Comparative evaluation of procoagulant and proinflammatory markers in drug naive versus oral contraceptive pill's (OCP's) treated Polycystic Ovary Syndrome (PCOS) women

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ABBREVIATIONS

ADMA	Asymmetric dimethyl arginine
AES	Androgen excess society
APC	Activated Protein C
APCR	Activated Protein C Sensitivity Ratio
APTT	Activated partial thromboplastin time
BMD	Bone mineral density
BMI	Body Mass Index
BP	Blood pressure
CAD	Coronary Artery Disease
CHOD-PAP	Cholesterol oxidase- Phenol +Amino Phenazone
Cm	Centimeter
CVD	Cardio vascular disease
DBP	Diastolic blood pressure
DHEAS	Dehydroepiandrosterone
DVT	Deep Vein Thrombosis
ECLIA	Electrochemilumniscence
EE	Ethinyl Estrogen
E+P	Estrogen + Progesterone
ELISA	Enzyme linked immunosorbent assay
ET-1	Endothelin-1
FGIR	Fasting Glucose to Insulin Ratio.
FG Score	Ferrimen Gall way Score

FSH	Follicle Stimulating Hormone
GnRH	Gonadotrphins release hormone
GOD-POD	Glucose oxidase peroxidase method
GPO-PAP	Glycerol -3-phosphate oxidase - Phenol +Amino Phenazone
HDL	High density lipoprotein
HOMA-IR	Homeostatic model assessment of insulin resistant
HRP	Horse radish peroxide
HRT	Hormone replacement therapy
Hs-CRP	High sensitivity-C reactive protein
IDF	International Diabetes Federation
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin-8
IMT	Intima media thickness
IRMA	Immunoradiometric assay
IU/L	International units per liter
µIU/ml	Micro international units per milliliters
Kg	Kilograms
Kg/m²	Kilogram per meter square
LDL	Low density lipoprotein
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LNG	Levonorgestrel
MCP-1	Monocyte chemo attractant protein-1

mg/dl	Milligrams per deciliters
mm Hg	Millimeter mercury
NCAH	Non classical congenital adrenal hyperplasia
ng/ml	Nanogram per milliliter
ng/dl	Nanogram per deciliter
NDDG	National diabetes data group
NF-κB	Nuclear factor kappa B
NICHHD	National Institute of child health and human development.
NIH	National Institute of Health
□C	Degree Celsius
OCP's	Oral Contraceptive Pill's
OGTT	Oral Glucose Tolerances Test
PAI-1	Plasminogen Activator Inhabitor-1
PCOS	Polycystic ovary syndrome
PCO	Polycystic ovaries
PE	Pulmonary Embolism
pg/ml	Pico gram per milliliter
PRL	Prolactin
QUICKI	Quantitative insulin sensitivity check index
RIA	Radio immuno assay
SBP	Systolic blood pressure
SD	Standard deviation
SHBG	Sex hormone binding globulin

SKIMS	Sher-i-Kashmir institute of medical sciences
SPSS	Statistical package for social sciences
s-ICAM	Soluble intercellular adhesion molecule
s-VCAM	Soluble Vascular cell adhesion molecule
T	Testosterone
T_4	Thyroxine
TG	Triglyceride
TMB	3,3'5,5' Tetramethyl-benzidine
TNF-α	Tumor necrosis factor-α
t-PA	Tissue type plasminogen activator
TSH	Thyroid stimulating hormone
T2DM	Type 2 diabetes mellitus
USG	Ultrasonography
u-PA	Urokinase type plasminogen activator
vWF	von Will brand factor

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ABSTRACT

BACKGROUND:

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of young women. There is a spectacular increase in the prevalence of PCOS all over world especially in Asia. The condition seems to be on rise in Kashmir valley although systemic studies on the subject are underway. First line of treatment is often the oral contraceptive pills (OCP's) as most of these women attend to various gynaecologists and they desire menstrual regulation. Since there is plethora of data that PCOS women have evidence of enhanced markers of inflammation and indicators of coagulation. Therefore treatment with OCP's may worsen the metabolic, inflammatory and coagulation parameters in this population. We undertook this study to investigate the effect of OCP treatment procoagulant and proinflammatory markers in women with PCOS. The results may therefore translate in to the clinical practice.

AIMS AND OBJECTIVES:

Estimate and compare the impact of OCP's on the proinflammatory and procoagulant activities in women with PCOS.

SUBJECTS:

The study was done as joint collaborative effort between departments of Endocrinology, Sher-i-Kashmir Institute of Medical Sciences, Soura and department of Clinical Biochemistry, University of Kashmir. We studied 51 women with a confirmed diagnosis of PCOS who were drug naive (controls) and compare them with 30 PCOS women who received Estrogen and progesterone (OCP's) as treatment modality for approximate period of six months (cases). We investigated and compared clinical, anthropometric, hemodynamic, hormonal, metabolic, procoagulant and proinflammatory parameters of cases and controls. Rotterdom 2003 Criteria was applied for making a positive diagnosis of PCOS after ruling out disorders such as hypothyroidism, hyperprolactinemia, Non classical congenital adrenal hyperplasia(NCAH), Cushing's syndrome, androgen excreting adrenal or

ovarian lesion and androgen intake,. An informed consent was obtained from all the participants and the study was approved by the Institutional Ethics Committee.

METHODS:

The clinical assessment included detailed menstrual history, quantitation of hyperandrogenism (hirsutism, acne vulgaris, and male pattern hair growth), anthropometric assessment (height, weight, waist and hip circumference). Biochemical assessment involved two hour OGTT (oral glucose tolerance test) for glucose and insulin in additions to lipids. Plasma levels of s-ICAM (Soluble intercellular adhesion molecule), MCP-1(Monocyte chemoattractant protein 1), TNF-α (Tumor necrosis factor-α), PAI-1(Plasminogen activator inhabitor-1) and factor VIII were done to estimate severity of inflammation and procoagulant activities. Hormonal evaluation included T4, thyroid stimulating hormone (TSH), cortisol (morning), luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), 17-hydroxyprogesterone (17-OHP), and total testosterone (T). The samples for LH, FSH, T, and 17-OHP were collected on days 3–7 (early follicular phase) of spontaneous cycle or medroxyprogesterone-induced menstrual cycle in amenorrheic patients. Overnight dexamethasone suppression test, if needed, was done after taking basal samples and performing OGTT.

ASSAYS:

Radioimmunoassay (RIA) was used to analyse T4, cortisol, 17-OHP, T and Immunoradiometric assay (IRMA) for TSH, LH, FSH, PRL using commercial kits in duplicate and according to supplier protocol {Diagnostic Product Corporation (DPC); USA for LH, FSH, PRL, DIASORIN ;North western Ave for T4 , cortisol and IMMUNOTECH; France for T, TSH and 17-OHP}. Plasma glucose (mg/dl) was measured by glucose oxidase peroxidase (GOD-POD) method {URILAB; India} Cholesterol was measured by CHOD-PAP method {DIALAB; Austria} TG was measured by GPO-PAP method {DIALAB; Austria} HDL and LDL was measured by New clearance method {RANDOX; UK} on Hitachi 912; Japan. Intra and inter-assay variations were within the limits permitted by the manufacturer. Plasma levels

of s-ICAM, MCP-1, TNF-α, PAI-1 were measured using ELISA kits and factor VIII was measured by Coagulometric method.

Statistical analysis was done using SPSS 11.5 software. In addition to descriptive statistics, student's t test and ANOVA was used to compare the groups.

RESULTS:

Among 51 women with PCOS without treatment (controls) and 30 women with PCOS on OCP's (cases), number of cycles/year were (9.12±3.88 vs. 9.90±3.30 P=0.358). Ferriman-Gallwey score was less in OCP treated PCOS subjects compared to drug naive (10.00±2.60 versus 12.27±4.71, P=0.017) indicating efficacy of the treatment. OCP treated PCOS patients showed increasing marginal rise in clinical parameters like weight in Kg (59.07±6.34 versus 58.57±8.52, P=0.782), waist hip ratio (0.92±0.06 versus 0.91±0.06, P=0.544) and BMI in Kg/m^2 (24.07±3.42 versus 23.66±3.43, P=0.606), systolic BP (123.00±7.21 versus 122.24±6.99 mm Hg, P=0.640), diastolic BP (79.46±4.29 versus 79.37±4.62 mm Hg, P=0.928). Metabolic parameters like fasting blood glucose levels were higher in OCP treated PCOS subjects compared to drug naive PCOS subjects (88.77±10.41 versus 87.75±19.91 mg/dl, P=0.795), as was total cholesterol (186.10±44.76 versus 155.08±28.86 mg/dl, P=0.000) and LDL (118.45±45.66 versus 83.20±27.63 mg/dl, P=0.000). Serum testosterone level decreased in OCP treated PCOS subjects compared to drug naive PCOS subjects (56.05±31.81 versus 63.31±31.30 ng/ml, P=0.320). Fasting insulin levels were higher in OCP treated PCOS subjects compared to drug naive PCOS subjects (16.23±24.72 versus 12.28±11.10, P=0.326), as was HOMA-IR (3.00±4.17 versus 2.73±2.64, P=0.335) indicating worsening of insulin resistance with OCP use. Accordingly QUICKI was lower in OCP treated PCOS subjects compared to drug naive PCOS subjects (0.513±0.013 versus 0.516±0.001, P=0.449).

Plasma levels of s-ICAM-1 (ng/ml) were higher in OCP treated PCOS subjects compared to drug naive PCOS subjects (417.03±131.62 versus 312.41±131.65, P=0.001) as were plasma levels of MCP-1(pg/ml) (464.82±91.19 versus 456.78±187.25, P=0.827) and TNF-α (pg/ml) (25.60±4.24 versus 22.85±5.19,

P=0.016) indicting negative impact on various inflammatory markers. Plasma levels of procoagulant marker PAI-1(ng/ml) were higher in OCP treated PCOS subjects compared to drug naive (1.10±0.59 versus 1.05±0.40, P=0.682) but in contrary plasma levels of factor VIII were higher in drug naive compared to OCP treated PCOS subjects (0.35±0.33 versus 0.68±0.32, P=0.000).

CONCLUSION:

These findings imply that although OCP treatment helps in menstrual cyclicity and hyperandrogenism in women with PCOS, metabolic parameters such insulin resistance indices, glucose tolerance and lipid profile of PCOS subjects worsens with their use. In line with objectives of the present study we observed elevation of plasma concentrations of procoagulant (PAI-1) and proinflammatory markers (s-ICAM, MCP-1, TNF-α) in PCOS subjects treated with OCP's. Interestingly our data did not suggest elevation of factor VIII levels (procoagulant marker) which looks intriguing. We don't have any apparent explanation for this contrary result at this moment and the finding needs to be looked at closely and in a systematic way to explain it. Although, we did not have a control group to see the pre-existing procoagulant and proinflammatory activity in PCOS women, our data suggests that OCP group had unfavorable profile as compared to full blown PCOS women who were not treated. This indicates that E+P use in PCOS women can be viewed as unsafe and their routine use may be avoided. More studies with an arm of healthy control women on a larger cohort and more detailed parameters of coagulation and inflammation with longitudinal follow up will likely answer the question.

Chapter 1

INTRODUCTION

Polycystic ovary syndrome (PCOS) is defined as hyperandrogenic chronic anovulation and accordingly women with PCOS have the symptoms of irregular menstrual cycles, hyperandrogenism, and polycystic enlarged ovaries. PCOS is a heterogeneous metabolic disorder affecting 4 to 10% of reproductive age women (1). The incidence of PCOS varies according to the diagnostic criteria employed. Polycystic ovaries (PCO) on ultrasound are noted in up to 25%-30% of reproductive aged women (2,3) and the vast majority of women with PCO do not have the syndrome. Women with unexplained hyperandrogenic, chronic anovulation (i.e. NIH criteria) make up approximately 7% of reproductive age women (4) while as the Rotterdam criterion being broader in view inclusion of sonographic evidence of cysts in the ovary increases the prevalence by 50% over the NIH criteria (5). The prevalence according to the Androgen Excess Society (AES) criteria is somewhere in between. There are no systematic prevalence studies from our country however, the condition seems to be very common than west and it seems to be on rise in our population. Women with PCOS usually present with symptoms of irregular menstrual cycles, acne, hirsutism, hyperandrogenism, obesity and enlarged PCO (6). As many as 30% or more of women with endocrinologically confirmed PCOS do not have PCO, on pelvic ultrasound (7). The genetic contribution to PCOS remains uncertain, and there is currently no recommended genetic screening test. PCOS phenotype is likely a complex genetic trait, and based on genome wide association studies of such diseases, for example type 2 diabetes, there are likely multiple variants. Also no specific environmental substance has been identified as a cause of PCOS, although certain medications such as valproate have been shown in vitro (8) or in clinical series in women with epilepsy to induce hyperandrogenism (9). As obesity increases its prevalence or exaggerates its phenotype, the epidemic of obesity is

accompanied by a parallel increase in PCOS prevalence. In 1935 Stein and Leventhal described PCOS as a syndrome characterized by obesity, hirsutism and infertility but now the definition of PCOS is controversial at best and task of making a positive diagnosis is difficult, thus hampering its scientific evaluation (10,11). Besides the pathogenesis of PCOS is complex and currently three putative mechanisms have been proposed as follows:

1. Primary Disordered Gondadotropin Secretion: The first biochemical abnormality that was identified in women with PCOS was disordered gonadotropin secretion, with a preponderance of luteinizing hormone (LH) to follicle stimulating hormone (FSH). This theory explained the morphology of the ovary, hirsutism, and anovulation. The excess circulating androgen leads to stimulation of the pilosebaceous unit that increases sebum production, induces terminal hair differentiation, and in rare instances in the scalp leads to pilosbaceous unit atresia and androgenic alopecia. Studies of gonadotropin secretion in women with PCOS have established that women have augmented release of LH in response to a GnRH (Gonadotrphins releasing hormone) challenge with appropriate levels of FSH secretion (12). Obesity tends to blunt baseline LH levels and GnRH stimulated levels in women with PCOS, though their response remains elevated when compared to appropriate age and weight matched control women (13).

2. Primary Ovarian and Adrenal Hyperandrogenism: Ovarian steroidogenesis is perturbed in the syndrome with increased circulating androgen levels. The intrafollicular androgen levels tend to be elevated in antral follicles supporting the fact that there is a lack of adequate granulosa aromatase activity (14). Thecal cells from PCOS women put into long term culture exhibit defects in steroidogenesis including hyper production of androgens, possibly a genetic defect in the cells (15). In 20-30% of women with PCOS have evidence of adrenal hyperandrogenism, primarily based on elevated levels of dehydroepiandrosterone (DHEAS) (an androgen marker of

adrenal function), suggesting that the defect in steroidogenesis is primary, and affects both androgen secreting glands, i.e. the ovary and the adrenal.

3. Primary Disorder of Insulin Resistance: Women with PCOS show multiple abnormalities in insulin action. Dynamic studies of insulin action, including hyperinsulinemic euglycemic clamps and frequently sampled intravenous glucose tolerance tests have shown that women with PCOS are more insulin resistant than weight matched control women, a defect primarily present in skeletal muscle (16,17). Hyperinsulinemia is linked to ovarian and adrenal hyperandrogenism in a number of disorders of inherited insulin resistance with compensatory hyperinsulinemia including leprechaunism, the Rabson Mendenhall syndrome, and the lipodystrophies (18). In vitro cultures of PCOS thecal cells have been found to overproduce androgens in response to insulin supplementation.

Thus, PCOS is now recognized to be a variant of metabolic syndrome which may include hyperinsulinemia, hyperlipidemia, diabetes mellitus, and possibly cardiac disease, as well as the more conventionally recognized hirsutism, ovarian follicular atresia with anovulation, infertility, elevated androgen levels, endometrial cancer and obesity (19). Therefore, PCOS now should be viewed not just as a gynecological or dermatological disorder, but a sex limited manifestation of metabolic syndrome that involves multiple body systems and probably stems from a key pathogenic element called hyperinsulinemia. There are multiple consensus conferences held so far, for purpose of establishing a diagnosis of PCOS. None of them has been good enough to withstand various validation techniques. Three main diagnostic criteria employed for diagnosis of PCOS are as follows;

A. The National Institute Of Health/National Institute Of Child Health and Human Development (NIH/NICHHD 1990) consensus conference criteria is the most commonly employed diagnosis for the PCOS and is as follows

1. Clinical and/or biochemical hyperandrogenism
2. Oligo-anovulution(oligomenorrhea/amenorrhea)

Exclusion of disorders like non-classical congential adrenal hyperplasia (NCAH), cushing syndromes, thyroid dysfunction, hyperprolactinemia and androgen producing tumors.

B. ROTTERDAM 2001 CRITERIA (20)

The Rotterdam criteria for the diagnosis of PCOS (2003) states 2 of the 3 features needs to be present to make the diagnosis and with the exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome). These features includes (1) Oligo- or anovulation (2) Clinical and/or biochemical signs of hyperandrogenism and (3) PCO (either 12 or more follicles measuring 2–9 mm in diameter, or an ovarian volume of >10 cm^3).

C. ANDROGEN EXCESS SOCIETY(AES) (21)

Diagnostic criteria lead down by (AES 2006) includes hyperandrogenemia and or hyperandrogenism plus one out of 2 remaining criteria of oligo/amenorrhea and polycystic ovaries on ultrasonography.

Obesity is the major accompaniment in 30-60% of the women with PCOS (22). Obesity promotes an unconventional, sub-clinical inflammation, mainly due to secretion of a battery of pro-inflammatory factors [e.g. Leptin, TNF-α, IL-6, IL-1β] (23,24). The proinflammatory nature of adipose tissue is heightened in proportion to fat accumulation and exhibits consistent positive correlations with increasing body mass index (BMI) and especially with visceral adiposity (23,24,25). Thus, central obesity appears to trigger and exacerbate an inflammatory cascade that initially evolves within fat depots.

Indeed, current evidence suggests that this obesity-related activation of inflammatory signaling pathways is linked to major cardio vascular disease (CVD) risk factors [e.g. Type 2 diabetes (T2DM) and atherosclerosis] (26,27). Obesity also induces multiple constitutional alterations in the micro-environment and cellular content of adipose tissue depots which collectively promote differentiation of pre-adipocytes, insulin resistance and proinflammatory responses (23,24). Weight gain enhances both lipogenesis and adipogenesis inside fat depots, as well as secretion of proinflammatory adipokines and chemokines [e.g. MCP-1, and IL-8] into the plasma. In response to such chemotactic stimuli mononuclear cells are recruited from the circulation and transmigrate into adipose tissue depots, increasing the number of resident macrophages (28,29). In turn, this growing local population of macrophages secretes cytokines, such as TNF-α, IL-1β and IL-6, which can potentially aggravate the proinflammatory and insulin resistant profile of adipocytes. Thus, sustained fat accumulation establishes an unremitting local inflammatory response within the expanding adipose tissue. Progressively this cascade transcends to a chronic low-grade generalized inflammatory state in obesity, mediated by persistent release of proinflammatory adipokines of either adipocyte or macrophage origin (30) with adverse effects on peripheral tissues and organs (e.g. liver, muscles, endothelium). These effects promote hepatic and skeletal muscle insulin resistance, hypertension, atherosclerosis and hypercoagulability. In fact, subclinical CVD and early impairment of endothelial structure and function have been previously reported in non-obese PCOS women (31-35). Insulin resistance has been associated with an increased incidence of CVD as atherosclerosis is now considered to be an inflammatory disorder (36,37).

