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INTERPLAY OF HORMONAL PARAMETERS & IL – 6: OBESE VS NON-OBESE PCOS

Obstetrics & Gynaecology

Bishal Kumar Dey Junior Research Fellow, Dept. of Anatomy, Diamond Harbour Govt. Medical College and Hospital

Dr. Arun Das Demonstrator, Dept. of Biochemistry, Diamond Harbour Govt. Medical College and Hospital

Dr. Sarmishtha Ganguly Associate Professor, Dept. of Biochemistry, Diamond Harbour Govt. Medical College and Hospital

Prof. Dr. Somajita Chakraborty Professor and Head, Dept. of Obstetrics & Gynaecology, Diamond Harbour Govt. Medical College and Hospital

Dr. Sanchita Roy* Associate Professor, Dept. of Anatomy, Diamond Harbour Govt. Medical College and Hospital*Corresponding Author

ABSTRACT

Background: Polycystic Ovary Syndrome, the most common endocrinological disorder among women in the reproductive age group which is characterized by chronic ovulatory dysfunction, certain hormonal disturbances as well as metabolic derangements. **Aim:** This study aimed to see a correlation between Luteinizing Hormone and Interleukine – 6 level in normal and high body mass index women with PCOS and to establish the importance of IL – 6 as a useful biomarker for predicting detrimental consequences of PCOS like the development of type 2 diabetes mellitus. **Methods:** Follicle stimulating hormone, Luteinizing hormone, Thyroid stimulating hormone and Interleukine - 6 have been estimated. Statistical analysis has been performed with SPSS software. Student's T-test is used to compare means between each of the test group with the control group and Karl Pearson's method of correlation is used to find out the correlation coefficient. **Results:** The study included 187 women, diagnosed with PCOS (normal BMI group: n = 25, and high BMI group: n = 31) and 100 age matched control. There were significant association found between LH and IL – 6 concentrations in these two groups of PCOS with respect to control. **Conclusion:** There is an association in between IL – 6 and LH concentrations in these two groups of PCOS with respect to control. IL – 6 is a marker for T2DM and the development of T2DM does not depends on BMI. Probably, LH in its elevated condition play a vital role to develop T2DM in females with PCOS.

KEYWORDS

polycystic ovary syndrome, interleukine – 6, leuteinizing hormone etc

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrinopathy affecting about 1 in 15 women worldwide with heterogeneous presentation and multifactorial etiology (Norman et al., 2007). According to the Rotterdam criteria hyperandrogenism, chronic anovulation and polycystic ovaries on USG are three main clinical features of PCOS patients ("Revised 2003 Consensus on Diagnostic Criteria and Long-Term Health Risks Related to Polycystic Ovary Syndrome.," 2004). This disorder is associated with infertility, obesity, insulin resistance (IR) and subsequent increased risk of type 2 diabetes mellitus (T2DM). Therefore, it is evident that individuals with PCOS have increased life time risk for cardiovascular diseases.

IL – 6 is a major pro-inflammatory cytokine in chronic inflammation which is secreted by the macrophages in response to any kind of inflammation or infection with other immune molecules (cytokines and chemokines) like TNF – α, IL – 8, IL – 18, MCP – 1 and MIP – 1 (Ali et al., 2019). Early research in vivo showed that human recombinant IL – 6 if infused could induce gluconeogenesis, subsequent hyperglycaemia and compensatory hyperinsulinemia (Stith & Luo, 1994). IL – 6 might play a key role in the development of cardiovascular diseases (CVD) through metabolic, endothelial and coagulant effect and elevated level of IL – 6 were reported to be associated with an increased risk of future myocardial infarction and atherothrombosis (Guldiken et al., 2008; Ridker et al., 2000; Yudkin et al., 2000).

As to reproductive processes it has been speculated that increased IL – 6 productions might alter vital steps in follicular maturation by paracrine and autocrine actions, ultimately contributing to ovarian dysfunction, failure of fertilisation and implantation (Sukhikh & Vanko, 1999). Obesity that aggravates both the metabolic sequelae and reproductive abnormalities was reported to be associated with increased IL – 6 levels (Roytblat et al., 2000; Vgontzas et al., 2006).

