correlation of C3 and FBS

DISCUSSION

In our study, we compared the two groups which were composed of PCOS and without PCOS morphology. PCOS patients had higher systolic/diastolic blood pressure, higher BMI, higher fasting blood glucose, and postprandial blood glucose was similar in both the group, higher total cholesterol, TG, LDH levels were seen in PCOS group compared to healthy controls and lower HDL level than the control group.

Out of the total cases almost half of the polycystic ovarian syndrome was found in the age group between 26-30 years which was similar to the study of Mellembakken *et al*. which indicated that the prevalence of PCOS increased with age and is peaked up in the early and mid-twenties of life [8].

Our study reported the systolic and diastolic blood pressure to be significantly higher in PCOS patients compared to healthy controls. The study also noted that the PCOS patients were notably obese and with high BMI which could be responsible for hypertension in the PCOS women compared to the healthy our study was similar to the study conduct by Lo JC and Barcellos *et al*. founded women with PCOS were 40% more likely to have elevated blood pressure than the non-PCOS women, independent of age, BMI, diabetes or dyslipidemia [9, 10].

In our study, the fasting and postprandial blood glucose level was significantly high in PCOS patients compared to the healthy women when comparison made by case and controls for the levels of fasting & postprandial blood sugar. The high level of glucose was mainly due to insulin resistance and as a result of diabetic mellitus this result agrees with study conduct by of Bu Z *et al*. which demonstrated that a majority of PCOS women had impaired glucose tolerance [11].

Our study also demonstrated that the body mass index was significantly correlated with the PCOS in both the group above 20kg/m^2 and below 20kg/m^2 ($p<0.001$) the study also showed mean BMI was higher in both groups of the case compared to control which was similar to the study conducted by Sharma *et al*. this study find BMI is more prevalent in women with PCOS

however, obesity is an independent and stronger risk factor for developing PCOS [12].

While taking account to C3, the level of c3 was higher in the case as compared to control and was statically significant. Our observation was different from the study conducted by the Wu Y *et al*. as they reported that the C3 levels were higher but not significantly different between premenopausal women with PCOS (2.1 g/L) and controls (1.8 g/L) [13]. The different behind this variation was, our study had some limitations firstly, the sample size was small which makes the results less generalized secondly, we did not measure visceral fat and waist-to-hip ratio which might be an adiposity and insulin resistance in PCOS women and control.

In our study, we did the comparisons between various parameters of lipid profile in PCOS patients which was statistically significant (P<0.001). Serum levels of total cholesterol, triglyceride, high-density lipoprotein and total cholesterol/high-density lipoprotein ratio between cases and controls group were compared. These findings showed a significantly higher level of different lipid parameters in PCOS patients as compared to healthy women. This study also highlighted that the TC levels were above the threshold cut-off value the and finding was similar to the observations in the study made by Kiranmayee *et al*. Maximum number of women with PCOS demonstrated abnormal anthropometric parameters, and in more than 70% of women, lipid abnormalities such as low levels of high-density lipoprotein (HDL) cholesterol and high levels of triglycerides and low-density lipoprotein cholesterol were seen. Significant positive correlations between triglycerides, high-density lipoprotein, and cholesterol were observed [14]. Another authore Halasawadekar *et al*. also reported that TG and TC/HDL ratio was significantly high in the PCOS group compared to the healthy control [15].

Pearson correlation of serum complement component 3 with clinical variables among PCOS patients has persistently higher than the prevalence rates of metabolic syndrome in control women. Our finding was Similar to the study done Snyder et al. where the Levels of C3 were positively correlated with BMI, waist circumference, SBP, fasting insulin, fasting glucose, triglycerides, total cholesterol, HDL-C, and LDL-C in women with PCOS and controls, all $p<0.05$. Additionally, mean levels of C3 were positively associated with increasing CAC in women with PCOS and controls [16].

In summary, our study strongly agrees that the complement activation and dysregulation was significantly associated with PCOS having obesity and insulin resistance. This study also shows that PCOS women had elevated levels of c3, BMI, fasting blood glucose. These disturbances have implications for lipid clearance, inflammation, insulin resistance, and obesity. Our findings suggest that C3 may be a strong predictor of PCOS among women in the general population.

CONCLUSION

In the present study, the role of inflammation in polycystic ovarian syndrome was considered. C3 levels in the blood were compared in freshly diagnosed cases of PCOS patients and healthy controls. It was found that a majority of the polycystic ovarian syndrome patients were obese and insulin resistant. The levels of C3, marker of chronic low-grade inflammation, were higher in freshly diagnosed patients compared to the controls. The C3 values correlated well with increased in BMI and Age. This could probably imply a state of insulin resistance, low grade inflammation, abdominal obesity and activation of the complement cascade in a majority of women with polycystic ovarian syndrome. Abdominal obesity, anovulation, hirsutism and irregular menstrual a common finding in women with polycystic ovarian syndrome can lead to increase in the release of the inflammatory mediators which can activate the complement cascade and thus show increase in the levels of C 3 in women with PCOS. Research has shown that Inflammation is strongly associated with metabolic dysfunction, obesity, anovulation, hirsutism and infertility. Thus, in the current study, the state of low-grade inflammation seen in cases of polycystic ovarian syndrome could strongly influence their risk for adverse polycystic ovarian syndrome and metabolic complications.

CONFLICT OF INTERESTS

Authors have no conflict of interests.

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