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ADIPOCYTOKINES IN OBESE AND NON-OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME

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ABSTRACT:
Aims and Objectives: Dysregulation of adipocytokines play a key role in the pathogenesis of polycystic ovary syndrome (PCOS). This study aimed to compare serum adiponectin, osteocalcin and resistin levels in obese PCOS (BMI≥25kg/m2) and non-obese PCOS (BMI<25kg/m2) subjects and then to compare with their age and BMI matched controls.
Materials and methods: A total number of 81 PCOS subjects (Obese-42; Non-obese-39) and controls (Obese-42; Non obese-44) were recruited in the study. A Rotterdam criterion was used for the diagnosis of PCOS. All patients underwent detailed clinical, anthropometric, hormonal assessment, insulin resistance indices and serum levels of adipocytokines (adiponectin, resistin and osteocalcin).
Results: Forty five percent of overall PCOS subjects (obese PCOS: 60% and non-obese PCOS: 30%) had features of either clinical or biochemical evidence of hyperandrogenism. Serum adiponectin levels were significantly ($p<0.05$) lower in subjects with obese PCOS (4.3 ± 2.0 mcg/ml) when compared to its controls (5.5 ± 2.8 mcg/ml). Serum osteocalcin levels were significantly lower ($p<0.005$) in non-obese PCOS subjects (14.4 ± 7.2 ng/ml) when compared to age and BMI matched controls (19.7 ± 8.4 ng/ml). Serum resistin was not significant in both the groups. Serum adiponectin levels were significantly lower ($p=0.02$) and serum resistin levels were significantly higher ($p=0.02$) in obese PCOS when compared to non-obese PCOS. Serum adiponectin and osteocalcin had significant negative spearman's correlation with free testosterone index. Serum osteocalcin and adiponectin had a significant positive correlation ($p<0.05$) with quantitative insulin sensitivity check index (QUICKI) whereas serum resistin had a significant negative correlation ($p=0.03$) with QUICKI.
Conclusion: Significant derangements in serum adipocytokines levels were noticed among the PCOS subjects. Significantly lower serum adiponectin levels in obese PCOS and significantly lower serum osteocalcin levels in non-obese PCOS were noticed when compared to their age and BMI matched controls. Serum resistin levels were not significantly different. Significantly lower serum adiponectin levels and higher resistin levels were noticed in women with obese PCOS when compared to non-obese PCOS.

KEYWORDS: Adiponectin; Osteocalcin; Resistin; Adipocytokines; Obese polycystic ovary syndrome; Non-obese polycystic ovary syndrome.
RUNNING TITLE: Adipocytokines in PCOS

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorder affecting women of reproductive age. It may have a very variable presentation that may include irregular menstrual cycles, infertility, features of hyperandrogenemism and various metabolic disturbances like insulin resistance, dyslipidemia, hypertension and diabetes mellitus. Its prevalence ranges from 4% to 11% in various western literatures (1) and from 13% to 22% in various South Asian studies.(2) (3) (4)
Various adipocytokines (adiponectin, leptin, resistin, osteocalcin, vifastin, irisin, chemerin, retinol binding protein-4, lipocalin-2 and omentin-1) have an interaction with various organs and may play an important role in the development of insulin resistance which is the key feature in PCOS leading to the risk of development of various metabolic complications. (5) (6) (7) (8) (9) (10) (11)

Adiponectin is the most abundant adipokine secreted by adipocytes and is one of the most important proteins involved in insulin resistance and other pathophysiologic features of polycystic ovary syndrome. It has actions at the various organ systems including liver, skeletal muscle, hypothalamus, pituitary and gonadal organs. (12) It leads to the activation of adenosine monophosphate-activated protein kinase (AMPK) pathway leading to inhibition of acetyl coenzyme A carboxylase and subsequent increased fatty acid beta oxidation. (13) It also acts as an insulin sensitizer by reducing hepatic glucose production and stimulation of glucose uptake by skeletal muscle. (14) It has a modulating role in the regulation of central reproductive endocrine axis by inhibiting luteinizing hormone (LH) release and gonadotropin releasing hormone (GnRH) stimulated LH release. (15) Adiponectin has also been shown to have a vital role in maintaining hyperandrogenism which is a key role in the pathogenesis of PCOS. It will have beneficial effects by reducing insulin mediated androstenedione and progesterone production by reducing the expression of various factors in theca cells like LHR, CYP11A1 and CYP 17A1. (16)