AIM

Several studies involving the relationship between hormonal parameters, IL – 6 and PCOS have been performed. However, the results remain inconclusive and controversial probably because of the varied population and limited sample sizes. So, this study was aimed to explore that IL – 6 level can be used as diagnostic and prognostic marker for development of metabolic disorders like T2DM and cardiovascular diseases in these women with PCOS.

MATERIALS & METHODS

Study design

This was a case control study conducted at Diamond Harbour Govt. Medical College and Hospital, West Bengal from November 2020 - October 2021 which is a tertiary healthcare system. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans and passed through the institutional review board.

Inclusion criteria

The study included women with history of amenorrhea or oligomenorrhea, clinical features of hyperandrogenism like hirsutism and furthermore patients were required to present with typical polycystic ovaries on ultrasound. Polycystic ovary morphology was determined on the basis of ovarian volume criteria and follicular size and number. Subjects were all free from medication at the time of the study. 100 healthy fertile women with no clinical or biochemical signs of hyperandrogenism and ultrasound exclusion of PCOS, took part as a control group. No participants manifested or referred recent clinical infections.

Exclusion criteria

Patients with other associated disorders as Cushing disease, hypothyroidism, hyperprolactinemia, adrenal hyperplasia, ovarian tumours or recent or chronic infection were excluded from the study. A total 56 patients met the above criteria in the prospective case-control study.

Study groups

The BMI was calculated as weight (kg.) divided by the height in meters squared (m^2). On the basis of BMI, 56 cases were categorized into two groups – I) Non-obese/ Normal BMI group with BMI < 27 and ii)

Obese / High BMI group with BMI ≥ 27. 100 age matched women without PCOS have been considered as control.

Data collection

Estimation of IL – 6 has been done along with hormonal estimation. We estimated only those hormones that regulate the normal development of the bilateral ovaries along with facilitation of ovulation and maintain the proper metabolism. Therefore, hormones like follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH) have been estimated. The questionnaire was prepared for keeping the patient's history. It included age, height, weight, BMI and remarks of USG report. Blood was collected in clot vials from the patients and hormonal and IL – 6 estimations were performed. Hormonal estimation and IL – 6 estimations were performed by using the autoanalyzer ADVIA Centaur® Immunoassay System. It is a typical autoanalyzer that can estimate the concentration for hormones and cytokines. The inter- and intra-assay coefficients are less than 15% and less than 10%, respectively.

Statistical Analysis:

Statistical analysis has been performed with SPSS software. After testing for normality with the Kolmogorov – Smirnov test the values were expressed as Mean ± S.D. Student's T-test is used to compare means between each of the test group with the control group. Outcomes was considered as significant at $p < 0.05$ (in case of few parameters, $p < 0.01$ and $p < 0.001$ considered more significant over $p < 0.05$). Correlation is used to find out the association between choices of interest parameters.

RESULT

Characteristics of the study population

The study included 56 women, diagnosed with PCOS and investigations were conducted between days 5 and 8 from the last menstrual cycle. The characteristics of the study population are shown in table 1. Comparison of hormone levels between non-obese and obese PCOS groups Both groups had comparable serum LH level, FSH level, TSH and IL – 6 level. Patients in the high BMI group had significantly higher serum levels of TSH compared to the non-obese BMI group ($p < 0.05$). but TSH concentration in both non-obese (5.4025 ± 0.905 mIU/L) and obese PCOS (10.265 ± 0.17 mIU/L) is significantly high compared to control ($p < 0.05$) as shown in table 2. The mean FSH level (4.145 ± 1.93 IU/L) in non-obese PCOS is decreased compared to controls (6.73 ± 2.21 IU/L) with $p < 0.01$ whereas in obese PCOS significant variation not found in FSH level compared to control. The mean LH/FSH ratio was significantly higher in obese PCOS group in comparison to non-obese PCOS group. The percentage of women with LH/FSH ratio > 2 was higher in high BMI group. The IL – 6 level were high in both non-obese and obese women with PCOS in comparison to control group but it is significantly higher in high BMI PCOS group than that of normal BMI PCOS group. A scatter plot of the correlation between LH and IL – 6 is shown in Figure 1 and Karl Pearson's method of correlation is used to find out the correlation coefficient. In that case, the obtained correlation coefficient is $r = 0.9497$ and the co-efficient lies between $0 < r < 1$ (Moderately Positive Coefficient). That means increase in the IL – 6 concentration influences the increase in the LH concentration. The correlation coefficient in case of non-obese PCOS between LH and IL – 6 is $r = 0.2039$ ($r < 1$). The correlation coefficient in case of obese PCOS between LH and IL – 6 is $r = 0.6036$ ($r < 1$). So, it can be assumed that rise in the level of both LH and IL – 6 are more associated with obese PCOS.

