POSSIBLE CORRELATION BETWEEN OBESITY AND INFLAMMATION IN PCOS – EVALUATION OF LEPTIN AND CRP

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1. Introduction

Leptin is known to play an important role in the regulation of food intake and energy expenditure, in hematopoiesis, angiogenesis, reproduction and its receptors are found in almost all the tissues. Reports have shown that the ob gene product, leptin could be an important determinant of inflammation. In PCOS, serum leptin level correlates well with body weight, BMI, percentage of body fat, serum levels of Tumor Necrosis Factor – α (TNF- α) and Interleukin-6 (IL-6). Also, increased leptin can lead to cytokine release from the adipose tissue and the reproductive tissues. Thus, Obesity can be regarded as a pro-inflammatory state contributing to a wide range of chronic diseases such as diabetes and cardiovascular disease (CVD) and in Polycystic ovarian syndrome (PCOS) this might act as a crucial risk factor for the development of CVD.

C-reactive protein (CRP) is an acute phase protein present in atherosclerotic lesions, more specifically in the vascular intima, where it co-localizes with monocytes, monocyte derived macrophages and lipoproteins (hinting at the possibility for the role of CRP in the progression of atherosclerosis. The mechanism linking increased fat mass with high CRP level is not known and hence the present study. CRP may be involved in atherogenesis by directly influencing processes like complement activation, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation and thrombosis. Recently, CRP has been reported to be an independent predictor of the risk of atherosclerosis, cardiovascular events, atherothrombosis, hypertension and myocardial infarction.

In PCOS, it has been of our concern to evaluate inflammatory cytokines such as TNF- α, IL-6, leptin and CRP and study its correlation to BMI. However, no reports relating the synthesis of these cytokines to the pathogenesis of CVD are available.

Therefore, we investigated the expression of leptin, IL-6 and TNF- α and also their signaling product CRP in the reproductive and the adipose tissues. In an independent study, serum leptin and CRP was measured (in a set of 12 control and 12 PCOS subjects) with a view to understand the role of obesity in inflammation and the onset of CVD in PCOS subjects.

2. Results:

Increased secretion of cytokines in PCOS could enhance the synthesis of CRP which in turn can promote atherogenesis in PCOS. In order to evaluate this, a study was undertaken in the serum of the PCOS subjects. 12 control and 12 PCOS subjects were analyzed and their serum biochemical profile, leptin and CRP levels were evaluated and the results obtained are indicated in table 1. We observed a strong positive correlation between their serum leptin and serum CRP level in the PCOS subjects under study. CRP level was estimated using Biolatex agglutination method and most of the obese control and PCOS subjects under the study were found to possess CRP level at the ratio of 1:16 dilution. Its level was found to be negative in subjects with normal BMI. Further detailed analysis is needed to understand the actual link between CRP and inflammatory cytokines in PCOS.

BMI was found to be higher in PCOS subjects (28.5 ± 1.3) when compared to that of the control subjects (22.7 ± 0.8) and also BMI was correlated to the serum leptin level. Most of the PCOS subjects under study are found to be overweight or obese. Their serum triglyceride and serum cholesterol levels were found to be 154 ± 12.74 mg/dl and 198.67 ± 12.1 mg/dl respectively and these values were lower than that of the control subjects which were found to be 165+5.6 mg/dl and 201.75 ± 6.1 mg/dl respectively. Though there was no significant increase in the serum triglyceride and the serum cholesterol level of PCOS subjects, their serum HDL level (39.5 ± 2.3 mg/dl) was found to be significantly lower ($p<0.05$) than that of the control subjects (52.75 ± 1.21 mg/dl)

PCOS subjects are characterized by insulin resistance, but still their serum glucose level (91.2 ± 4.9 mg/dl) was found to be lower than that of the control subjects (101.25 ± 3.78 mg/dl) indicating that PCOS subjects might possess impaired glucose tolerance. In addition, they are also found to possess significantly high systolic blood pressure (124.31 ± 4.15), TG/HDL ratio (4.26 ± 0.78) and CHO/HDL ratio (5.4 ± 0.74) when compared with the control subjects having the corresponding values of 98.45 ± 3.4, 3.14 ± 0.12 and 3.82 ± 0.13. Most of the PCOS subjects under study were found to be hypertensive irrespective of their BMI.

3. Discussion:

Using the data from 12 control and 12 PCOS subjects in an independent study, we found a profound positive correlation between the serum leptin and BMI in PCOS subjects. Serum leptin level was also found to be highly correlated to CRP values in PCOS subjects with CRP being present in 1:16 dilution in most of the obese subjects under study. Leptin is the key hormone coupling the

immune system and the energy balance (Brian N et al., 1998) and it has been found to increase the CRP synthesis, which in turn can promote atherogenesis by augmenting the immune response to certain antigens. Data obtained from the present study suggest that elevated levels of serum leptin in PCOS subjects might promote their risk of getting CVD by stimulating CRP production. PCOS subjects have also been found to possess a high TG/HDL ratio, CHO/HDL ratio, an increased systolic blood pressure and a low HDL level, all of which suggest the risk of their acquiring CVD.