Osteocalcin is a biomarker synthesized by osteoblasts as under-carboxylated osteocalcin (ucOC) precursor molecule which matures to form carboxylated osteocalcin (cOC) after posttranslational modification by vitamin D dependant gamma glutamyl carboxylation. (17) (18) UcOC has an impact on glucose metabolism by several mechanisms. It stimulates the expression of several beta-cell proliferation genes, promotes the expression of the insulin gene and increases the insulin production within the beta-cells. It also acts as an insulin secretagogue and positively control energy metabolism through the release of adiponectin.(17) Osteocalcin also shows a significant correlation with body fat, body mass index and glycemic status. (19) (20) Hence Osteocalcin being a metabolic marker has a potential implication in the pathogenesis of PCOS. .

Resistin, named after its relation to the property of insulin resistance, is a small cysteine-rich protein primarily secreted by mature white adipocytes. (21) (22) (23) Resistin by virtue of its actions like activation of suppressor of cytokine signalling-3 (SOCS-3), activation of mitogen activated protein kinase (MAPK), inhibitory effects on the insulin signalling pathway may ultimately lead to insulin resistance.(24) (25) (26)

Adiponectin, osteocalcin and resistin play an important role in the pathogenesis of insulin resistance in PCOS. Both the western and Indian literature shows conflicting results with regard to serum levels of adiponectin, osteocalcin and resistin in PCOS subjects. Hence this study was aimed for assessment of serum levels of adiponectin, osteocalcin and resistin in PCOS subjects. In our previous published study we had studied the body composition, metabolic characteristics and insulin resistance in obese and non-obese women with PCOS. (27)

MATERIALS AND METHODS:

Subjects:

This is a cross sectional study conducted from Aug 1, 2016 to March 31, 2018 over a period of 20 months, at a tertiary care centre in southern India. “Cases” were defined as women with PCOS attending the Endocrinology and Reproductive Medical Unit clinic. For comparison with cases healthy women without PCOS from the community were regarded as “controls”. Written informed consent was taken from all the women enrolled in the study. The study protocol was approved by the institutional review board.

All women with 18–35 years of age with phenotypic features of PCOS and fulfilling the Rotterdam criteria (28) for the diagnosis of PCOS were included in the study. The secondary causes of oligo-anovulation and PCOS were excluded by detailed history taking, physical examination and appropriate hormonal blood investigations. Women with history of intake of drugs which can alter the plasma concentration of androgen levels or can cause hirsutism were excluded. Those subjects with pre-existing diabetes mellitus were also excluded from the study.

Women who had a history of intake of drugs which can alter the plasma concentration of androgen levels (oral contraceptive pills, metformin, thiazolidinediones, or spironolactone for more than the preceding 3 months) or history of intake of drugs which can cause hirsutism (androgens, valproic acid, cyclosporine, diazoxide, or minoxidil) were excluded from the study. Women with pregnancy or with pre-existing diabetes mellitus were also excluded from the study.

Study design:

PCOS subjects were classified based on the body mass index (BMI) (29) (30) as Obese PCOS (BMI ≥25 kg/m2) and Non-obese PCOS (BMI <25 kg/m2) respectively. Similarly controls were classified as Obese controls (BMI ≥25 kg/m2) and Non-Obese controls (BMI <25 kg/m2). All control subjects were age and BMI matched.