Insulin resistance has recently been associated with increased levels of inflammatory mediators in the blood (38, 39). Studies have therefore been conducted to look at inflammation in PCOS. TNF-α has been shown to play

an important role in initiation of inflammation (40). The TNF-α signaling pathway is mediated by nuclear factor kappa B (NF-KB) and is responsible for the expression of adhesion molecules such as soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble Intercellular adhesion molecule-1 (sICAM-1) in the endothelium (41). Gonzelez et al noted increased levels of TNF-α, the cytokine which causes insulin resistance and is secreted by the adipose tissue in PCOS women as compared to controls (42).

As is generally accepted that atherosclerosis is an inflammatory disease, the initiating factor is considered to be endothelial dysfunction (43, 44). This is followed by inflammatory cell infiltration and lipid accumulation; which in turn stimulate vascular smooth muscle cell proliferation, migration and extra-cellular matrix production. Normally, the vascular endothelium resists the binding of leucocytes, presenting a barrier to their infiltration into the sub-endothelial layer. However, a variety of stimuli may impair endothelial function, rendering it susceptible to leucocyte adhesion, and potentially initiating atherogenesis. Similar processes are also implicated in the acute manifestations of atherosclerosis (45). The predominant mechanism in the pathogenesis of the acute coronary syndromes is atherosclerotic plaque rupture. Histological evidence confirms that this is characterized by infiltration of leucocytes. These release proteolytic enzymes that weaken the extracellular matrix destabilizing the plaque, and increasing the risk of exposure of its thrombogenic sub endothelial matrix and lipid core. Endothelial erosion, a less common but important cause of unstable atherosclerotic disease, particularly in younger patients, is also associated with dysfunction and destruction of endothelial cells (46). Once again the adhesion of leucocytes is a fundamental component of this process. The binding of leucocytes to vascular endothelium is mediated by a variety of cell surface adhesion receptors–principally the selectins and integrins. The selectins are the initiators of adherence, but the binding they induce is weak, allowing

leucocytes to roll along the vessel wall. This contact enables leucocyte and endothelial integrins to interact, permitting the more secure binding that precedes spreading and infiltration. The main endothelial integrin receptors sVCAM-1 and sICAM-1, which bind to leucocyte β 1 (α4β1) and β2 (αLβ 2 and αMβ 2) integrins, respectively. Normal vascular endothelium expresses little or no sVCAM-1 or sICAM-1. They are, however, induced by endothelial dysfunction, suggesting that they may represent biological 'markers' of atherosclerosis and might predict an increased risk of its acute manifestations. Certainly, focal endothelial sVCAM-1 and sICAM-1 have been demonstrated overlying areas of early atherogenesis (47, 48) and within atherosclerotic plaques (49-51). Several data support the hypothesis that soluble cell adhesion molecule levels may reflect the extent of atherosclerotic disease. Patients with CVD or peripheral vascular disease have higher levels of sICAM-1 than healthy controls (52). Likewise, several large epidemiological studies have demonstrated that apparently healthy subjects with elevated levels of sICAM-1 are at increased risk of developing overt coronary artery disease (CAD), peripheral vascular disease, carotid atherosclerosis and stroke (53-58). As Rizzoni and colleagues report, many risk factors for atherosclerosis, including diabetes and hypertension, are generally (though not always) associated with increased levels of sICAM-1 and sVCAM-1 (59-66). It is unclear whether the observed elevations are because of direct effects or to subclinical atherosclerosis. Certainly, elevated levels of both sICAM-1 and sVCAM-1 have been documented in patients with essential hypertension without overt atherosclerotic disease (63) although in this cohort of elderly (mean age 69 years) hypertensive men, it seems likely that some would have in apparent disease. The data from Rizzoni et al (59) showing significant elevations of sICAM-1 and sVCAM-1 in patients with hypertension and normal intima–media thickness, do, however, suggest that hypertension may play a direct role. In PCOS, significantly higher levels of

sICAM-1 than in healthy women were found. sICAM-1 levels correlated with body composition, lipids and insulin secretion, but not with insulin resistance (67).

Women with PCOS are characterized by increased levels of procoagulant markers like fibrinogen and PAI-1 both of which promote atherogenic processes and increase the risk of CVD (68-71). Fibrinogen is synthesized by hepatocytes and holds a pivotal role in the coagulation cascade, being a major determinant of plasma viscosity and platelet aggregation. Expression of fibrinogen in the liver is up-regulated by IL-6 during the acute phase reaction and various studies have documented an association between elevated fibrinogen levels and increasing body mass index (BMI). Notably, fibrinogen has also been shown to predict weight gain in middle-aged adults (72). PAI-1 levels are increased in various disease states (some forms of cancer, obesity and the metabolic syndrome has been linked to the increased occurrence of thrombosis in patients with these conditions. In inflammatory conditions where fibrin is deposited in tissues, PAI-1 appears to play a significant role in the progression to fibrosis (pathological formation of connective tissue). Presumably, lower PAI levels would lead to less suppression of fibrinolysis and conversely a more rapid degradation of the fibrin. Women with PCOS may have an imbalance in the plasminogen activator system that is tilted toward a reduced production of the proteolytic enzyme plasmin. Systemically, this may increase their risk of CVD but at cellular level in the ovaries, it may result in impaired follicular rupture and anovulation (73). Glueck et al demonstrated that PAI-1 activity was an independent risk factor for miscarriages in PCOS (74). PAI-1 regulates the endogenous fibrinolytic system and constitutes the main inhibitor of fibrinolysis by binding and inactivating the tissue plasminogen activator (tPA), thus increased PAI-1 activity leads to decreased clearance of clots. Elevated PAI-1 levels have been associated with increasing BMI and visceral adiposity, as well as with

metabolic syndrome components (75-78). Thus, obesity especially abdominal adiposity influence the abnormalities in blood coagulation found in PCOS patients, as has been also demonstrated for many other CVD risk factors frequently associated with this prevalent disorder (79,80). Data in young PCOS subjects suggest that it is not only associated with abnormalities in fibrinolysis (81), but also with thrombophilia in conceptual agreement with the effect of insulin resistance on coagulation previously reported in the general population (82).

High factor VIII levels are a common risk factor for venous thrombosis (83-85) and may also be associated with the risk of arterial thrombosis in CAD (86,87) and stroke (88). The regulation of plasma factor VIII levels is complex. Most factor VIII circulates as a complex with von Willebrand factor (vWF) (89,90) the levels of which are known to be dependent on factors such as blood group (91-93) and endothelial stimulation (94,95). After its activation by thrombin, factor VIIIa dissociates from vWF to form a complex with factor IXa, which will result in marked acceleration of the activation of factor X (96). Activated factor X then converts prothrombin into thrombin, which in turn converts soluble fibrinogen into insoluble fibrin. It is possible that high factor VIII levels just increase the rate of thrombin and fibrin formation (in plasma, there is a large molar excess of factor IX over factor VIII). Another possibility is that high factor VIII levels influence thrombotic risk via an effect on the activated protein c sensitivity ratio (APCR). It has been shown that (in the absence of factor V Leiden) the thrombosis risk for the lowest quartile of normalized APCR (0.92) is 4.4-fold higher than that for the highest quartile {1.05}(97). For these measurements, “first generation” APC-resistant tests were used (no dilution of the sample with factor V–deficient plasma). This explains the finding that high factor VIII levels are associated with a reduced sensitivity for APC in the absence of factor V Leiden (98-100). After adjustment for factor VIII levels, the thrombosis risk associated with a

normalized APCR, 0.92 fell from 4.4- to 2.5 fold, indicating that factor VIII has a strong confounding effect on the thrombosis risk of a low APC ratio. Vice versa, it is also possible that high factor VIII exerts a thrombotic risk through the associated decreased responsiveness to APC. BMI (positively correlated with factor VIII levels) and higher levels of glucose, insulin, fibrinogen, and triglycerides are also associated with increased factor VIII levels (87,101,102).

The underlying etiology of PCOS remaining elusive, most of the therapeutic approaches in the past would focus on inhibiting or decreasing androgen production by various modalities {ovarian wedge resection, laproscopic ovarian drilling, LHRH (leutinizing hormone releasing hormone) analogues, aromatase inhibitors, estrogen-progesterone combinations, or anti-androgens} (103-109). The antiandrogen, spironolactone, a steroid chemically related to mineralocorticoid, aldosterone is used as a diuretic as well as antiandrogen (110, 111). Dual blockade by spironolactone anti-androgen and synthesis inhibitor (112,113), makes it suitable for long term treatment of hyperandrogenism (primarily hirsutism) and an-ovulation (114,115,117,118). Although the experience of spironolactone in PCOS is limited, it has a good safety record when used in smaller doses (117-122). The drug has been used as a sole agent, in combination or in head to head comparison with other agents (114, 117, 122-123). Treatment of PCOS by insulin sensitizers, like thiazolidinediones and metformin has generated significant interest in recent years, keeping in view the fundamental pathogenic factor of insulin resistance. Metformin has also been shown to directly inhibit human thecal cell androgen synthesis, suggesting an insulin independent mechanism (123-126). Many publications have demonstrated the efficacy of metformin in improving menstrual cyclicity, metabolic parameters, ovulation, cervical scores, and pregnancy outcomes both spontaneous and assisted (127-144). Among many metanalysis using metformin the results were favourable (145-147). In the

recent metanalysis of 27 trials using metformin involving 2150 women authors concluded that although metformin does not improve live birth rate but improves clinical pregnancy and ovulation rates (148). Other insulin sensitizers alone in combination with anti-androgens have been used with variable success (149-152).

In the chronic treatment of PCOS, OCP's are commonly used to induce regular menstrual cycle, protect the endometrium and ameliorate androgenic symptoms. OCP's typically contain the estrogen component (ethinyl estradiol) or its precursor mestranol which is metabolized into ethinyl estradiol and a progestin component which is variable according to the preparation (153). Estrogen component (ethinyl estradiol) of OCP's has a role in suppression of FSH, stabilization of endometrium, potentiation of progestin action, suppression of dominant follicle formation, increase in sex hormone binding globulin (SHBG) and decrease in free androgen (154). Progestin component has a role in suppression of LH, inhibition of LH surge, unreceptive endometrium, hostile cervical mucus, decrease in ovarian androgen secretion and androgen blocking effect (155).

The OCP's are categorized according to when they were approved or introduced as follows:

First generation; Norethindrone, Norethindrone acetate.

Second generation; Norgestrel, Levonorgestrel, Ethynodiol diacetate.

Third generation; Norgestimate, Desogestrel. Others Drospirenone, Dienogest (156).

The commercially available OCP'S in India are given in a following table:

Estrogen (µg)	Progestin (mg)	Commercial name
Ethinyl estradiol (35)	*Norethindrone (1)*	*Orthonovum1/35,Necon 1/35*
Ethinyl estradiol (35)	*Norethindone (0.4)*	*Ovcon 35*
Ethinyl estradiol (35)	*Ethynodiol (1)*	*Demulen 1/35*
Ethinyl estradiol(35)	*Norgestimate (0.25)*	*Orthocyclen*
Ethinyl estradiol (30)	*Norethindrone (1.5)*	*Loestrin 21 1.5/30*
Ethinyl estradiol (30)	*Norgestrel (0.3)*	*Lo-ovral*
Ethinyl estradiol(30)	*Desogestrel (0.15)*	*Desogen, Marvelon*
Ethinyl estradiol (30)	*Levonorgestrel (0.15)*	*Levlen, Nordette*
Ethinyl estradiol (30)	*Gestodene (0.075)*	*Gynera, Minulet*
Ethinyl estradiol (30)	*Drosperinone (3)*	*Yasmin, yamini*
Ethinyl estradio (20)	*Norethindrone (1)*	*Loestrin 21 (1/20)*
Ethinyl estradiol (20)	*Levonorgestrel (0.1)*	*Alesse, Levlite*
Ethinyl estradiol (20)	*Desogestrel (0.15)*	*Mircette, Mercilon*
Ethinyl estradiol(20)	*Gestodene (0.075)*	*Meliane, Harmonette*
Ethinyl estradiol (20)	*Drosperinone (3)*	*Yaz*
Ethinyl estradiol 15	*Gestodene (0.06)*	*Melodia*

PCOS women treated with OCP's have a lower incidence of ovarian cysts, and ovarian volume decreases with their use (157,158). OCP's decrease the body's production of androgens, which can reduce and slow hair growth and acne (159-160). Although there are some positive benefits of OCP use in women with PCOS, some of the disadvantages may contribute to the worsening of the disease process, which include increase in insulin resistance, blood coagulation, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol levels and triglycerides (TG) with no change of the total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios (161-166). OCP's increase the risk of venous thrombo embolism including deep vein thrombosis (DVT) and pulmonary embolism (PE),risk of first ischemic stroke and current use

significantly increases the risk of cardio-vascular disease among those at high risk (167-169). Women taking third generation OCP's had significantly higher C-reactive protein, fibrinogen, plasma viscosity, and HDL-cholesterol concentrations compared to non-users. Potentially harmful effects of OCP's may arise from their positive association with the acute phase response. There is a close relationship with inflammatory markers in particular in women taking third generation OCP's, which may, at least in part, contribute to the increased atherothrombotic risk, reported specifically in these women (170). Therefore pro-inflammatory state and a prothrombic / pro-coagulant state are individually a cause of concern in PCOS women using OCP's. Thus conventional treatment with OCP's may worsen the already existing procoagulant and proinflammatory state indicating higher cardiovascular risk in these women. In view of the above, we plan to study procoagulant markers and proinflammatory markers, in drug naive PCOS women and compare them with PCOS women treated with OCP's for varying duration of time that will help to know about the impact of OCP's on the existing CVD risk in PCOS women.

Chapter 2

REVIEW OF LITERATURE

In **1921** Archard and Thiers published a famous report of the "bearded diabetic women" pointing towards the association between glucose intolerance and hyperandrogenism. Since then glucose intolerance and insulin resistance has been intensively studied among the metabolic abnormalities (171).

First to report PCOS in **1935** was published by Stein and Leventhal. Since then, its prevalence data has remained variable because of the lack of well-accepted criteria for diagnosis. PCOS prevalence as per previous studies was mostly based on the demonstration of histopathological evidence of polycystic ovaries upon oophorectomy or wedge resection by Goldzieher and Green (1962) had shown presence of corpus luteum in 16% of surgical specimens (172).

Ferriman and Gallwey in **1961** did assessment of hirsutism in a systematic manner first time when they scored density (0-4) of hair in 11 body areas (upper lip, chest, upper back, sacrum, upper back, upper abdomen, lower abdomen, arm, forearm, thigh and lower leg) in 161 women aged 18-38 years (430 control women aged 15-74 years). They found a score of 5, 7 and 10 was respectively found in 9.9%, 4.3% and 1.2%. They also concluded that forearm and lower leg areas were less sensitive (173).

Peterson in **1973** in a Swedish population-based study estimated that 4.4 % of female population had a secondary amenorrhea of more than 3 months duration (174).

In **1976** Kahn and colleagues reported the existence of severe insulin resistance and acanthosis nigricans in three lean adolescent women. This type of insulin resistance soon became known as type A syndrome-similar to what is called as HAIR-AN syndrome (hyperandrogenism, insulin resistance and acanthosis nigricans)(175).

In **1980** Burghen and colleagues reported that women with PCOS have basal and glucose-stimulated hyperinsulinemia compared to weight-matched (control) women. They noted significant positive linear correlation between insulin and androgen levels in plasma (176).

Hatch R et al in 1981 suggested a simplified scoring system including only four important areas of assessment viz. sideburn area, lower jaw, upper neck, and buttocks (177).

Evans D J et al in **1983** showed the android type of obesity is associated with insulin resistance, glucose intolerance and atherogenic lipid profile in PCOS and was attributed to hyperandrogenism (178).

Penttila IM et al in **1983** measured lipid and protein levels in serum of healthy women during treatment with a new OCP combination .Treatment lasted for 3 months. At the end of treatment the serum total cholesterol, HDL-cholesterol and TG was increase in all women. Also lipid and protein levels returned to initial levels 2 months after treatment stopped (179).

Wild et al in **1985** demonstrated that the lipid abnormalities consisting of high total cholesterol, high LDL, high TG and lower HDL in PCOS females (180).

Dalhgren et al in **1985** reported that the lipid derangements and obesity in polycystic ovary syndrome patients as expected should increase the cardiovascular risk (181).

Orisni L et al in **1985** also studied ultrasound in the diagnosis of PCOS and suggested its clinical relevance (182).

Mathews D R et al in **1985** devised model for quantitating insulin resistance by Homeostatic model assessment for insulin resistance (HOMA) (183).

Cullberg G et al in **1985** performed lipid metabolic studies in women with PCOS during treatment with low dose desogestrel-ethinylestradiol (EE) combination. They found that only moderate changes were induced in lipid and lipoprotein patterns by the combination of desogestrel and EE (184).

Mauvais-Jarvis P et al in **1986** said the common areas affected are face and chin with a tendency towards male escutcheon. This is related to high levels of serum androgens or increased local skin 5-α reductase activity or both (185).

Dunaif et al in **1987** found that obese women with PCOS had significantly increased glucose values during an OGTT compared to age and weight matched ovulatory hyperandrogenic and control women. Twenty percent of obese women with PCOS had impaired glucose tolerance or frank type 2 DM by National Diabetes Data Group (NDDG) criteria (186).

Polson et al in **1988** conversely said that many patients with clinical evidence of PCOS may not demonstrate cysts in the ovaries (187).

Robinson GE et al in **1990** studied changes in metabolism induced by oral contraceptives desogestrel and gestodene in older women. Women taking the combined pill showed increases in fibrinogen and factor X and a reduction in antithrombin III when compared with their control values. There were also small but significant increases in triglycerides and triglyceride-rich lipoproteins. These changes in lipids and lipoproteins would not appear to increase the risk of cardiovascular disease; however the effects of the increase in the pro-coagulant factors are uncertain (188).

Wild R A in **1990** et al in a small study reported higher prevalence of coronary artery disease due to clinical excess of androgens (189).