Hypertension is a major cause for endothelial dysfunction, which in turn augments pathways crucial for atherothrombosis. Increased evidence has indicated that hypertension, through the vasoactive peptides, such as angiotensin and endothelin-1 can promote and accelerate the atherosclerotic process via inflammatory mechanisms (Li Jian-Jun, Chen Ji-Lin 2005). A significant decrease in the serum HDL level and a significant increase in the systolic blood pressure and CHO/HDL suggest that PCOS subjects are more prone for plague rupture, which can in turn lead to arterial thrombosis.

To conclude, our study for the first time proclaim that adipose tissue, endometrium and the ovarian cortex of the PCOS subjects express high level of leptin as well as IL-6 and TNF-alpha mRNA. Also, a strong positive correlation is observed between serum leptin, BMI and CRP in PCOS subjects. CRP values correlate well with BMI in most of the subjects under study. Biochemical profiles were also found to exert a pathological trait in them. All these facts suggest the influence of inflammatory cytokine over CRP synthesis which could in turn exert its proinflammatory cytokine and proatherogenic effects in the endothelial cells (Vincenzo Pasceri et al., 2000). The level of cytokine including leptin reveal that PCOS subjects are at a high risk of getting inflammation associated CVD with obesity acting as an added risk factor for the same.

4. Materials and Methods:

SUBJECTS:

In order to study the correlation between serum leptin and CRP, a study was undertaken in a population consisting of 12 control and 12 PCOS subjects in the age group 20-30 years of age. PCOS women with polycystic ovarian morphology were confirmed by visual inspection of the ovaries by laporatomy, laparoscopy or by ultrasound examination. There was no evidence for hyperprolactinemia, Cushing's syndrome, congenital or non-classical adrenal hypoplasia and hormone secreting tumors.

Control women were healthy with regular menstrual cycle and there was no evidence of hyperandrogenism, polycystic ovaries, endometriosis or abnormal uterine bleeding. The subjects considered were devoid of smoking habits and alcoholism.

Neither PCOS nor control women had taken medication within 60 days of blood collection. After obtaining written consent, fasting blood was drawn at the Institute of Obstetrics and Gynecology (Madras Medical College, Chennai) and University centers (Institute of Basic Medical Sciences). The subjects were allowed to fast for 12h and then 2ml of blood was collected from them. The blood was allowed to clot and it was retracted and separated by centrifugation at 2000g for 15 min. Serum was separated and stored at –80ºC for further analysis.

Anthropometric variables, including height and weight were measured using standard protocols. Height was measured to the nearest 0.1cm motionless on a scale and weight was measured to the nearest 0.1kg on a portable stadiometer. Waist circumference measurement was taken at the level of umbilicus and hip measurement was taken at the maximum circumference of the buttocks with the subjects in the relaxed standing posture. Blood pressure was measured using the mercury sphygmomanometer.

ELISA METHOD FOR MEASURING LEPTIN CONCENTRATION: Serum leptin concentration was measured using the Sandwich Leptin Elisa kit (Diagnostics Biochem Canada Inc.) according to the manufacturer's protocol.

AGGLUTINATION METHOD FOR SERUM CRP CONCENTRATION: Circulating CRP concentration was measured using bio-latex agglutination method according to the manufacturer's instruction.

SERUM BIOCHEMICAL ANALYSIS: Serum glucose was estimated by glucose oxidase-peroxidase method, serum cholesterol was measured by the cholesterol oxidase-peroxidase method, serum high density lipoprotein (HDL)-cholesterol was estimated by the glycerol-3-phosphate oxidase-peroxidase-N-ethyl-methyanilinpropan-sulphonate sodic method, serum triglyceride was measured using GPO-POD-ESPT method using autoanalyzer (BAYER RA 50; Bayer Company India, India).

STATISTICAL ANALYSIS: For each experimental series, data are presented as mean$\pm$SEM. Statistical significance ($P<0.05$) for each variable was estimated using t-test carried out

using a software program (SPSS 7.5 for windows student version).

DECLARATION OF INTEREST:

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Table 1: Biochemical profile of control and PCOS subjects under study.

S.No.	BIOCHEMICAL PARAMETERS	CONTROL SUBJECTS	PCOS SUBJECTS
1.	BMI	22.7±0.8	28.5±1.3
2.	Glucose (mg/dl)	101.25±3.78	91.2±4.9
3.	Triglycerides (mg/dl)	154.0±12.74	165.0±5.6
4	Cholesterol (mg/dl)	198.67±12.1	201.75±6.1
5	HDL (mg/dl)	39.5±2.3	52.75±1.21
6	TG/HDL ratio	3.14±0.12	4.26±0.78
7	CHO/HDL ratio	3.82±0.13	5.4±0.74
8	Systolic Blood Pressure	98.45±3.4	124.31±4.15

9	Diastolic blood Pressure	118±2.5	123±3.2
10	Leptin (ng/ml)	6.9±1.4	14.7±1.3