All women were subjected for anthropometric measurements (height, weight, BMI and waist-hip ratio). All women underwent biochemical tests for assessment of adipocytokines, hyperandrogenism and for exclusion of secondary causes of PCOS. The assay methods for adipocytokines were as follows; adiponectin (sandwich assay ELISA; DIA source, S.A KAPME09; intra-assay CV 4.7%), osteocalcin (electrochemiluminisence immunoassay; Roche Elecsys 1010/2010; intra-assay CV 3.8%) and resistin (sandwich assay ELISA; DIA source, S.A KAPME50; intra-assay CV 5%). Parameters of hyperandrogenism which are 8 am total testosterone, free testosterone, sex hormone binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEAS) were assessed by chemiluminescenceimmunoassay. All women also underwent biochemical tests

(prolactin, thyroid stimulating hormone, 17 hydroxyprogesterone, and post 1 mg dexamethasoneserum cortisol) for the exclusion of secondary causes of PCOS. Insulin resistance was assessed by various insulin resistance indices namely fasting insulin level (cut-off value; ≥ 12.2 μ U/ml) homeostasis model assessment-insulin resistance (HOMA-IR) (cut-off value; ≥ 2.5) and quantitative insulin sensitivity check index (QUICKI) (cut-off value; <0.33). (31) (32) (33) (34) (35). All the parameters including anthropometric, parameters of hyperandrogenism along with adipocytokines were compared between PCOS women and their age and BMI matched controls.

Sample size calculation:
The sample size was calculated based on the data published by Remsberg et al. (36). A part of this study has already been published. (27) With 80% power, and alpha error of 5%, the sample size required was 36 in each of obese and non-obese PCOS group. An equal number of patients were required in each of the control groups (obese controls and non-obese controls) for comparison between cases and controls.

Statistical analysis:
Statistical analysis was done using SPSS for Windows, Version 16.0. (SPSS Inc., Chicago, USA). Two-sample t-test was used to compare the mean differences in continuous outcome variables between the groups. Similarly, the Chi-square test was used for the association between the categorical variables, as appropriate. Spearman's and Pearson's correlations were used for nonparametric and parametric correlations, respectively.

RESULTS:
A total of eighty one PCOS subjects (Obese-42; non-obese-39) and eighty six controls subjects were recruited in the study. Overall 45% of PCOS subjects had hyperandrogenism either clinical or biochemical (obese-60%; non-obese-30%). Baseline characteristics and demographic features of PCOS women and controls are shown in Table 1. Comparison of serum adipocytokine levels (adiponectin, osteocalcin and resistin) between obese and non-obese PCOS with age and BMI matched controls are shown in Table 2. Serum adiponectin levels were lower in subjects with obese PCOS (4.3 ± 2.0 mcg/ml) when compared to its controls (5.5 ± 2.8 mcg/ml) and was statistically significant ($p<0.05$). Serum osteocalcin levels were lower in non-obese PCOS subjects (14.4 ± 7.2 ng/ml) when compared to age and BMI matched controls (19.7 ± 8.4 ng/ml) and was statistically significantly ($p=0.005$). Serum resistin was not significant in both the groups. A comparison of serum adipocytokine levels (adiponectin, osteocalcin and resistin) among obese and non-obese PCOS are shown in Table 3. Serum adiponectin levels were significantly lower ($p=0.02$) and serum resistin levels were significantly higher ($p=0.02$) in obese PCOS when compared to non-obese PCOS. Correlation between parameters for hyperandrogenism, insulin resistance indices with adipocytokines in PCOS women and controls are shown in Figure 1 and Table 4, 5.

DISCUSSION:
This study attempted to evaluate the serum levels of adiponectin, osteocalcin and resistin in obese and non-obese PCOS subjects in comparison with their age and BMI matched controls.