Discussion

PCOS is a complex and heterogeneous disorder associated with reproductive and metabolic disturbances. Although a majority of cases with PCOS are obese/overweight and several studies suggested an increased risk for developing metabolic disturbances in these obese PCOS patients, a small but significant proportion of patients with PCOS present with normal body mass index (BMI ≤ 27kg/m2) that makes diagnostic work up and treatment approach more difficult. These cases are termed as non-obese PCOS. We observed in our study that the PCOS patient with normal BMI are having late onset presentation than that with high BMI. So delayed diagnosis in non-obese PCOS may affect the diseased individual silently from detrimental effects. So, awareness should be developed among the clinician regarding the early diagnosis of these cases of non-obese PCOS. In our study we have seen that woman with PCOS both non-obese and obese are associated with higher TSH levels when compared to age matched controls without PCOS. The findings of the present study are in consistent with study by Dahiya et al., Kedar KV et al. and Moustafa MM et al. (Dahiya et al., 2012; Kedar et al., 2019; Moustafa et al., 2019). Although the etiopathogenesis of hypothyroidism and PCOS is completely different, these two entities have many features in common. Whether this is due to some common factors predisposing an individual to both disorders, or due to a pathophysiological connection between the two disorders has not yet been clearly understood. It is hypothesized that there is presence of thyroid hormone receptors on human oocytes and thyroid hormones are involved in the gonadotropin induced estradiol and progesterone secretion by human granulosa cells. So, hypothyroidism would interfere with ovarian function. Wakim et al. in their research reinforced the hypothesis that hypothyroidism worsens PCOS by further decreasing sex hormone binding globulin levels, increasing the conversion of androstenedione to testosterone and aromatisation to estradiol and reducing the metabolic clearance rates of androstenedione and estrone (Wakim et al., 1995). High level of testosterone contributes to PCOS symptoms like infertility, polycystic ovaries, hirsutism, male pattern hair loss and acne. Increased estrogen again increases the levels of thyroid binding globulin and mask the activity of free thyroid hormones. Thus, the clinical features of PCOS gets aggravated due to hypothyroidism and vice-versa.

In PCOS women the normal gonadotrophin axis is disturbed, therefore LH levels increase, and FSH levels decrease, leading to a reversal of LH/FSH ratio (Balen et al., 2003). Our results are concordant with the study of Lal et al. who also found significant differences between obese and non-obese women regarding LH, FSH, and LH/FSH ratio (Lal et al., 2017). But study done by Alnakash et al. and Zaheera Saadia showed no differences in the LH, FSH and LH/FSH ratio between obese and non-obese PCOS individual (Alnakash & Al-Tae'e, 2007; Saadia, 2020). Upon these findings, it can be said that the geographical and dietary variation may play a vital role in the hormone levels of PCOS women and in our population obese women should be advised to try to lose weight and change their lifestyle for reversal of LH/FSH ratio.