Dahlgren et al in **1992** have calculated via a risk model analysis that patients with PCOS had a 4 to 7-fold higher risk of myocardial infarction compared to age matched controls. In a study of 143 women undergoing cardiac catheterization, aged 60 years or younger, PCOS was detected in 42% of women on pelvic ultrasonography. Patients with PCOS exhibited coronary artery segments with >50% stenosis with significantly greater clinical heart disease than women with normal ovaries on ultrasonography (190).

Talbott and coworkers in **1995** reported lipid abnormalities consisting of high total cholesterol, high LDL, high TG and lower HDL in PCOS females. They

also observed that these lipid abnormalities persisted on adjusting for BMI (191).

Quehenberger P et al in **1996** studied the effect of OCP treatment on selected factors involved in the activation i.e. circulating activated factor VII (cFVIIa), and in the inhibition of blood coagulation, i.e. plasma protein S activity and circulating thrombomodulin (cTM). They reported the increased level of activated factor VII and decreased plasma protein S activity and circulating thrombomodulin during use of oral contraceptives (192).

Solomon CG in **1998** showed that the menstrual disturbances in the form of irregular and unpredictable uterine bleed attributed to unopposed estrogen mediated endometrial proliferation are common. In amenorhoeic females it has been estimated that about 30-40% may actually have PCOS (193).

Bloemenkamp KW et al in **1998** investigated 99 pre-menopausal women, age 15-49 years, who had used OCP at the time of a first, objectively confirmed episode of deep-vein thrombosis (DVT). The following hemostatic variables were measured: APTT, factor VII, factor VIII, factor XII, fibrinogen, prothrombin, total antithrombin, normalised activated protein C sensitivity ratio (n-APC-sr), protein C, protein S and free protein S. They found marked and significant effects of oral contraceptive use on the levels of several clotting factors, with an increase in factor VII, factor XII, protein C and a decrease in antithrombin, n-APC-sr and protein S (166).

Gonzalez F et al in **1999** in a study was undertaken to determine the status of circulating TNF-α and the relationship of TNF-α with insulin levels, body weight, or both in women with PCOS. They conclude that serum TNF-α is increased in normal-weight women with PCOS and is even higher in obese individuals regardless of whether they have PCOS; factors other than obesity are the cause of elevated serum TNF-α in normal-weight women with PCOS; and whereas increased circulating TNF-α may mediate insulin resistance in obesity, which may in turn promote hyperandrogenism in obese women with

PCOS, it remains to be demonstrated whether this is also the case in normal-weight women with PCOS (194)

Van Rooijen M et al in **2002** compared the effects of two different combined OCP on levels of plasma lipoproteins and coagulation factor VII. A significant rise in plasma triglyceride levels was obtained with both preparations, although the increase was more pronounced with ethinyl estradiol/desogestrel. Plasma concentrations of factor VII and activated factor VII were increased significantly only with ethinyl estradiol/desogestrel than with ethinyl estradiol/levonorgestrel (195).

Sundararaman PG et al in **2003** reported the increased risk of atherosclerosis in women with PCOS. They conducted a cross-sectional case control study, they assessed insulin resistance and carotid IMT (Intima media thickness) in 40 women presenting with hyper androgenic features of PCOS. Insulin resistance was assessed by fasting glucose/insulin ratio and IMT by the doppler system with electrical linear transducer midfrequency of 12 MHz Women with PCOS had higher fasting insulin levels, higher insulin resistance and greater IMT. They concluded that South Indian women with the reproductive abnormalities of PCOS have greater insulin resistance and IMT, and therefore they must be advised about lowering the risk of future vascular disease (196).

Ganie MA et al in **2004** in a study carried out to estimate the prevalence of glucose intolerance and insulin sensitivity in young women with PCOS examined 168 young women who attended AIIMS endocrine center for hirsutism and / or oligomenorrhea were enrolled for the study. NICHHD consensus conference criteria were used for diagnosis of PCOS. Results indicated higher prevalence glucose intolerance even at younger age in PCOS females (197).

Ganie MA et al in **2004** on comparison of efficacy of spironolactone with metformin in the management of PCOS found that spironolactone appears

better than metformin in the treatment of hirsutism, menstrual cycle frequency and hormonal derangements and is associated with fewer adverse events (198).

Vrbikova J, Cibula D in **2005** suggested that common oral contraceptives (COC's) are the primary treatment for contraception and regulation of menses in women with PCOS. COC's also offset the effect of estrogen on the endometrium and improve acne, hirsutism and oligomennorhea (199).

In a recent study Apridonidze et al in **2005** hypothesized that the metabolic syndrome is prevalent in PCOS and those women with both conditions will have more hyperandrogenism and menstrual cycle irregularity than women with PCOS only .Women with PCOS and the metabolic syndrome had significantly higher levels of serum free testosterone and lower levels of serum SHBG than women with PCOS without the metabolic syndrome. No differences in total testosterone were observed between the groups. They concluded that the metabolic syndrome and its components are common in women with PCOS, placing them at increased risk for cardiovascular disease (200).

J Vrbikova et al in **2005** reported significantly higher levels of ICAM-1 than in healthy women were found. ICAM-1 correlated with body composition, lipids and insulin secretion, but not with insulin resistance (67).

EvanthiaDiamanti-Kandarakis et al in **2006** reported higher plasma levels of hs-CRP(mg/l), SICAM-1, E-slectin in PCOS compared to the controls.VCAM-1 did not differ significantly in two groups. Significant reduction in VCAM-1 and hs-CRP was achieved after 6 months of metformin administration .These findings imply the presence of chronic inflammation in women with PCOS. Metformin decreased the level of plasma inflammatory indices (201).

Anuradhakalra et al in **2006** in the prospective study calculated the body mass index (BMI) and waist hip ratio of 65 women with PCOS. Fasting glucose,

insulin and lipid profiles were also estimated in each case. Insulin resistance was defined by fasting glucose-to-insulin ratio £ 4.5. The association of obesity markers and insulin resistance with lipid parameters was thenstudied.They reported insulin resistance is associated with dyslipidemia in women with PCOS independent of obesity (202).

E. Diamanti-Kandarakis et al in **2006** conducted a study to investigate the coexistence of active inflammation markers and endothelial dysfunction in young women with PCOS, and their relationship with metabolic and hormonal abnormalities of the syndrome. They concluded that PCOS patients had statistically higher levels of endothelin 1(ET-1), sICAM-1, sVCAM-1 andhsCRP (P = 0·01). This study demonstrates that endothelial dysfunction coexists and is influenced by the presence of increased serum levels of inflammation and endothelial activation markers in young women with PCOS. These parameters appear to be interrelated with hyperandrogenaemia in this insulin-resistant population (203).

Ganie MA et al in **2008** showed that there is the high prevelance of PCOS characteristic in girls with euthyroid chronic lymphocytic thyroiditis (204).

Kulshreshtha B etal in **2008** reported that women with PCOS had an exaggerated insulin response to glucose (205).

Luque Ramirez M et al in **2009** compared the effects of anti- androgenic OCP's with metformin on blood coagulation tests and endothelial function in women with PCOS as a function of presence of obesity and smoking. They concluded that oral contraceptives and metformin may exert deleterious effects on blood clotting tests of PCOS women, yet the effects of metformin appears to be milder. As smoking potentiates some of these effects and deteriorates endothelial function, smoking cessation should be promoted in PCOS patients and the possible adverse impact of OCP's on the already unfavorable prothrombotic state of PCOS patients should be considered (206).

Scholes D et al in **2010** reported that prolonged use of today's OCPs, particularly <30 mcg EE, may adversely impact young adult women's bone density while using these agents. They conducted a cross-sectional study of 606 women aged 14-30 years, examined both OCP duration and estrogen dose and their association with bone mineral density (BMD) at the hip, spine, and whole body (dual-energy X-ray absorptiometry).They found that among 389 OCP users and 217 nonusers enrolled, 50% were adolescents (14-18 years). Of OCP users, 38% used "low-dose" OCP's [<30 mcg ethinyl estradiol (EE)]. In adolescents, mean BMD differed by neither OCP duration nor EE dose. However, 19- to 30-year-old women's mean BMD was lower with longer OCP use for spine and whole body (p=.004 and p=.02, respectively) and lowest for >12 months of low-dose OCP for the hip, spine and whole body (p=.02, .003 and .002, respectively) (207).

Kriplani A et al in **2010** conducted a prospective randomized trial to compare efficacy of a drospirenone-containing combined oral contraceptives (COC) with desogestrel-containing COC in women with PCOS not desirous of child-bearing. Sixty women were randomized into study group [ethinylestradiol (EE) 30 mcg+drospirenone 3 mg] and control group (EE 30 mcg+desogestrel 150 mcg), treated for 6 months and followed up at 1 month, 3months, 6 months, during treatment and 3 and 6 months post-treatment. They reported that in women with PCOS, a drospirenone containing COC has better outcome in terms of persistent regular cycles, anti-androgenic effect, fall in BMI and BP, better lipid profile, favorable glycaemia and hormonal profile than desogestrel-containing COC (208).

Halperin et al in **2011** studied the association between the COC and insulin resistance, dysglycemia and dyslipidemia in women with PCOS and concluded that COC use was significantly associated with an increase in HDL-C and triglycerides. Significant heterogeneity was found in glucose, cholesterol,

HDL-C, LDL-C, triglycerides, fasting glucose to insulin ratios and homeostatic model assessment –IR (209).

Kilic et al in **2011** in a study to evaluate the optimal treatment strategy addressing cardiovascular risk in obese and non-obese patients with PCOS concluded that metformin treatment leads to improvement in hormonal and metabolic parameters and decreases asymmetric dimethyl arginine (ADMA) and homocysteine levels possibly independent of BMI. However, the use of oral contraceptives in obese and non-obese patients with PCOS with impaired glucose tolerance increases ADMA and hs-CRP levels and creates an increase in the metabolic risk (210).

Ekaterini Koiou et al in **2012** evaluated PAI-1 antigen levels in women with PCOS and different levels of adiposity and PCOS phenotypes. They studied 199 women with PCOS and 50 age-matched healthy women divided in normal weight (n=100 and n=25, respectively) and overweight/obese (n=99 and n=25, respectively). Normal weight and overweight/obese patients with PCOS were further divided in patients diagnosed according to the 1990 criteria (i.e. with anovulation and hyperandrogenemia; 1990 criteria group) and in patients with the additional phenotypes introduced in 2003 (i.e. with polycystic ovaries and either anovulation or hyperandrogenemia; additional 2003 criteria group). In normal weight subjects, plasma PAI-1 levels did not differ between women with PCOS (regardless of group) and controls, or between the 1990 criteria and the additional 2003 criteria groups of PCOS. In overweight/obese subjects, plasma PAI-1 levels were higher in both the 1990 criteria and the additional 2003 criteria groups of PCOS compared with controls ($p<0.001$ and $p=0.004$, respectively), but did not differ between the 1990 criteria and the additional 2003 criteria groups of PCOS. In conclusion, plasma PAI-1 levels are elevated in overweight/obese women with PCOS but not in normal weight women with this syndrome. Plasma PAI-1 levels do not differ between the phenotypes of PCOS (211).

Chapter 3

AIMS & OBJECTIVES

To study and compare the levels of following procoagulant and the proinflammatory markers

1. Tumor necrosis factor α (TNF-α).
2. Soluble Intercellular cell adhesion molecule-1 (sICAM-1).
3. Antihaemophilic factor (Factor VIII).
4. Plasminogen activator inhibitor-1 (PAI-1).
5. Monocyte chemo attractant protein-1 (MCP-1).

in drug naive and OCP treated women with PCOS.

Chapter 4
MATERIALS
&
METHODS

4.1 MATERIALS & METHODS:

4.11 History and general examination

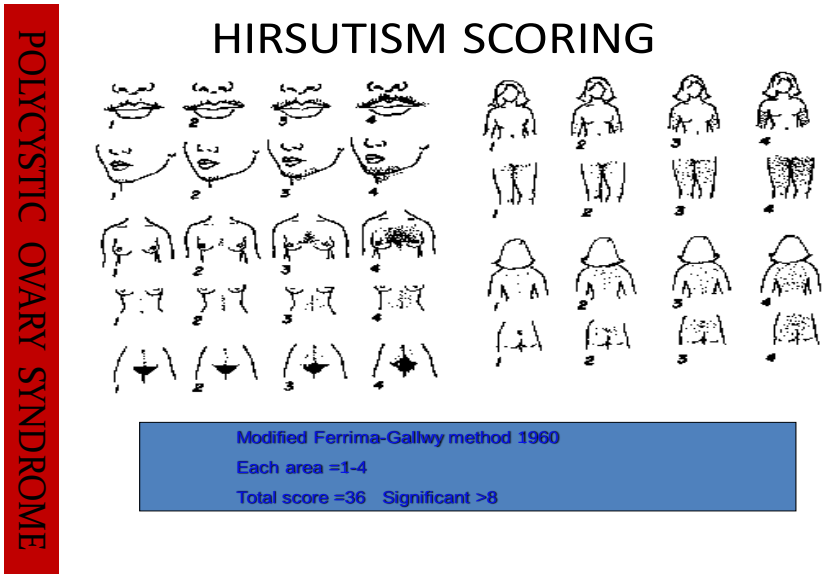
All women who qualified Rotterdam 2003 criteria for a diagnosis of PCOS were informed about the study. The first step was consent attainment. The women who gave informed consent were enrolled in the study. Once enrolled all women were interviewed to furnish a detailed account of medical facts with special reference to the menstrual history, duration and extent of hair growth, weight gain, acne etc. The details of menstrual history included age of menarche, regularity, duration, dysmenorrhea, flow and number of menstrual cycles per year. Oligomenorrhea was defined as an inter-menstrual interval of >35 days or a total of <8 menses per year and amenorrhea as absence of menstruation during last 6 months. Note was made of family history of hirsutism, infertility, menstrual disorders, diabetes mellitus or glucose intolerance, coronary artery disease and obesity at least in three generations. The subjects were randomly selected from the two categories:

1. **Cases -** The women taking OCP's (estrogen + progesterone) from various gynecology clinics for the treatment of Rotterdam 2003 criterion based PCOS diagnosis. The women had been taking OCP's for a period of 24 + 2 weeks was taken as cases.
2. **Controls –** The women who qualified Rotterdam 2003 criteria for diagnosis of PCOS and had not received any drug so far were taken as control group. All these women who consented were subjected to a positive diagnostic criterion as follows: The Rotterdam criteria for the diagnosis of PCOS (2003) states 2 of the 3 features needs to be present to make the diagnosis and with the exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome). These features includes (1) Oligo- or anovulation (2) Clinical and/or biochemical signs of hyperandrogenism and (3) PCO

(either 12 or more follicles measuring 2–9 mm in diameter, or an ovarian volume of >10 cm^3. Non classic adrenal hyperplasia (NCAH), Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, and androgen-producing tumors will be ruled out by doing relevant investigation.

4.12 Physical examination

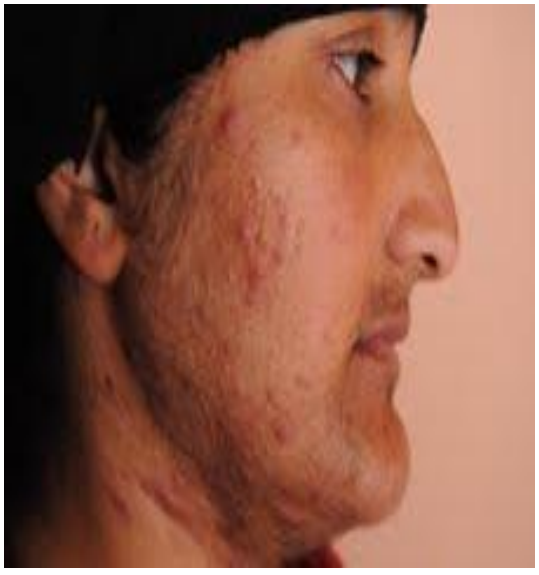
- All women underwent anthropometric assessment like measurement of height, weight, waist-hip circumference ratio, blood pressure recording, and detailed systemic examination. Hirsutism assessment was done using modified Ferriman-Gallwey score by counting nine specified body areas **(Pic A).** A score of > 8 out of a total of 36 will be taken as significant **(Pic A1).**



Pic A

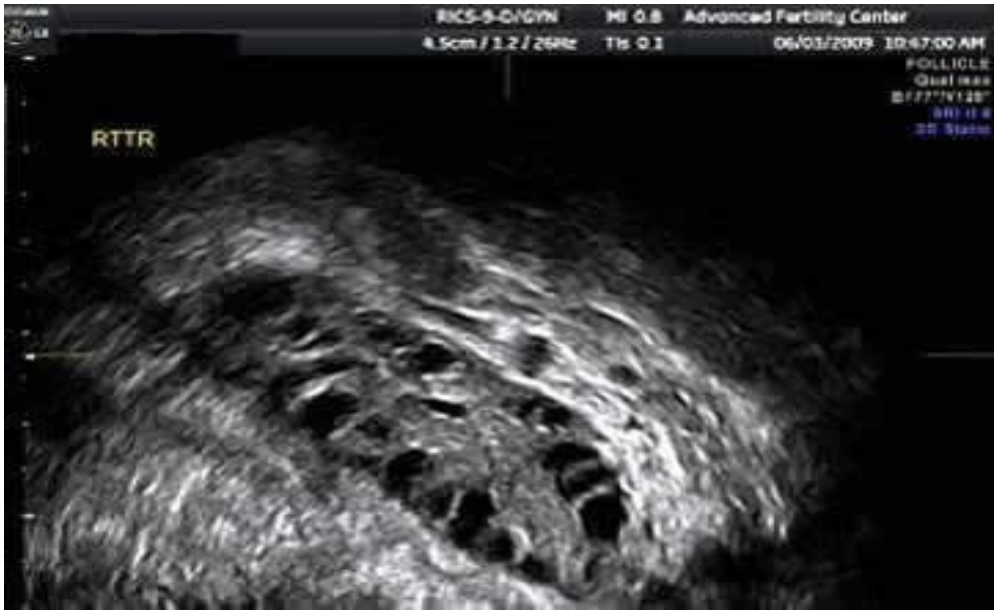
Pic A1

- Acne vulgaris will be scored using a four point scale :0,no acne:1,minor acne on face only:2,moderate acne on face only:3,severe acne on face, back and or chest. Moderate to severe acne was taken as a clinical feature of hyperandrogenemia **(Pic A2).**



Pic A2

- All patients were subjected to transabdominal ultrasonography (USG) by a single observer. The USG was done to measure and to record typical features of PCOS (multiple small peripheral cysts, increased ovarian volume and thecal hyperechogenecity) and to rule out any adrenal or ovarian mass lesion **(Pic A3).**



Pic A3

4.13 Investigations and assays

On the day of study, a fasting (10-14 hour) blood samples were taken for

- Triglycerides (TG)
- Low density lipoprotein (LDL)
- High density lipoprotein (HDL)
- Total cholesterol

Samples were estimated on a fully automated chemistry analyzer [Hitachi-912] by using standard commercially available kits or a standard methodology was adopted wherever necessary **(Pic B).**

Pic B: Fully automated chemistry analyzer [Hitachi-912] at department of biochemistry, SKIMS.