Serum adiponectin levels were significantly lower in subjects with obese PCOS when compared to its controls ($p=0.04$). However it was not significant when it was compared with non-obese PCOS and their controls. When compared among the obese and non-obese PCOS subjects, it was statistically significant ($p=0.02$) and lower levels in the obese group. Serum adiponectin had a significant positive correlation ($p<0.05$) with QUICKI. Serum adiponectin levels have been shown be to lower in PCOS patients when compared to age and BMI matched controls in majority of the studies including a meta-analysis. (37) (38) (39) (40) However few studies had documented similar levels of serum adiponectin levels in PCOS subjects and controls.(41) In our study, we noticed significant lower levels of adiponectin in obese PCOS subjects when compared to controls and among obese vs non-obese PCOS subjects. Since the adiponectin levels are reduced in obesity per se (42) and PCOS subjects are more prone for development of obesity, this could have led to lower levels more in the obese group rather than the non-obese group. Adiponectin is directly involved in insulin resistance and hyperandrogenism which are the vital features of PCOS. (12) (13) (14) Lower adiponectin levels in PCOS subjects compared to controls have a direct role in the pathophysiology of PCOS.

Serum osteocalcin levels were lower in the PCOS subjects (obese and non-obese group) when compared to controls; however it was statistically significant only in the non-obese PCOS subjects when compared to their age and BMI matched controls. It did not show any significance when it was compared among obese and non-obese PCOS subjects. Serum osteocalcin had a significant positive correlation ($p<0.05$) with QUICKI. A Greek study (43) showed significant lower serum osteocalcin levels in in PCOS subjects when compared to control group however they were not significant in another Romanian study. (18) Osteocalcin being an insulin secretagogue has an impact on glucose and energy metabolism. (17) (18) Thus osteocalcin has an vital endocrine cross talk between skeleton and energy metabolism forming bone pancreas loop. Lower serum osteocalcin levels are detrimental for PCOS subjects.

Serum resistin levels were significantly higher in obese PCOS group when compared to non-obese PCOS group. However it was not significant when these PCOS subjects were compared with their age and BMI matched controls. Serum resistin had a significant negative correlation ($p=0.03$) with QUICKI. Studies

regarding the resistin levels in patients with PCOS are conflicting. Few studies from western and Indian literature have documented higher levels of resistin in PCOS. (7) (44) (45) However the other studies have noticed a similar value which was statistically insignificant when compared to age and BMI matched controls. (46) (47) (48) Resistin by virtue of its name plays a vital role in the development of insulin resistance in PCOS. It acts on various insulin signalling pathways leading to insulin resistance. (24) (25)

Serum osteocalcin and adiponectin levels had a significant negative correlation with serum free testosterone levels in subjects with PCOS. SHBG had significant positive correlation with serum adiponectin levels. Serum resistin levels did not have any correlation with the parameters of hyperandrogenism. So adipocytokines may have a significant role in a subset of PCOS subjects who have features of hyperandrogenism either by clinical or biochemical criteria.

Our study has several merits. It is the first Indian study which has looked into the osteocalcin levels in PCOS subjects and compared with age and BMI matched controls. It is also the first Indian study which has looked at all the three important adipocytokines (adiponectin, osteocalcin and resistin) in a single study which will help in better understanding of the pathophysiology of adipocytokines in PCOS. This study also attempted at correlation of these adipocytokines with various insulin resistance indices for assessing their pivotal role in insulin resistance. However there were a few limitations. A BMI cut-off value of <25 kg/m2 was considered for non-obese group which includes both normal weight subjects (BMI<23 kg/m2) and overweight subjects (BMI ≥23 kg/m2 but ≤25 kg/m2) due to the inadequate number in the normal weight PCOS group. Ideally for better interpretation and proper understanding of pathophysiology there should have been three groups with adequate number namely normal weight (BMI<23 kg/m2), overweight (BMI ≥23 kg/m2 but ≤25 kg/m2) and obese (BMI >25 kg/m2) in both PCOS group and control group.

CONCLUSION:

Serum adiponectin levels were significantly lower in obese PCOS and serum osteocalcin levels were significantly lower in non-obese PCOS when compared to their age and BMI matched controls. Serum resistin levels were not significantly different in PCOS when compared to its age and BMI matched controls. A lower serum adiponectin levels and higher resistin levels were noticed in women with obese PCOS when compared to non-obese PCOS.

Financial support and sponsorship:

This study was approved and funded by the fluid research grant, CMC, Vellore (IRB. Min No. 9553).

Conflicts of interest: There are no conflicts of interest.

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