The development of T2DM has been considered to be associated with chronic inflammation in different studies and thus markers of activated inflammatory processes like IL – 6 has been shown to predict the risk and prognosis of T2DM (Pradhan et al., 2001; Spranger et al., 2003). In our study we have seen that woman with PCOS both non-obese and obese are associated with higher IL – 6 levels when compared to age matched controls without PCOS which is consistent with the study by CC Kelly et al. and in our cohort, it is significantly high in obese PCOS than that of non-obese PCOS (Kelly et al., 2001). But in another studies plasma IL – 6 level were not increased when compared with age and BMI matched controls and the hypothesis that PCOS is associated with chronic inflammation, were nullified (Möhlig et al., 2004; Villuendas et al., 2002). Though In some researches it has been found that obesity itself associated with elevated level of IL – 6 level (Escobar-Morreale et al., 2003; Sukhikh & Vanko, 1999) but Alexandra N. Vgontaz et al. showed elevated IL – 6 level in PCOS independent of obesity (Roytblat et al., 2000). Aboedldalyl S et al. also established in their study the role of chronic inflammation in PCOS (Aboeldalyl et al., 2021). In our study population we have noted that the IL – 6 level has positive correlation with the LH concentration in both non-obese and PCOS individual. Thus, increase in IL – 6 level can be potential biomarker for predicting detrimental consequences of PCOS. Further experimental studies are required with more sample size to establish the biological and clinical relevance of IL – 6 level in PCOS patients. The hormonal and metabolic factors in PCOS subjects may be strongly associated with insulin resistance that may lead to a condition of low-grade inflammation eliciting an overproduction of IL – 6. Whether this is following any microbial inflammatory stimulus, further studies should be undertaken, particularly in view of the number of different microbial agents to which an individual is exposed during her life.

Table: 1 – Characteristics of study population. All the data has been expressed in mean ± standard deviation format.

Sl. No.	Variables	Control [n = 100]	High BMI [n = 31]	Normal BMI [n = 25]
01.	Age, years	21.63 ± 2.341	20 ± 1.87†	22 ± 2.44
02.	Height, meter	1.524 ± 0.343	1.54 ± 0.106	1.546 ± 0.014

03.	Weight, kilogram	57.031 ± 2.322	63.66 ± 3.858†	47 ± 3.559†
04.	BMI, kg/m2	26.935 ± 2.28	27.033 ± 1.924	21.1325 ± 2.409†

'†'symbolizes that test data has a significance at < 0.001 with respect to control.

Table: 2 – Parameters measured for the High and Normal BMI-PCOS females and age matched control females. Parameters were the concentrations of FSH, LH, TSH and IL – 6. The test data were compared with the control data for establishing the significance by statistical methods.

Sl. No.	Parameters	Control [n = 100]	High BMI [n = 31]	Normal BMI [n = 25]
01.	FSH, IU/L	6.73 ± 2.21	7.48 ± 1.726	4.145 ± 1.93†
02.	LH, IU/L	5.629 ± 0.216	26.15 ± 3.566†	14.89 ± 1.442†
03.	LH/FSH (ratio) > 2	0.9 ± 0.22	4.0241 ± 0.313 (77%)	3.595 ± 0.785 (68%)
04.	TSH, mIU/L	2.59 ± 1.352	10.265 ± 0.17†	5.4025 ± 0.905†
05.	IL – 6, pg/	6.73 ± 2.21	28.03 ± 4.361†	7.9 ± 1.10†

'†'symbolizes that test data has a significance at < 0.001 with respect to control.

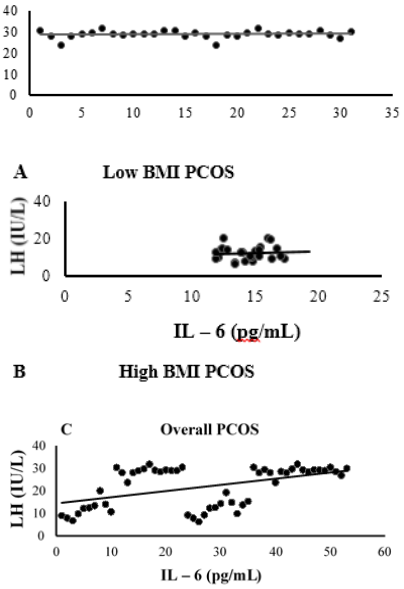

Fig: 1 – Scatter plot was done in between IL-6 (pg/mL) vs. LH (IU/L) – A) Normal BMI PCOS, B) High BMI PCOS and C) Overall PCOS. In each case, data show a positive correlation.

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