- The OGTT was performed after 8 hour overnight fasting with 75 grams of oral anhydrous glucose load dissolved in 300ml of water. Blood samples for glucose and insulin were collected at 0-minutes, 60-minutes and 120-minutes after the glucose load. Glucose was estimated on a fully automated chemistry analyzer [Hitachi-912] by using standard commercially available kits or a standard methodology was adopted wherever necessary. The serum insulin was estimated by Electrochemilumniscence (ECLIA) using Cobas e 411 (Roche diagnostics).

  Hormonal analysis was estimated by RIA/ IRMA in the department of Clinical immunology and Molecular Medicine SKIMS. The hormone estimation included:

- 17-OHP – to rule out non classical Congenital adrenal hyperplasia

- T4 – to rule out hypothyroidism
- TSH – to rule out hypothyroidism
- PRL- to rule out prolactinoma
- LH
- FSH
- Cortisol -to rule out Cushing's syndrome
- Testosterone- to diagnose hyperandrogenism and to rule out androgen secreting ovarian or adrenal tumours.

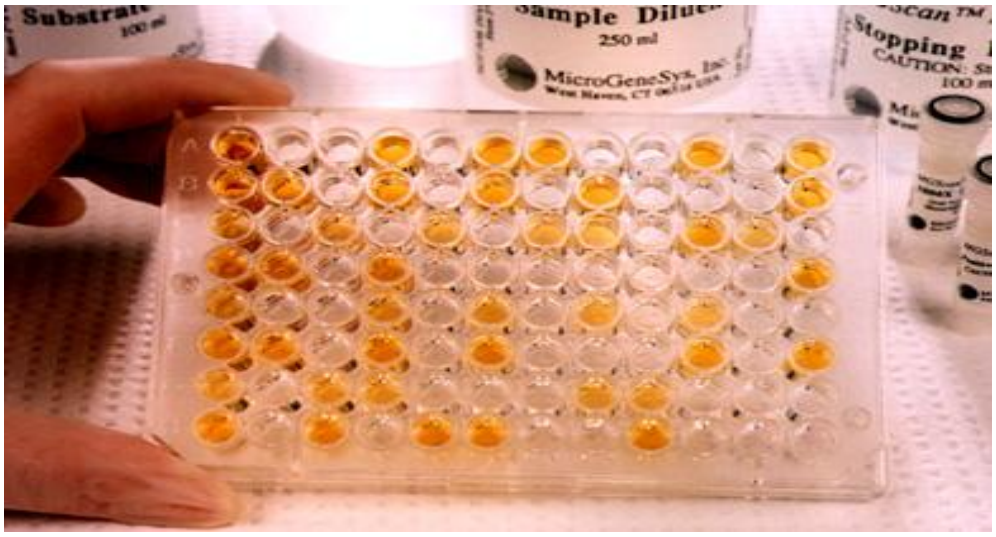
The sampling was arranged in such a way so that the LH, FSH, 17-OHP and testosterone was collected on 3rd to 7th day of the follicular phase of either spontaneous or progesterone induced menstrual cycle. Hormonal assays was done by (RIA) radio immunoassay (T4, testosterone, 17-OHP, and cortisol) and immuno-radiometric assays (IRMA)(TSH, LH, PRL, and FSH) using commercial kits in duplicate and according to supplier protocol **(Pic C).**

Pic C: Gamma counter for RIA at department of immunology SKIMS

- The aliquots for procoagulant markers and proinflammatory markers in the plasma were separated from basal samples. Procoagulant markers (PAI-1) and proinflammatory markers (s-ICAM-1, TNF-α, MCP-1) in the plasma were assayed using ELISA (212,213) kits according to the respective protocols.
- **ELISA Assay:** The assay used was quantitative sandwich ELISA. The micro titer plate is provided in the kit which is pre-coated with a monoclonal antibody specific to the procoagulant or proinflammatory marker. Standards or samples are then added to the appropriate micro titer plate wells with a biotin-conjugated polyclonal antibody preparation specific for the marker and incubated. Marker if present, will bind and become immobilized by the antibody pre-coated on the wells and then be "sandwiched" by biotin conjugate. The micro titer plate wells are thoroughly washed to remove unbound marker and other components of the sample. In order to quantify the amount of marker present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin and a TMB (3, 3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain marker, biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the

addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm

(Pic D and Pic E).



Pic D: Microplate ELISA: coloured wells indicate reactivity. The darker the colour, the higher the reactivity.

Pic E: ELISA Reader at department of immunology, SKIMS

- Factor VIII in plasma was estimated using Coagulometric method as per given protocol
- **Coagulometric Assay:** Plasma deficient in factor VIII, comprising the intrinsic pathway results in a prolonged partial thromboplastin time (APTT).Coagulation factor deficient plasma is used to confirm the factor VIII deficiency, in general and to identify and quantify coagulation factor deficiency in patient plasma. A mixture of the factor VIII deficient plasma and a patient plasma is tested in APTT assay and the result is interpreted using the references curve obtained with the dilution of standard human plasma or a normal plasma pool mixed with deficient plasma .A patient plasma deficient in specific factor is not able to compensate for the absence of factor in the corresponding coagulation factor deficient plasma and therefore results in prolonged APTT **(Pic F)**

PIC F: showing automated coagulation analyzer in department of hematology, SKIMS

4.14 Equipments:

EQUIPMENTS	SOURCE
Centrifuge	AICIL
Coagulation Analyzer	SYSMEX CA 550
Deep freezer (-70□c)	REVCO
ELISA Reader	BIO-RAD
Gamma counter	STRATEC
Incubator	AICIL
Refrigerator	Kelvinator
Vortex mixer	REMI

4.15 Glass and plastic ware used:

Glass And Plastic Ware	SOURCE
Appendrof tubes	Tarson
Beakers	Borosil
Clot activator vial	AcCuvet-plus
Glass pipettes	Borosil
Measuring tubes	Borosil
Micro tips	Tarson
Test tube stand	Prolab
Tip boxes	Axygen
Tri sodium citrate vials	AcCuvet-plus

4.16 Kits:

Kits	**Method**	**Source**
Cholesterol	CHOD-PAP	DIALAB; Austria
Cortisol	RIA	DIASORIN ;North western Ave
Factor VIII	Coagulometric method	SIEMENS ;USA
FSH	IRMA	DPC;USA
Glucose	GOD-POD	URILAB; India
HDL	New clearance method	RANDOX;UK
LDL	New clearance method	RANDOX;UK
LH	IRMA	DPC;USA
MCP- 1	ELISA	PUREGENE; Minneapolis, USA
PAI-1	ELISA	SYMANSIS; New zealand
PRL	IRMA	DPC;USA
s-ICAM	ELISA	GEN-PROBE;US
T4	RIA	DIASORIN; North western Ave
Testosterone	RIA	IMMUNOTECH ;France
TG	GPO-PAP	DIALAB; Austria
TNF-α	ELISA	INVITROGEN; California
TSH	IRMA	IMMUNOTECH ;France
17-OHP	RIA	IMMUNOTECH ;France

Chapter 5
RESULTS

A total of 81 subjects with PCOS participated in the study. 30 subjects who received OCP's were taken as cases and were compared with 51 subjects with PCOS who received no treatment (drug naive) and were considered as controls. All subjects met the Rotterdam 2003 criteria for a positive diagnosis of PCOS.

5.1 CLINICAL PARAMETERS

The mean age of controls and cases was (21.92±5.83 vs. 21.63±4.18 years) respectively and was comparable (p=0.813) as shown in Fig 1 & Table 1. Among the controls, 17 (33.3%) subjects were in the age group of 13-18 years, 20 (39.2%) were in the age-group of 19-24years, 14 (27.4%) subjects were in the age group of 25-30 years. Among cases 8 subjects (26.6%) were in the age-group of 13-18 years, 12 subjects (40%) were in the age group of 19-24 years and 10 subjects (33.3%) were in age group 25-30 years.

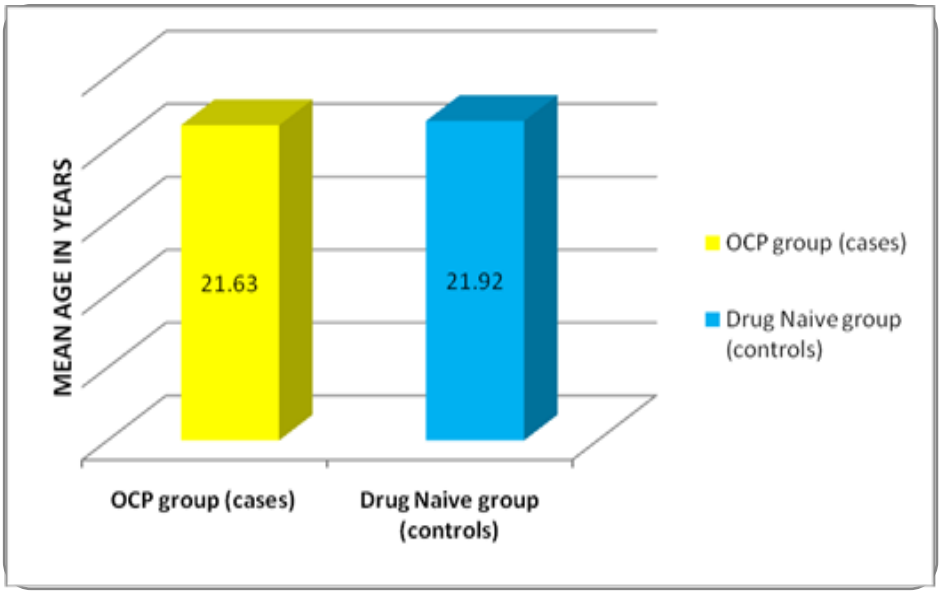


Fig 1. Showing comparison of mean age in years between cases vs. controls in years

The mean age at menarche for controls and cases was (12.96±1.49 vs. 13.13±1.27 years) respectively and was comparable (p=0.597) shown in Fig 2

& Table 1. Among the controls 6 subjects had (11.7%) menarche at 11 years of age,16 subjects (31.3%) had menarche at 12 years of age,14 subjects (27.4%) at 13 years of age, 8 subjects (15.6%) at 14 years of age, 5 (9.8%) subjects at 15 years of age, and 2 (3.9%) subjects had menarche at 16 years of age. Similarly among cases 2 subjects (6.6%) had age of menarche at 11years of age, 6 subjects (20%) had age of menarche at 12 years of age, 14 subjects (46.6%) had at 13 years, 5 subjects (16.6%) had at 14 years, and 3(10%) had age of menarche at 15 years of age.

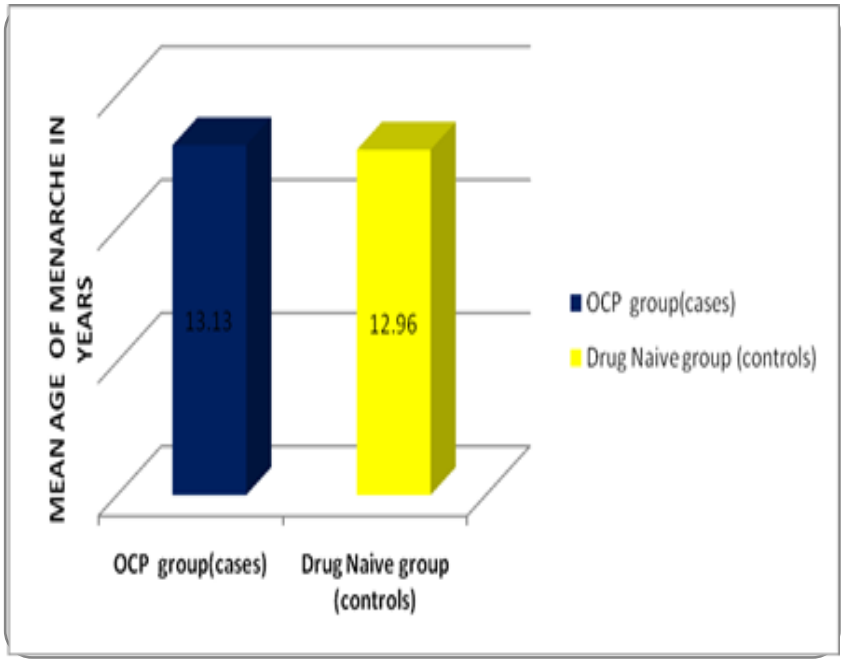


Fig 2.Showing comparison of mean age of menarche in years between cases and controls in years.

Table 1. Showing comparison of mean age and age of menarche between cases and controls.

Parameters	**Drug naive group (Controls) (Mean±SD) N=51**	**OCP group (Cases) (Mean±SD) N=30**	**p-value**
Mean Age (years)	21.92±5.83	21.63±4.18	0.813
Age of menarche(years)	12.96±1.49	13.13±1.27	0.597

Mean number of menstrual cycles per year for controls and cases was (9.12±3.88 vs.9.90±3.30) respectively and was comparable (p=0.358) shown in Fig 3. & Table 2. Among controls 19 (37.2%) subjects had < 8 cycles per year, 25 (49%) subjects had 8-12 cycles per year and 7 (13.7%) subjects had greater than 12 cycles per year. Among cases 5 subjects (16.6%) had < 8 cycles per year while 25 subjects (83.8%) had 8 -12 cycles per year and none of the subjects had greater than 12 cycles per year.

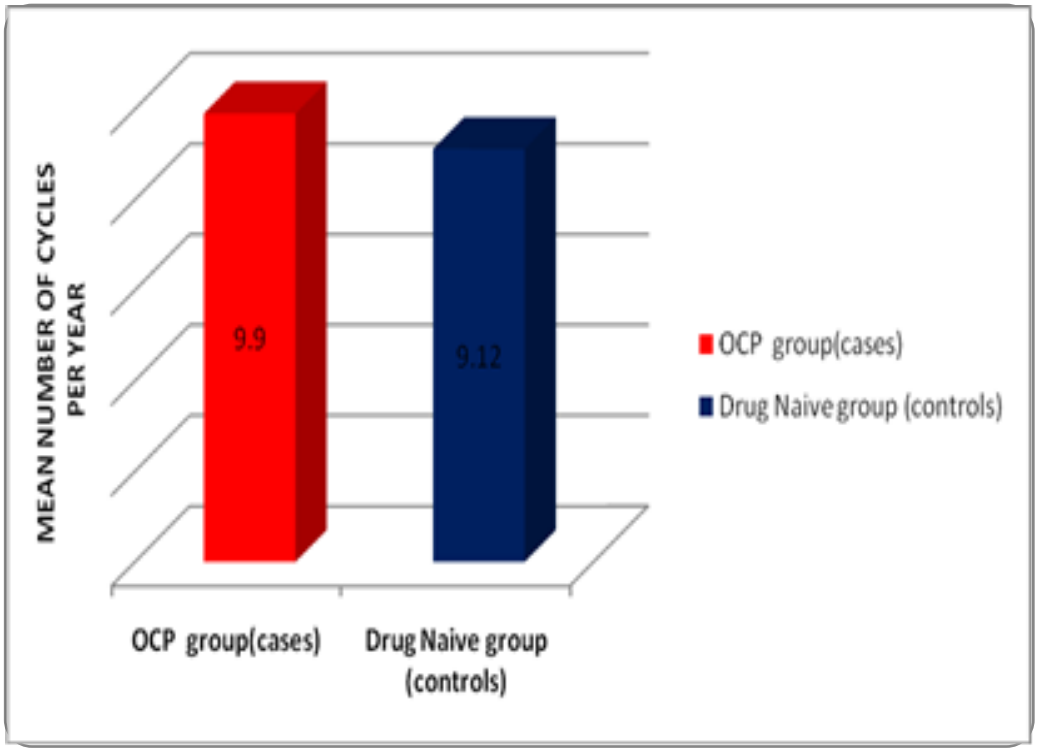


Fig 3. Showing comparison of number of cycles per year between cases and controls.

Mean Ferriman-Gallwey (FG score) of the controls and cases was (12.27±4.71 vs. 10.00±2.60) respectively and was statistically significant (p=0.017) shown in Fig 4 & Table 2. The FG score of >8 was seen in 10 (33.3%) cases and 15 (29.4%) controls while as a score of >10 was seen in 15 (50%) cases and 34 (66.6%) controls.

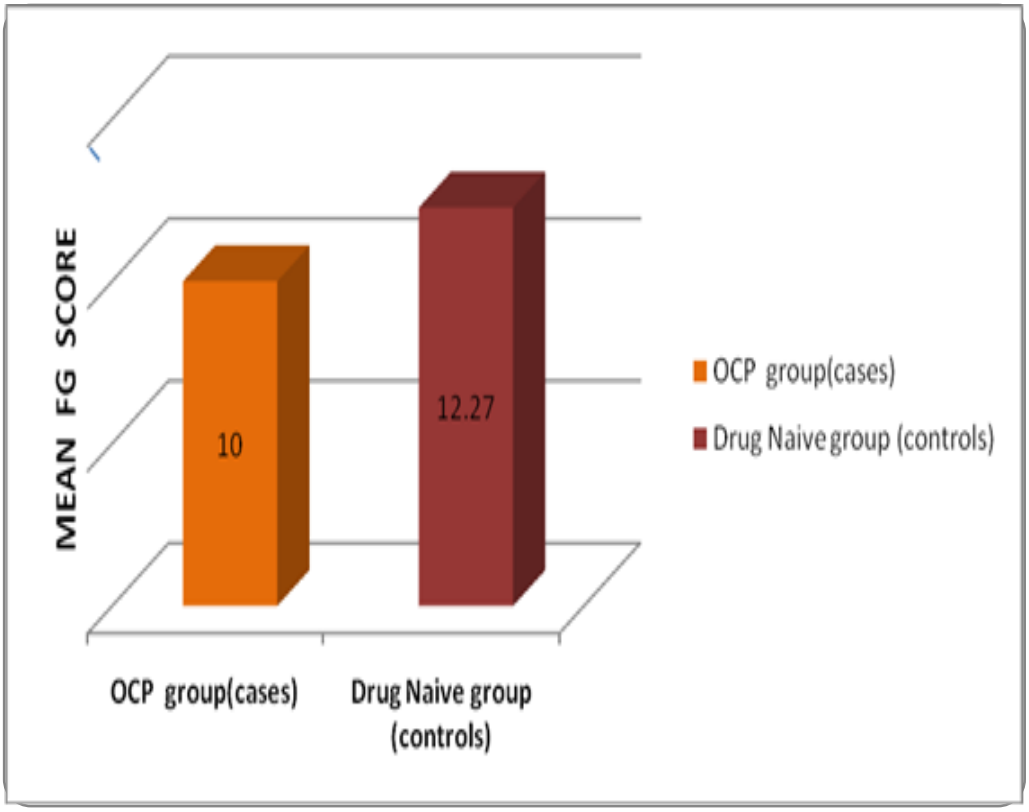


Fig 4. Showing comparison of mean Ferrimen-Gallwey score (FG score) between cases and controls.

Table 2**.** Showing comparison of mean number of menstrual cycles per year and FG score between cases and controls.

Parameters	**Drug naive group (Controls) (Mean±SD) N=51**	**OCP group(Cases) (Mean±SD) N=30**	**p-value**
No of cycles per year	9.12±3.88	9.90±3.30	0.358
Ferriman-Gallwey score (FG-score)	12.27±4.71	10.00±2.60	0.017

5.2 ANTHROPOMETRIC PARAMETERS

Mean weight of controls and cases was (58.57±8.52 vs 59.07±6.34 kg) which was comparable (p=0.782) shown in Fig 5 & Table 3.

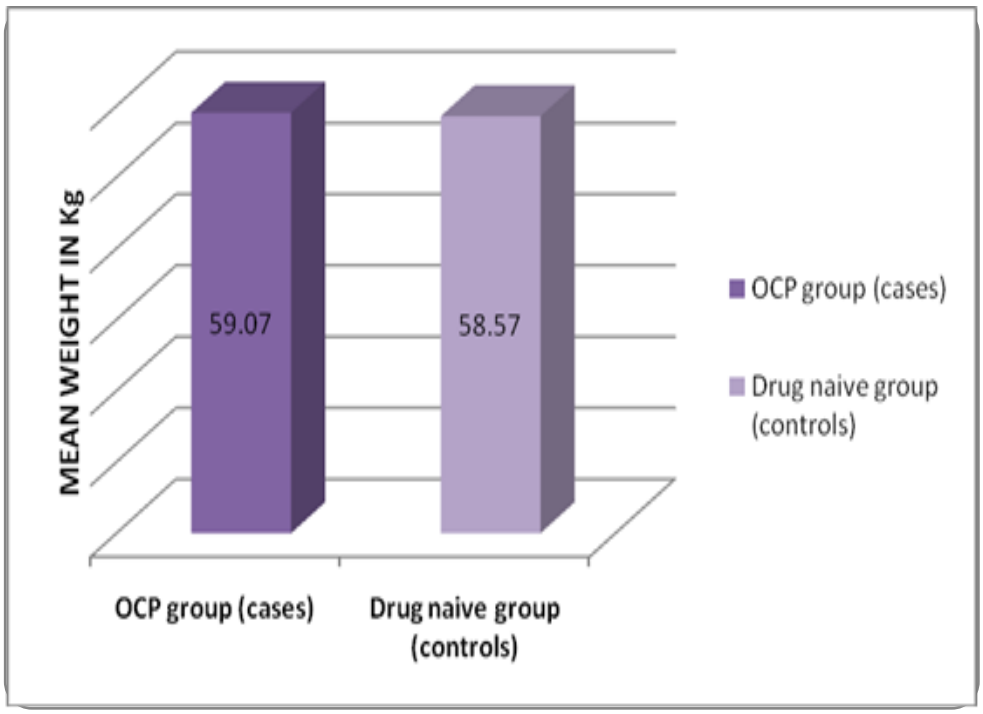


Fig 5. Showing comparison of body weight in kg between cases and controls.

Mean Body mass index (BMI) of controls and cases was (23.66±3.43 vs. 24.07± 3.42 Kg/m²) and was comparable (p=0.606) as shown in Fig 6 &Table 3. As per International Diabetes Federation (IDF), among controls 33 subjects (64.7%) had BMI less than 25 kg/m^2 (optimal weight),14 (27.4%) had BMI between 25-30 kg/m^2 (over weight), 4 (7.8%) were having BMI greater than 30 kg/m^2 (obese).Among the cases 19 subjects (63.3%) had BMI less than 25 (optimal weight), 9 (30%) had BMI between 25-30 Kg/m^2 (overweight) and 2 subjects (6.6%) had BMI greater than 30 Kg/m^2 (obese).

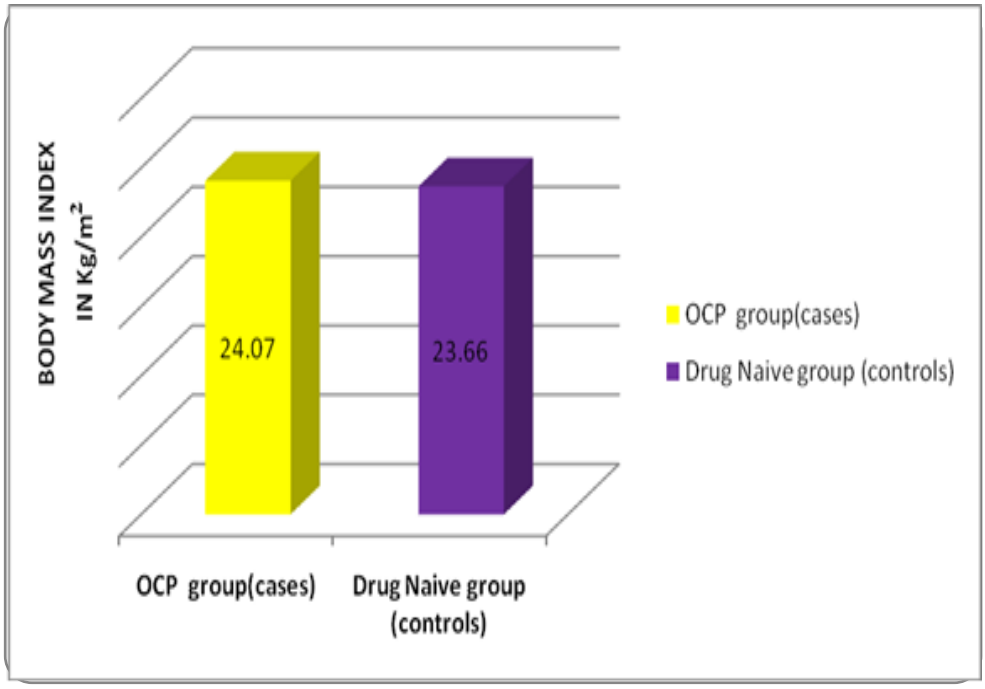


Fig 6. Shows comparison of mean BMI in kg/m^2 between cases and controls.

Mean waist circumference of controls and cases was (79.96±10.15 vs. 78.20±8.49 cm) respectively which was comparable (p=0.427) as shown in Fig 7 &Table 3. As per National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) 2001 criteria waist circumference of greater than 88 cm represents central obesity. Among controls 13 subjects (25.4%) had waist circumference greater than 88 cm, 38 (74.50%) have waist circumference less than 88 cm. Among cases 4 females (13.33%) had waist circumference greater than 88 cm and 26 (86.6%) had waist circumference less than 88 cm.

As per European Group for Study of Insulin Resistance (EGIR) 1999 criteria waist circumference of greater than 80 cm represents central obesity. Among controls 25 subjects (49.0%) had waist circumference greater than 80 cm, 26 subjects 50.9% had waist circumference less than 80 cm. Among cases 11 subjects (36.6%) had waist circumference greater than 80 cm and 19 subjects (63.33%) had waist circumference less than 80 cm.

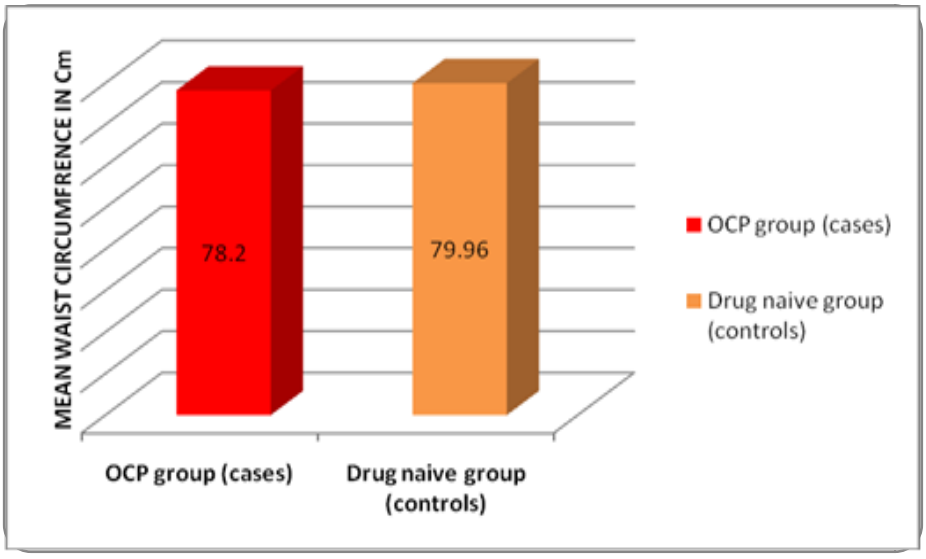


Fig 7.Showing comparison of mean waist circumference in cm between cases and controls.

Mean waist hip ratio for controls and cases was (0.91±0.06 vs. 0.92±0.06) respectively which was comparable (p=0.544) and is shown in Fig 8 &Table 3. Waist hip ratio of greater than 0.85 is central obesity. Among controls 44 (86.2%) subjects have Waist hip ratio greater than 0.85 and 7 subjects (13.7%) had waist hip ratio less than 0.85. Among 30 cases 27 (90%) subjects had waist hip ratio of greater than 0.85 and 3 subjects (10%) had waist hip ratio less than 0.85.

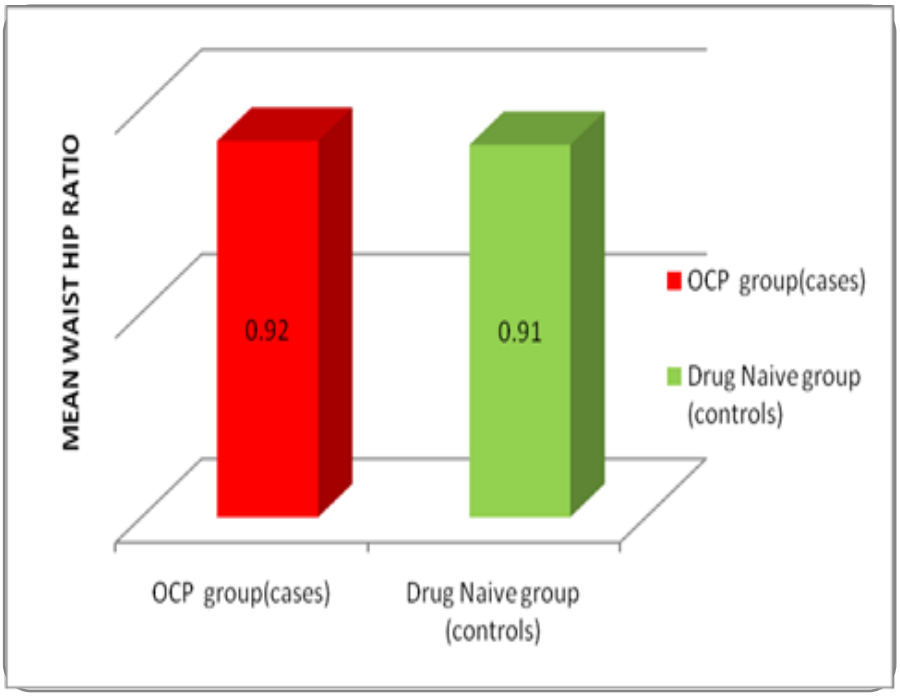


Fig 8. Showing comparison of mean waist hip ratio between cases and controls.

5.3 HEMODYNAMIC PARAMETERS

Mean of systolic blood pressure in controls and cases was (122.24±6.99 vs 123.00±7.21 mm of Hg) which was comparable (p=0.640) shown in Fig 9 &Table 3.As per International Diabetes Federation (IDF) systolic blood pressure of greater than 130 mm Hg is diagnosed as systolic hypertension. Among controls 11(21.5%) subjects have systolic blood pressure greater than 130 mm Hg and 40 (78.4%) subjects have systolic blood pressure within the normal range. Among cases 9 (30%) subjects have systolic blood pressure greater than 130 mm Hg and 21 (70%) subjects have systolic blood pressure within the normal range.

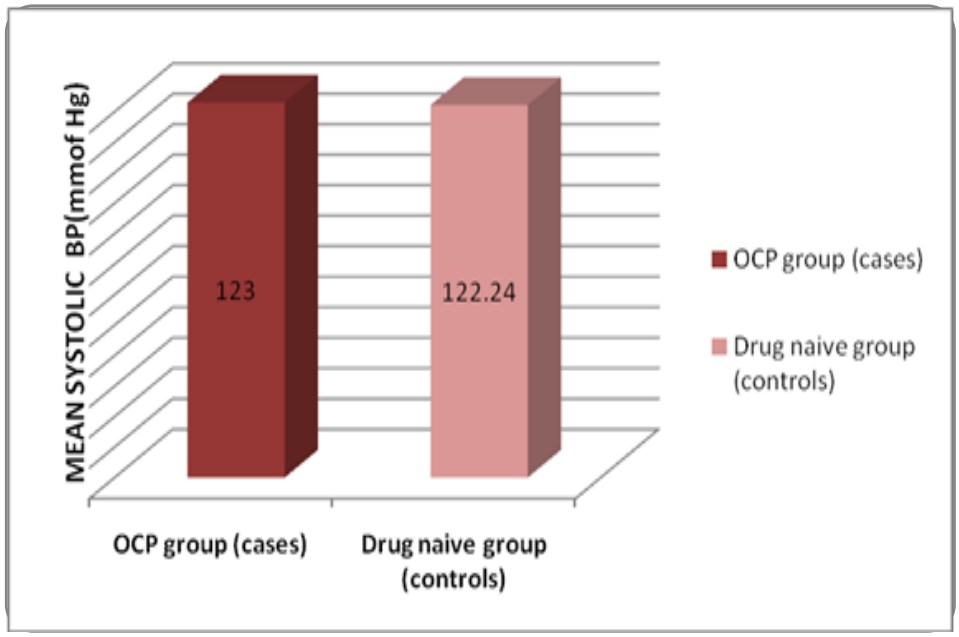


Fig 9. Showing comparison of mean systolic BP in mm Hg between cases and controls.

Mean diastolic blood pressure in controls and cases was (79.37±4.62 vs 79.46±4.29 mm of Hg) which was comparable (p=0.928) shown if Fig 10 &Table 3.As per International Diabetes Federation (IDF) diastolic blood pressure of 85 mm Hg is diagnosed as diastolic hypertension..

Among controls 8 (15.6%) women have diastolic blood pressure greater than 85 mm of Hg,43 subjects (84.3%) have diastolic blood pressure within the normal range. Among cases 4 (13.3%) women have diastolic blood pressure greater than 85mm Hg and 26(86.6%) diastolic blood pressure within the normal range.

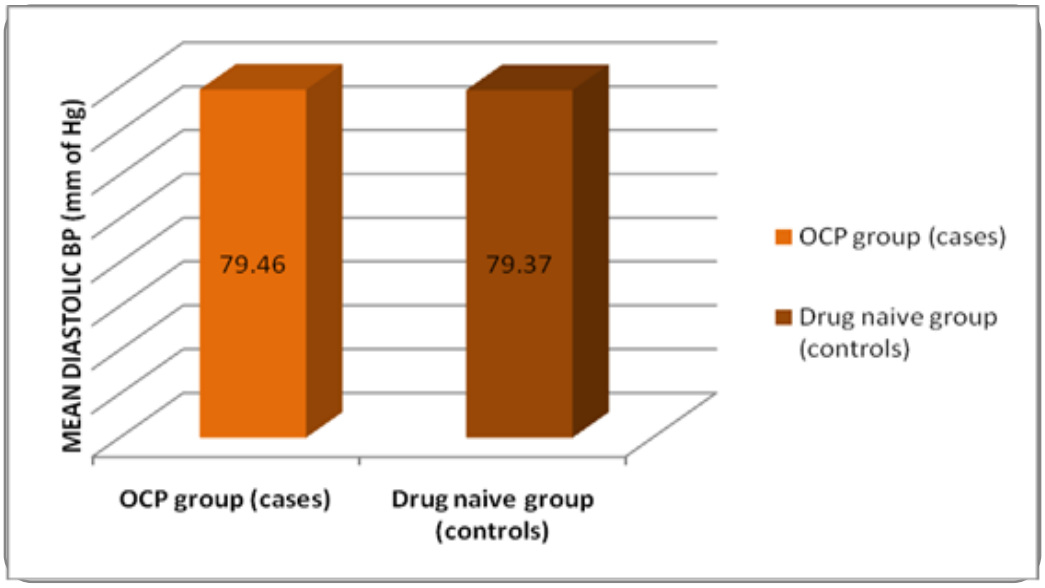


Fig 10.Showing comparison of mean diastolic BP in mm Hg between cases and controls.

Table 3**.** Showing comparison of anthropometric variables and hemodynamic parameters (BP) between cases and controls.

Parameters	**Drug naive group (Controls) (Mean±SD) N=51**	**OCP group (Cases) (Mean±SD) N=30**	**p-value**
Weight(kg)	58.57±8.52	59.07±6.34	0.782
Waist(cm)	79.96±10.15	78.20±8.49	0.427
Waist-Hip ratio (WHR)	0.91±0.06	0.92±0.06	0.544
BMI (Kg/m^2)	23.66±3.43	24.07±3.42	0.606
Systolic BP (mm of Hg)	122.24±6.99	123.00±7.21	0.640
Diastolic BP(mm of Hg)	79.37±4.62	79.46±4.29	0.928

5.4 BIOCHEMICAL PARAMETERS

Mean fasting blood glucose in control and cases who participated in our study was (87.75±19.9 vs. 88.77±10.41 mg/dl) respectively which was comparable (p=0.795) as shown in Fig 11 & Table 4. As per Modified US National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria, fasting glucose equal to or greater than 100 mg/dl is diagnosed as impaired fasting glucose. Among controls 6 (11.7%) subjects had fasting glucose equal to or greater than 100 mg/dl (impaired fasting glucose). Among cases 3 (10%) subjects have fasting glucose equal to or greater than 100 mg/dl (impaired fasting glucose).

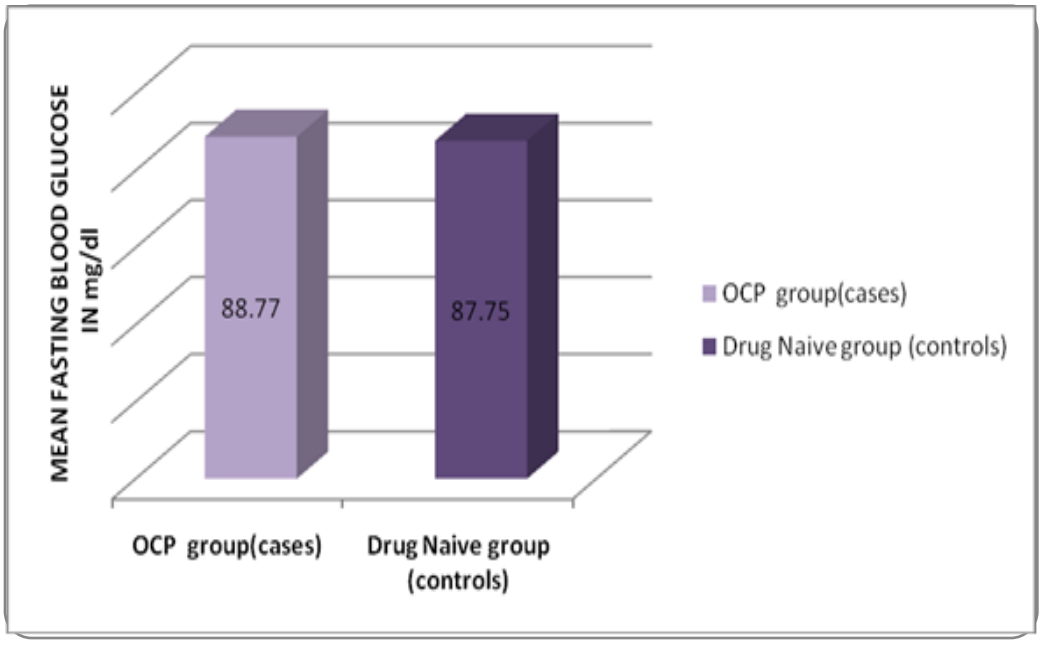


Fig 11. Showing comparison of fasting blood glucose in mg/dl between cases and controls.

Mean blood glucose 2 hr in controls and cases was (109.27±43.9 vs.108.77±25.70 mg/dl) respectively which was comparable (p=0.954) as shown in Fig 12 & Table 4. Among controls 3 subjects (5.8%) have 2 hr glucose between140-199 mg/dl that is impaired glucose tolerance as per World Health Organization (WHO) criteria while as 48 females (94.11%) had 2 hr glucose within normal range. Among cases 4 (13.3%) subjects had 2 hr glucose between 140-199 mg/dl and 26 subjects (86.6 %) had 2 hr glucose within normal range.

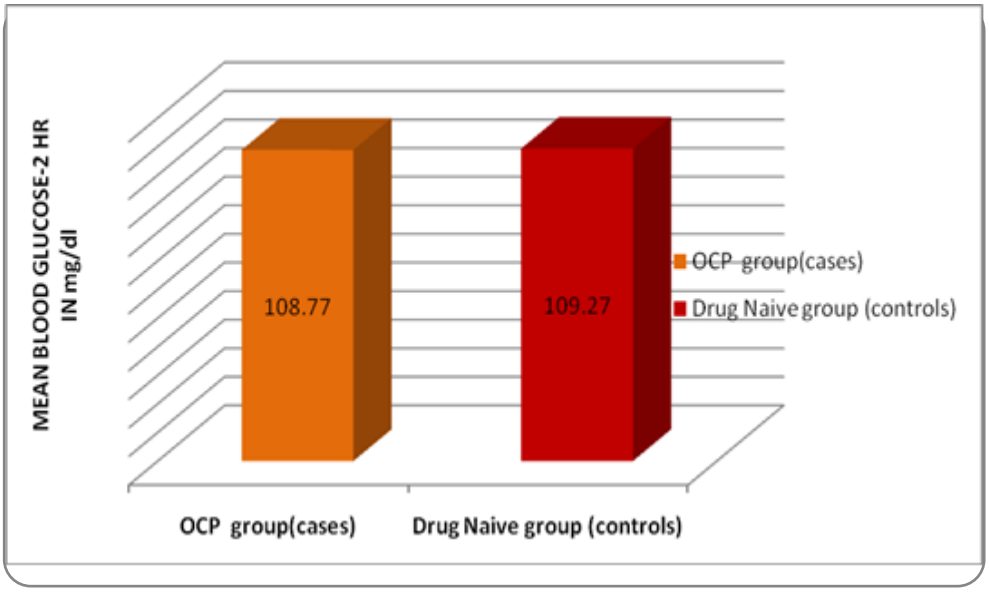


Fig 12. Showing comparison of blood glucose 2 hr in mg/dl between cases and controls.

As per WHO criteria fasting glucose equal to or greater than 126 mg/dl and/or glucose 2 hr equal to or greater than 200 mg/dl is diagnosed as diabetes mellitus. Among controls 2 subjects (3.92%) have fasting glucose greater than 126 mg/dl and 1 subject (1.9%) had 2hr glucose greater than 200 mg/dl.

Mean serum cholesterol level among controls and cases was (155.07 ±28.86 vs. 186.10±44.76 mg/dl) respectively which was statistically significant (p=0.000) shown in Fig 13 & Table 4. As per International Diabetes Federation (IDF) 2006, serum cholesterol greater than 200mg/dl is diagnosed as dyslipidemia. Among controls 7 subjects (13.72%) have cholesterol greater than 200 mg/dl, 44 females (86.2%) have cholesterol in the normal range. Among cases 9 subjects (30%) have cholesterol greater than 200 mg/dl and 21 subjects (70%) have cholesterol within normal range.

Mean serum triglyceride levels in controls and cases was 113.00±46.47 vs. 118.17±41.14 mg/dl) respectively which was comparable (p=0.616) shown in Fig 13 &Table 4. As per International Diabetes Federation (IDF) 2006 serum triglycerides greater than 150 mg/dl is diagnosed as dyslipidemia. Among controls 7 subjects (13.72%) have triglycerides greater than 150 mg/dl, 44 subjects (86.2%) have triglycerides in normal range. Among cases 5 subjects (16.6%) have triglyceride greater than 150 mg/dl and 25 subjects (83.33 %) have triglyceride within normal range.

Mean HDL cholesterol in controls and cases was (45.35±9.99 vs. 48.90±11.90 mg/dl) which was comparable (p=0.155) shown in Fig 13 & Table 4. As per International Diabetes Federation (IDF) 2006, HDL cholesterol less than 50mg/dl is diagnosed as dyslipidemia. Among controls 42 subjects (82.35%) have HDL less than 50mg/dl, 9 subjects (17.64%) have in normal range. Among cases 21 subjects (70%) have HDL less than 50 mg/dl and 9 (30 %) have HDL within normal range.

Mean LDL value in controls and cases was (83.20±27.6 vs. 118.45±45.66 mg/dl) respectively which was statistically significant (p=0.000) shown in Fig

13 & Table 4. As per International Diabetes Federation (IDF) 2006, LDL cholesterol greater than 100 mg/dl diagnosed as dyslipidemia. Among controls 11 subjects (21.5%) have LDL greater than 100mg/dl, 40 subjects (78.43%) have LDL in normal range. Among cases 18 (60%) have LDL greater than 100 mg/dl and 12 subjects (40 %) have LDL within normal range.

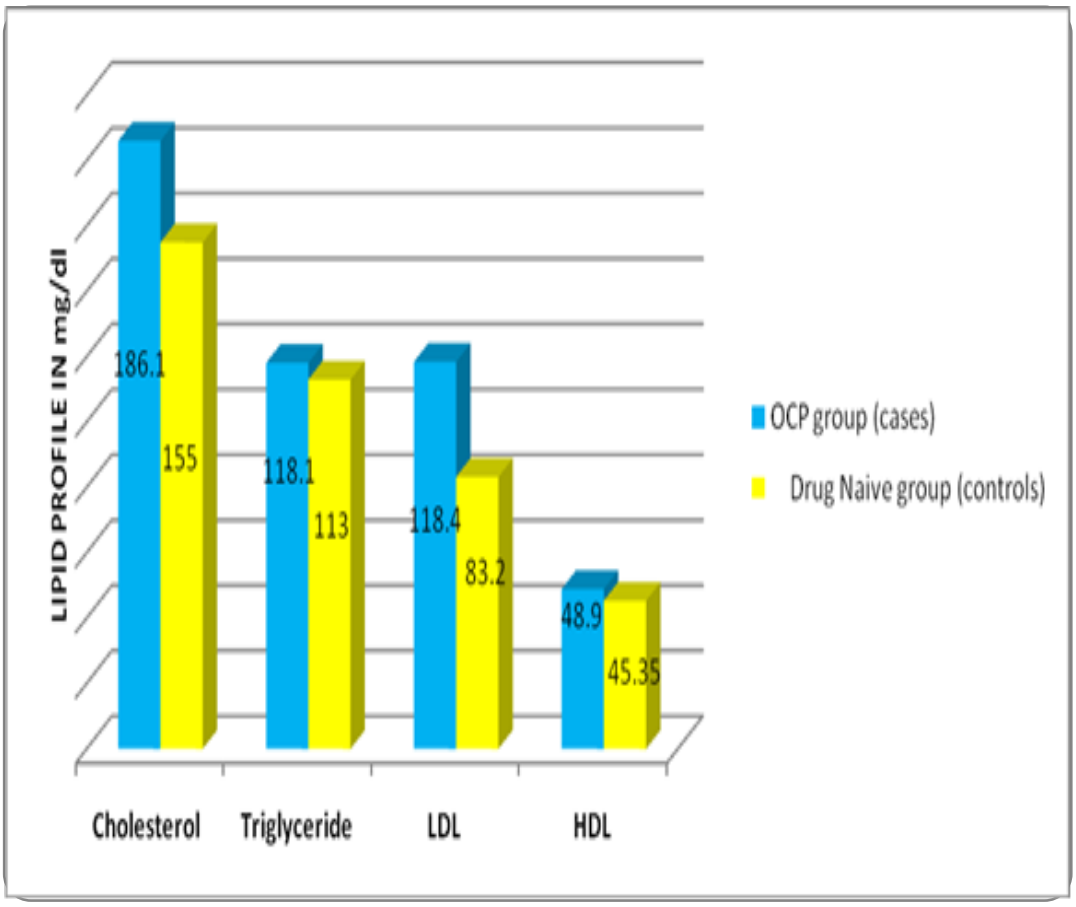


Fig 13. Showing comparison of lipid profile in mg/dl between cases and controls.

Table 4.Showing comparative description of OGTT derived blood glucose values and lipidogram of cases and controls.

Parameters	**Drug naive group (Controls) (Mean±SD) N=51**	**OCP group (Cases) (Mean±SD) N=30**	**p-value**
Blood glucose- Fasting (mg/dl)	87.75±19.91	88.77±10.41	0.795
Blood glucose-1 hour	122.06±45.46	124.60±28.93	0.784
Blood glucose-2hour(mg/dl)	109.27±43.95	108.77±25.70	0.954
Serum Total cholesterol (mg/dl)	155.08±28.86	186.10±44.76	0.000
Serum Triglycerides (mg/dl)	113.00±46.47	118.17±41.14	0.616
Serum HDL(mg/dl)	45.35±9.99	48.90±11.90	0.155
Serum LDL(mg/dl)	83.20±27.63	118.45±45.66	0.000

5.5 INSULIN SENSITIVITY PARAMETERS

Mean fasting insulin levels in controls and cases were (12.28±11.10 vs. 16.23±24.72 µIU/ml) which were comparable (p=0.326) shown in Fig 14 & Table 5. Among controls 27 females (52.94%) have fasting insulin greater than 10 µIU/ml and 24 females (47.05%) have fasting insulin within the normal range. Among cases 14 subjects (46.6%) have insulin greater than 10 µIU/ml and 16 subjects (53.3%) have insulin within the normal range.

Insulin one hour in controls and cases was found to be (65.89 ± 43.97 vs. 59.42 ±34.76 µIU/ml) respectively which was comparable (p=0.493) shown in Fig 14 &Table 5.

Insulin two hour in controls and cases was (43.43±36.07 vs. 65.69±63.67 µIU/ml) respectively which was comparable (p=0.48) shown in Fig 14 &Table 5.

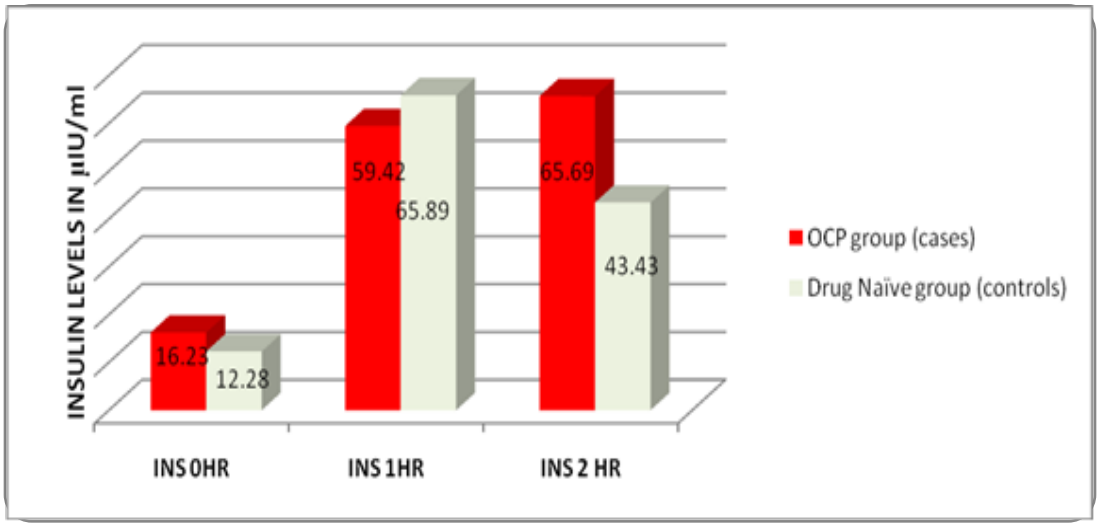


Fig 14.Showing comparison of fasting, 1 hour, 2 hour insulin levels in µIU/ml between cases and controls

FGIR (Fasting glucose to insulin ratio) in controls and cases was found to be (10.03±5.34 and 11.60±9.3) respectively which was comparable (p=0.336) shown in Fig 15 & Table 5.

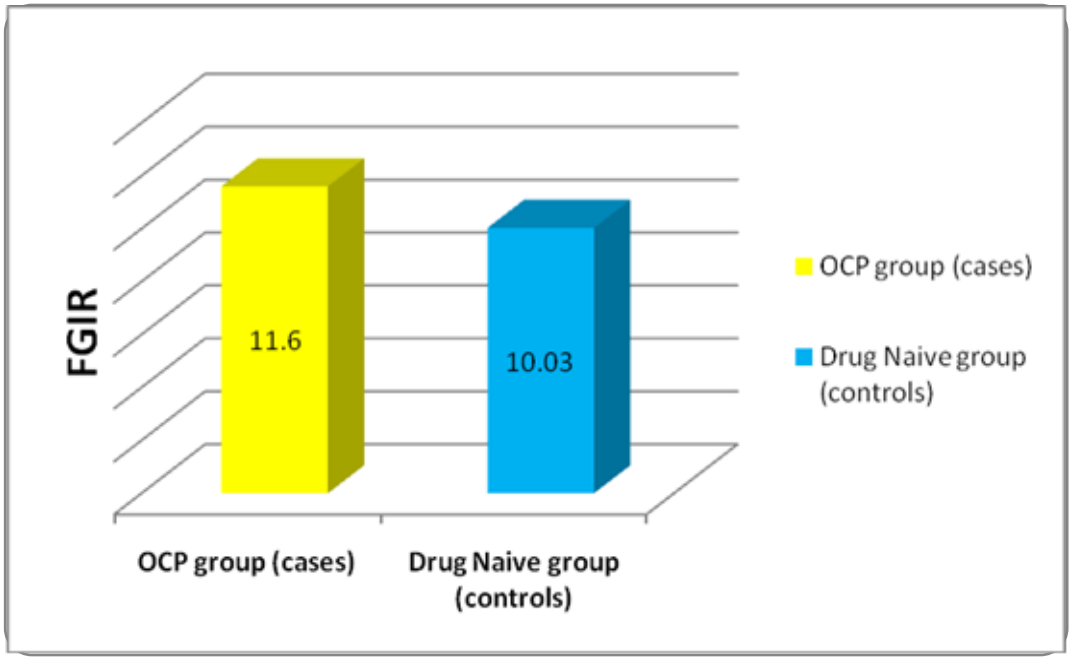


Fig 15.Showing comparison of mean FGIR between cases and controls.

HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) in controls and cases was found to be 2.73±2.64 vs. 3.00±4.17 which was comparable (p=0.335) shown in Fig 16 &Table 5.

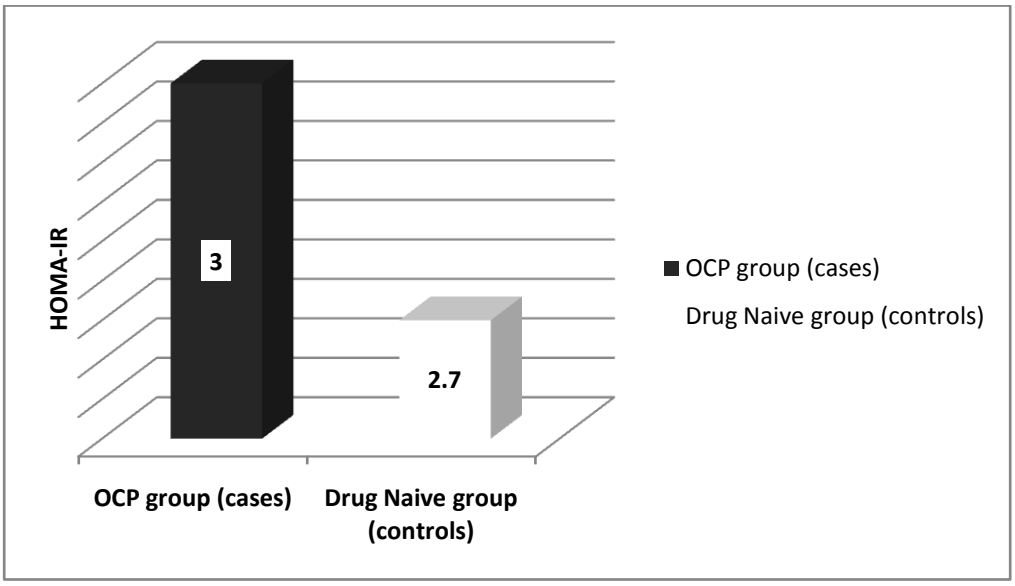


Fig 16.Showing comparison of HOMA-IR between cases and controls.

QUICKI (Quantitative Insulin Sensitivity Check Index) in controls and cases was found to be (0.516±0.01 vs. 0.513±0.013) respectively which was comparable (p=0.449) shown in Fig 17 & Table 5.

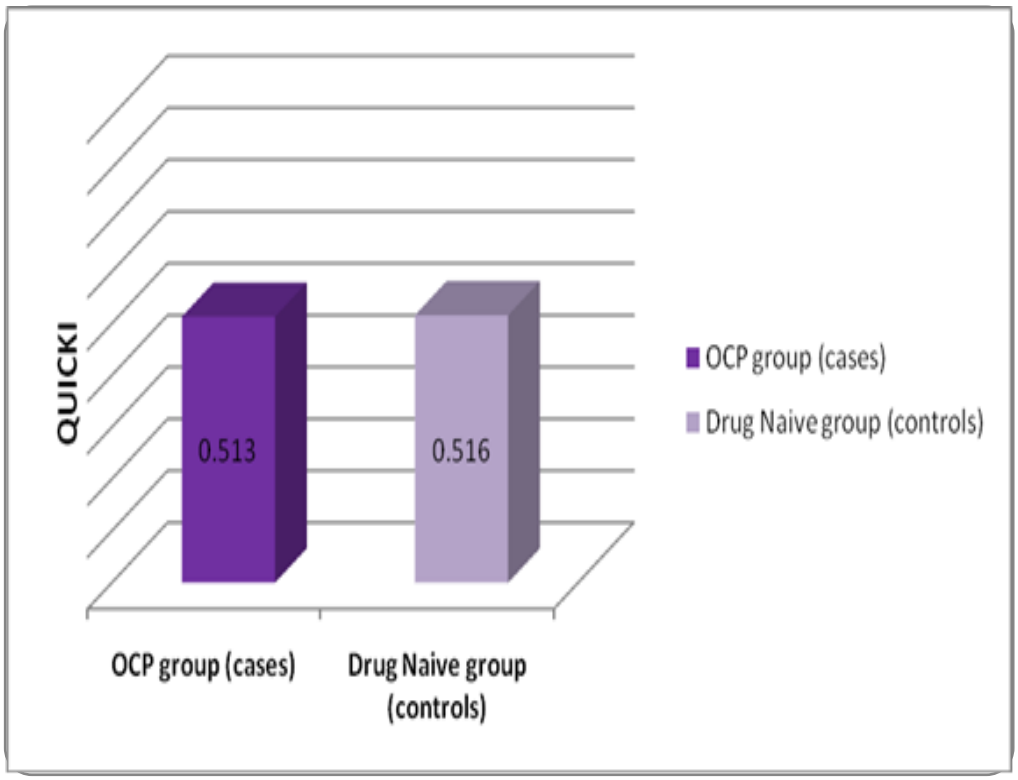


Fig 17. Showing comparison of QUICKI between cases and controls.

Table 5. Showing comparison of OGTT derived insulin sensitivity parameters among cases and controls

Parameters	**Drug naive group (Mean±SD) N=51**	**OCPgroup (Mean±SD) N=30**	**p value**
Serum insulin- Fasting (µIU/ml)	12.28±11.10	16.23±24.72	0.326
Serum insulin -1hour (µIU/ml)	65.89±43.97	59.42±34.76	0.493
Serum insulin-2hour(µIU/ml)	43.43±36.07	65.69±63.67	0.48
FGIR	10.03±5.34	11.60±9.30	0.336
HOMA-IR	2.73±2.64	3.00±4.17	0.335
QUICKI	0.516±0.01	0.513±0.013	0.449

5.6 HORMONAL PARAMETERS

Mean luteinizing hormone (LH) value of controls and cases was found to be (8.17±6.14 vs. 6.75±6.26 IU/L) respectively which is comparable (p=0.322) shown in Fig 18 &Table 6. Among controls 10 females (19.6%) have LH/FSH ratio greater than 2, while 41 subjects (80.3%) have normal LH/FSH ratio. Among cases 7 subjects (23.3%) have LH/FSH ratio greater than 2 while 23 subjects (76.6%) have normal LH/FSH ratio.

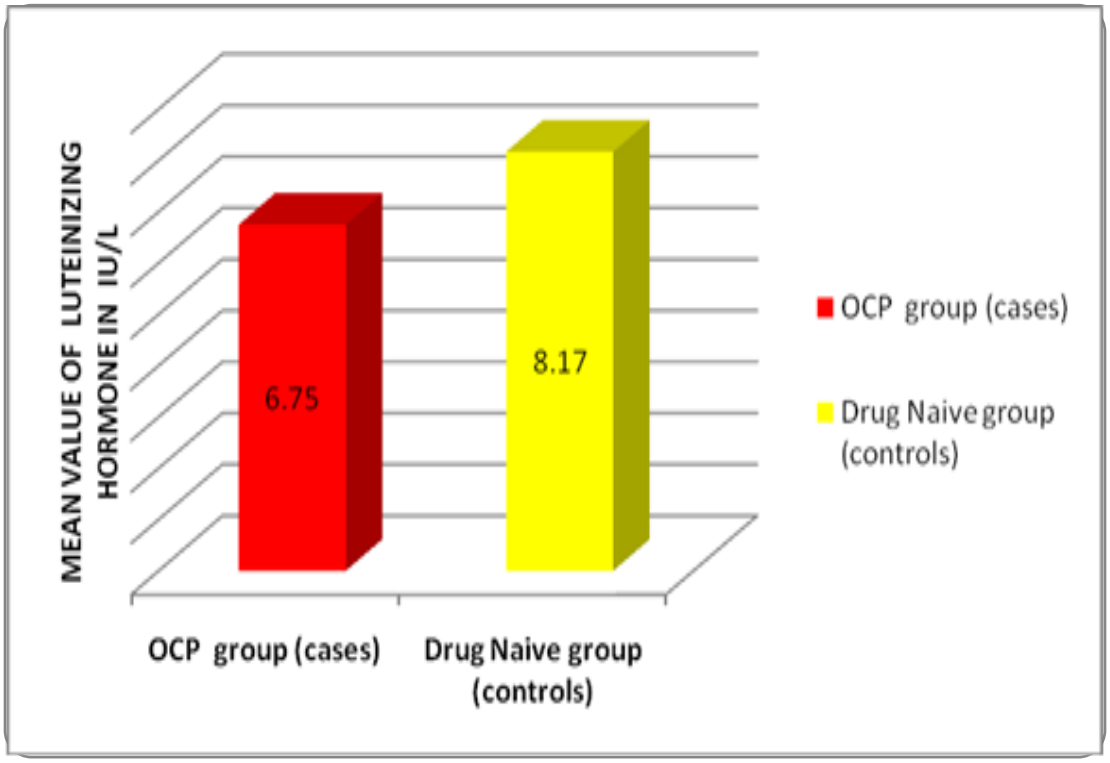


Fig 18.Showing comparison of mean LH values in IU/L between cases and controls.

Mean FSH value in the two groups of controls and cases was found to be (6.17±2.21 vs. 4.88±2.17 IU/L) which is statistically significant (p=0.013) shown in Fig 19 & Table 6.

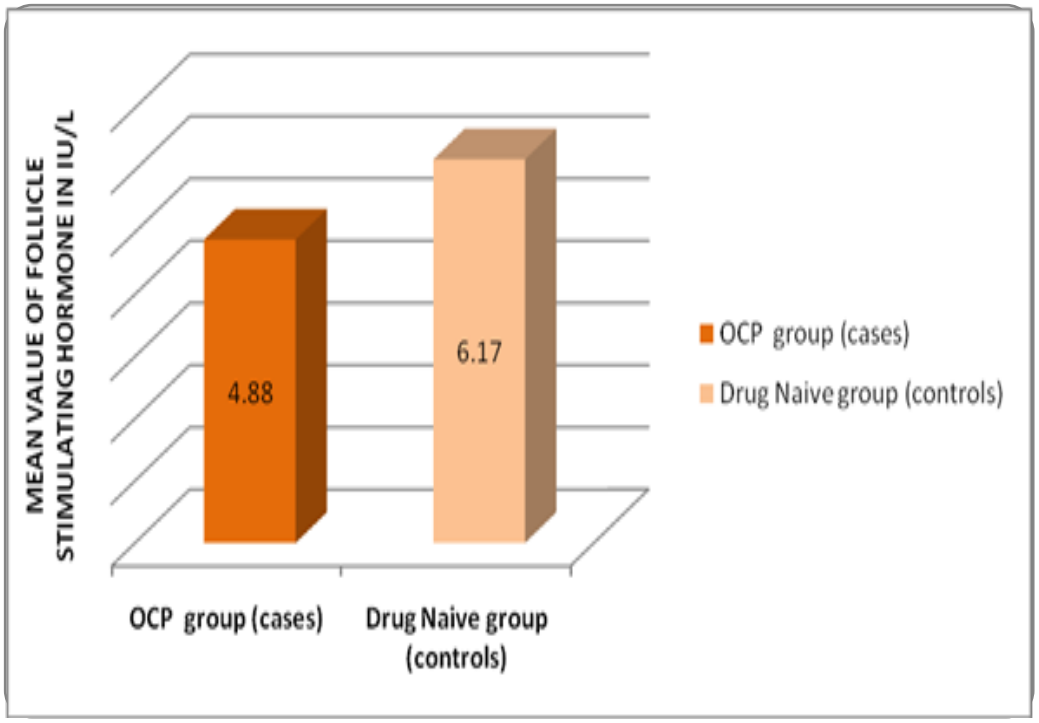


Fig 19. Showing comparison of mean FSH levels in IU/L between cases and controls.

Mean testosterone levels of controls and cases was found to be (63.31±31.30 vs. 56.05±31.81 ng/dl) respectively which was comparable (p=0.320) shown in Fig 20 & Table 6.Among controls 26 subjects (50.9%) had testosterone >65 ng/dl, while 25 subjects (49%) have testosterone within normal range. Among cases 7 subjects (23.3%) have testosterone >65 ng/dl while 23 subjects (76.6%) have testosterone within normal range.

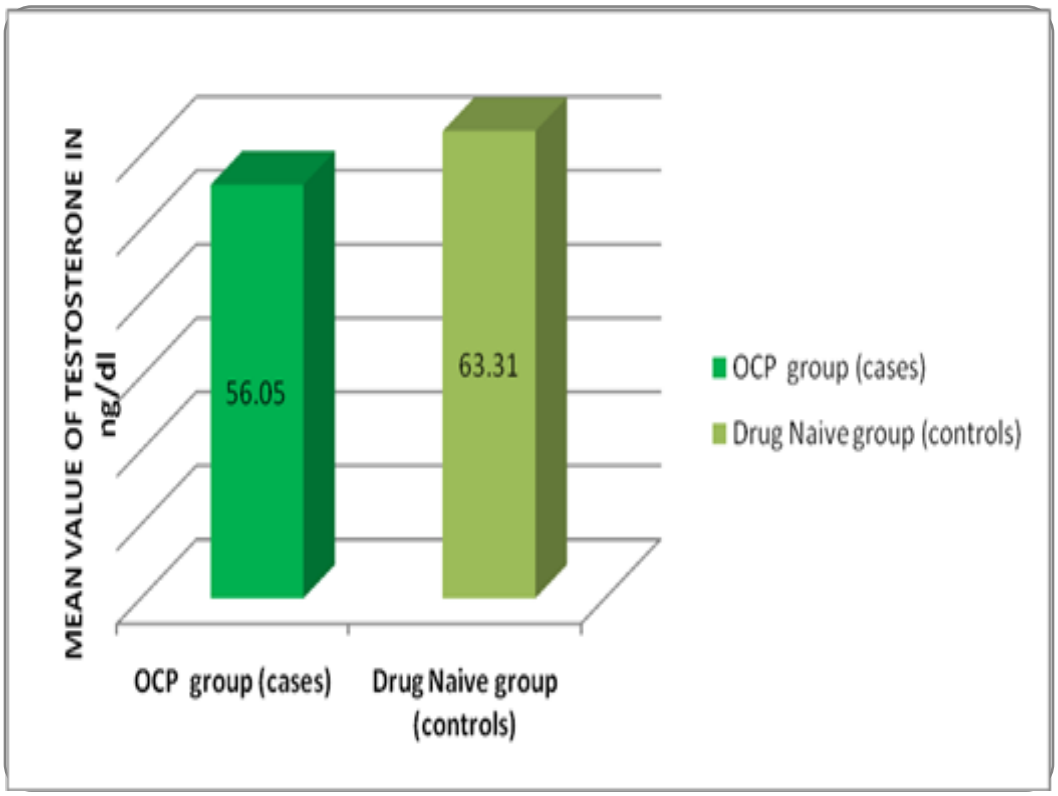


Fig 20. Showing comparison mean total serum testosterone levels in ng/dl between cases and controls.

Table 6 . Showing comparative description of mean serum gonadotrophins and the mean total serum testosterone levels in ng/dl between cases and controls .

Parameters	**Drug naive group (Mean± SD) N=51**	**OCP group (Mean± SD) N=30**	**P value**
Serum LH (IU/L)	8.17±6.14	6.75±6.26	0.322
FSH (IU/L)	6.17±2.21	4.88±2.17	0.013
Serum total testosterone (ng/dl)	63.31±31.30	56.05±31.81	0.320

5.7 PROCOAGULANT AND PROINFLAMMATORY MARKERS

Mean plasma soluble intercellular adhesion molecule-1 (s-ICAM-1) values in controls and cases were found to be (312.41±131.65 vs. 417.03±131.65 ng/ml) respectively and was statistically significant (p=0.001) shown in Fig 21 & Table 7.

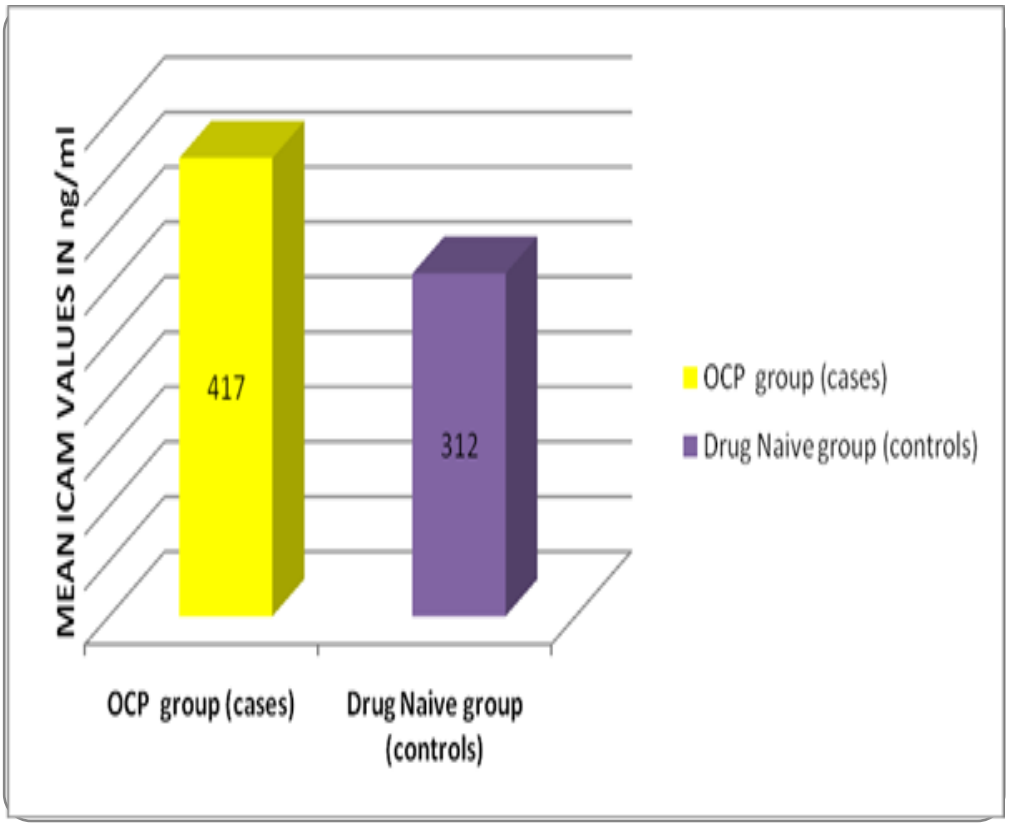


Fig 21. Showing comparison of soluble ICAM-1 in ng/ml values between cases and controls

Mean Monocyte chemo attractant protein-1 (MCP-1) value in controls and cases was found to be (456.78±187.2 vs. 464.82 ± 91.19 pg/ml) which was comparable (p=0.827) shown if Fig 22 & Table 7.

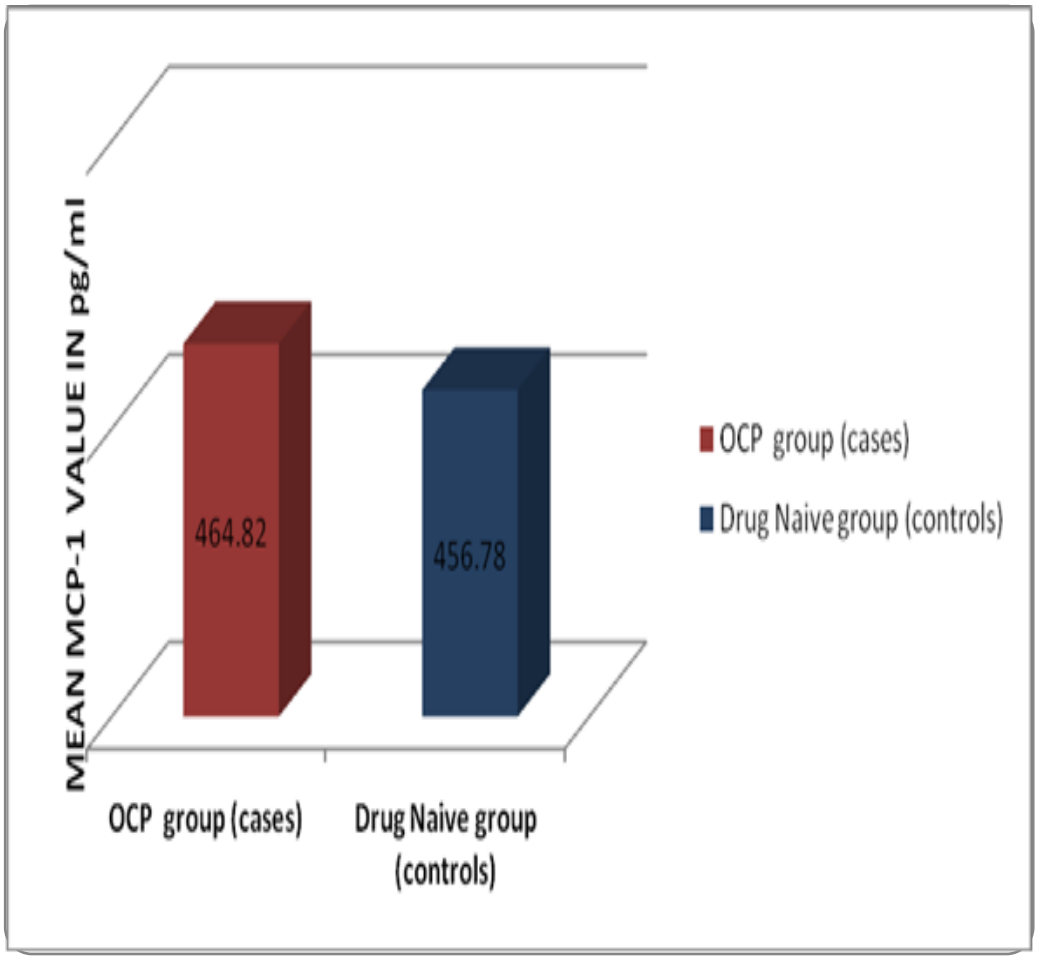


Fig 22.Showing comparison of MCP-1 pg/ml between cases and controls.

Mean TNF-α levels in controls and cases was found to be 22.85±5.19 vs. 25.60±4.24 which is statistically significant (p=0.016) shown in Fig 23 and Table 7.

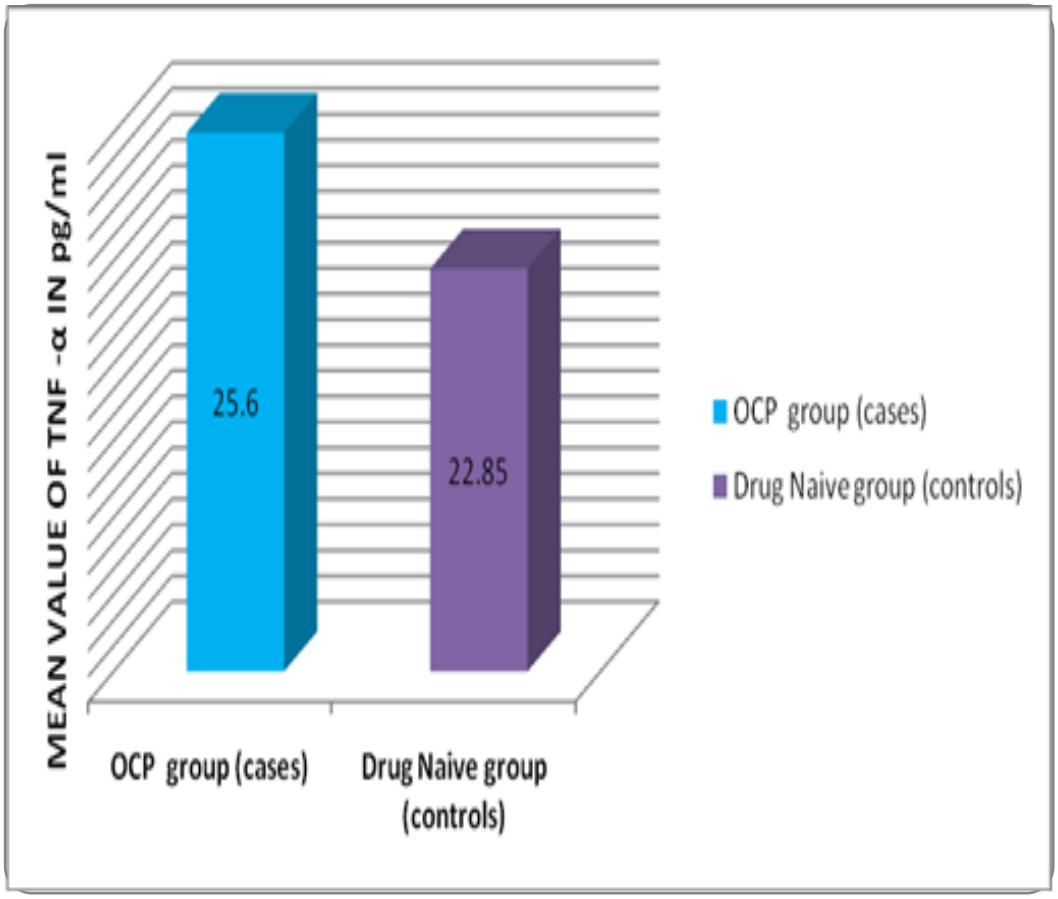


Fig 23. Showing comparison of TNF-α level in pg/ml between cases and controls.

Mean PAI-1 levels in controls and cases was found to be (1.05±0.40 vs. 1.10±0.59 ng/ml) which is comparable (p=0.682) shown in Fig 24 & Table 7.

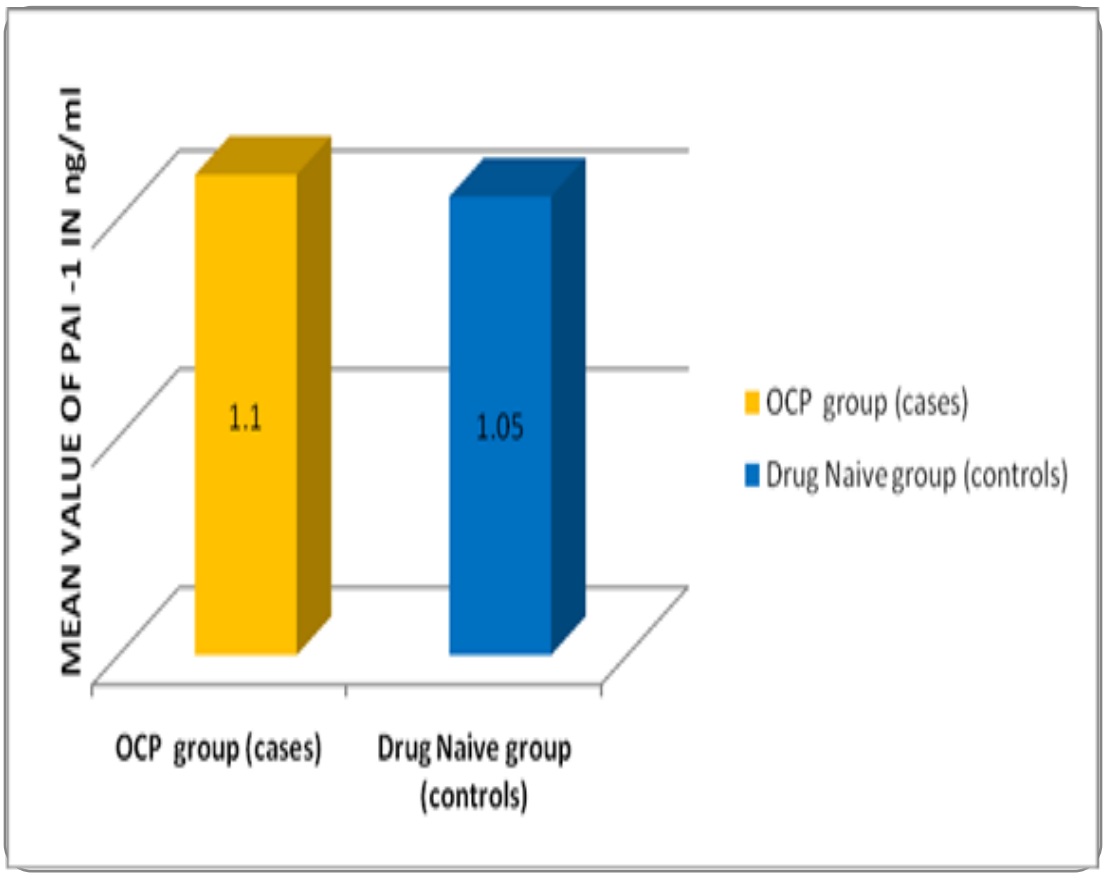


Fig 24. Showing comparison of PAI -1 levels in ng/ml between cases and controls

Mean factor VIII levels in controls and cases was found to be 0.685±0.32 and 0.350±0.33 which is statistically significant (p=0.00) shown in Fig 25 & Table 7.

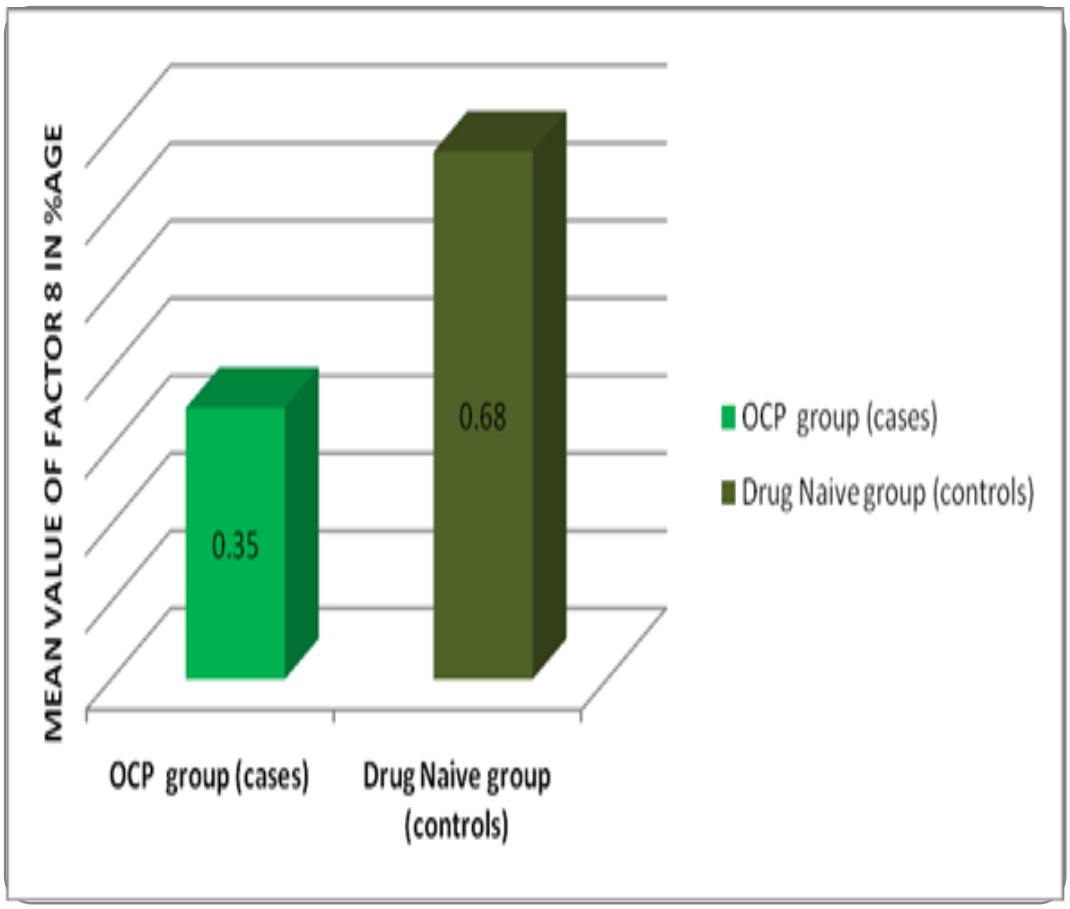


Fig 25. Showing comparison of Factor VIII levels between cases and controls

Table 7. Showing comparison of s-ICAM-1, MCP-1,TNF-alpha, Factor VIII and serum PAI-1 levels between cases and controls

Parameters	**Drug naive group (Mean±SD) N=51**	**OCP group (Mean±SD) N=30**	**p-value**
s-ICAM-1 (ng/ml)	312.41±131.65	417.03±131.62	0.001
MCP-1(pg/ml)	456.78±187.25	464.82±91.19	0.827
TNF-α (pg/ml)	22.85±5.19	25.60±4.24	0.016
FACTOR VIII	0.68±0.32	0.35±0.33	0.000
PAI-1 (ng/ml)	1.05±0.40	1.10±0.59	0.682

5.8 CORRELATION

In Pearson's correlation between the procoagulant, proinflammatory markers and different anthropometric, biochemical & hormonal parameters, statistically significant and negative correlation was found between factor VIII and INS 0 hr (r=-0.234, p=0.036), HOMA-IR (r=-0.233, p=0.037) and s-ICAM-1 (r=-0.239, p=0.032) shown in table 8-10.

Table 8: Showing correlation of Factor VIII with clinical and anthropometric parameters.

Parameters	r	p
Age	0.125	0.266
Menarche	-0.055	0.625
Cycles/year	-0.053	0.637
FG score	0.077	0.496
Wt	0.014	0.898
BMI kg/m^2	0.056	0.620
Waist (cm)	0.061	0.586
hip (cm)	-0.023	0.839
Waist hip ratio	0.147	0.191
SBP mm of Hg	-0.162	0.149
DBP mm of Hg	-0.144	0.201

Table 9: Showing correlation of Factor VIII with different hormonal, biochemical and insulin sensitivity parameters.

Parameters	r	p
LH (IU/L)	-0.031	0.781
FSH (IU/L)	-0.072	0.522
Serum total testosterone (ng/ml)	0.123	0.276
Blood glucose fasting (mg/ml)	-0.026	0.818
Blood glucose -1 hr(mg/dl)	0.085	0.450
Blood glucose -2 hr (mg/dl)	0.062	0.580
Plasma insulin fasting (µIU/ml)	-0.234*	0.036
Plasma insulin -1 hr (µIU/ml)	0.027	0.812
Plasma insulin -2hr (µIU/ml)	-0.197	0.078
HOMA-IR	-0.233*	0.037
FGIR	0.044	0.696
QUICKI	0.109	0.334

Table 10: Showing correlation of Factor VIII with different lipid parameters, pro- coagulant and inflammatory markers

Parameters	r	p
Total cholesterol (mg/dl)	-0.199	0.076
Triglycerides (mg/dl)	-0.092	0.416
HDL (mg/dl)	-0.155	0.167
LDL (mg/dl)	0.174	0.`119
PAI (ng/ml)	0.013	0.907
TNF-α (pg/ml)	-0.158	0.158
MCP-1 (pg/ml)	-0.033	0.767
s-ICAM-1 (ng/ml)	-0.239*	0.032

PAI-1 showed statistically significant and positive correlation with blood glucose one hour (r=0.233, p=0.036)(fig 26), blood glucose two hour (r=0.258,p=0.020)(fig 27),Insulin two hour (r=0.309,p=0.005) and triglyceride level (r=0.334,p=0.002)(fig 28). PAI-1 showed negative and statistically significant correlation with age of menarche (r=-0.295,p=0.007) shown in table 11-13.

Table 11: Correlation of Plasminogen Activator Inhibitor-1 (PAI-1) with different clinical and anthropometric parameters.

Parameters	**r**	**p**
Age	0.064	0.571
Menarche	-0.295**	0.007
Cycles/year	-0.071	0.529
FG score	-0.127	0.257
Wt (kg)	0.063	0.574
BMI (kg/m^2)	0.142	0.205
Waist (cm)	0.203	0.070
Waist hip ratio	0.142	0.206
SBP (mm Hg)	-0.117	0.299
DBP (mm Hg)	-0.159	0.157

Table 12: Correlation of Plasminogen Activator Inhibitor-1 (PAI-1) with different hormonal, biochemical and insulin sensitivity parameters.

Parameters	r	P
LH (IU/L)	0.098	0.382
FSH (IU/L)	0.085	0.448
Serum total testosterone (ng/ml)	0.036	0.751
Blood glucose fasting (mg/ml)	0.194	0.082
Blood glucose -1 hr(mg/dl)	0.233*	0.036
Blood glucose -2 hr (mg/dl)	0.258*	0.020
Plasma insulin fasting (µIU/ml)	0.085	0.453
Plasma insulin -1 hr (µIU/ml)	0.117	0.300
Plasma insulin -2hr (µIU/ml)	0.309**	0.005
HOMA-IR	0.115	0.307
FGIR	-8.020	0.862
QUICKI	-0.181	0.105

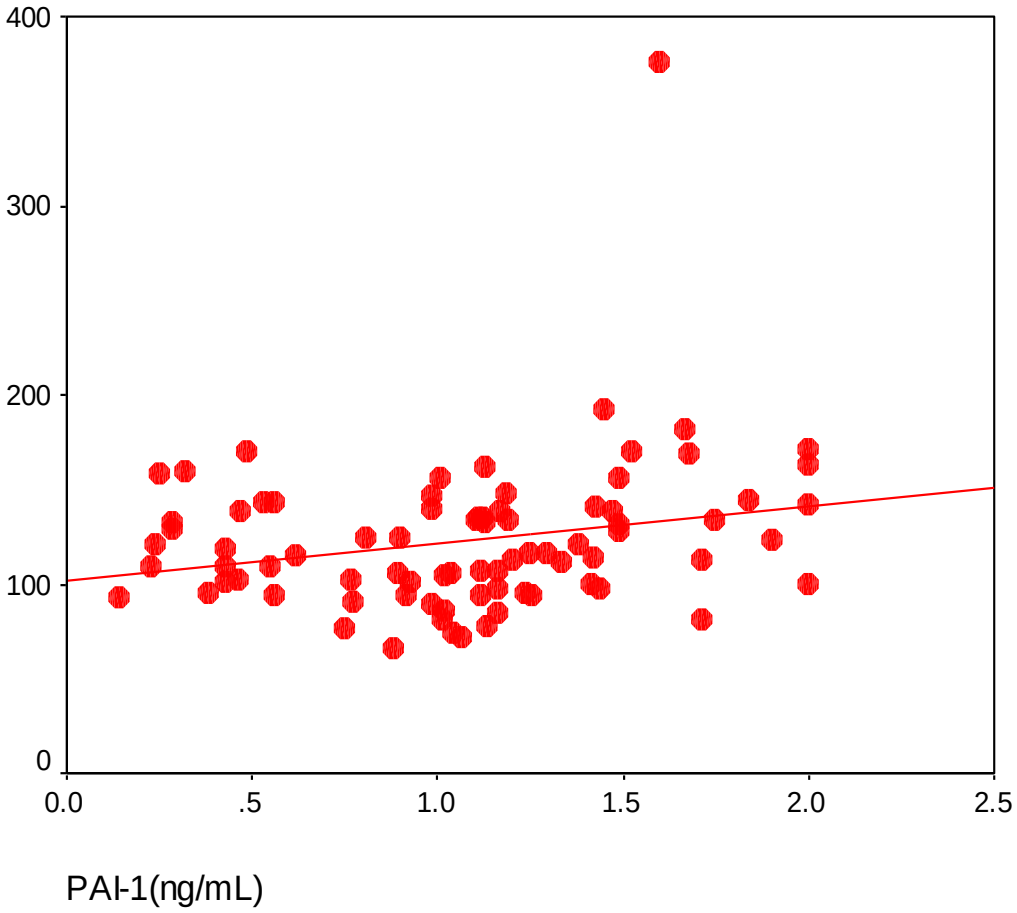


Fig 26. Graph showing correlation between PAI-1 and blood glucose 1hr

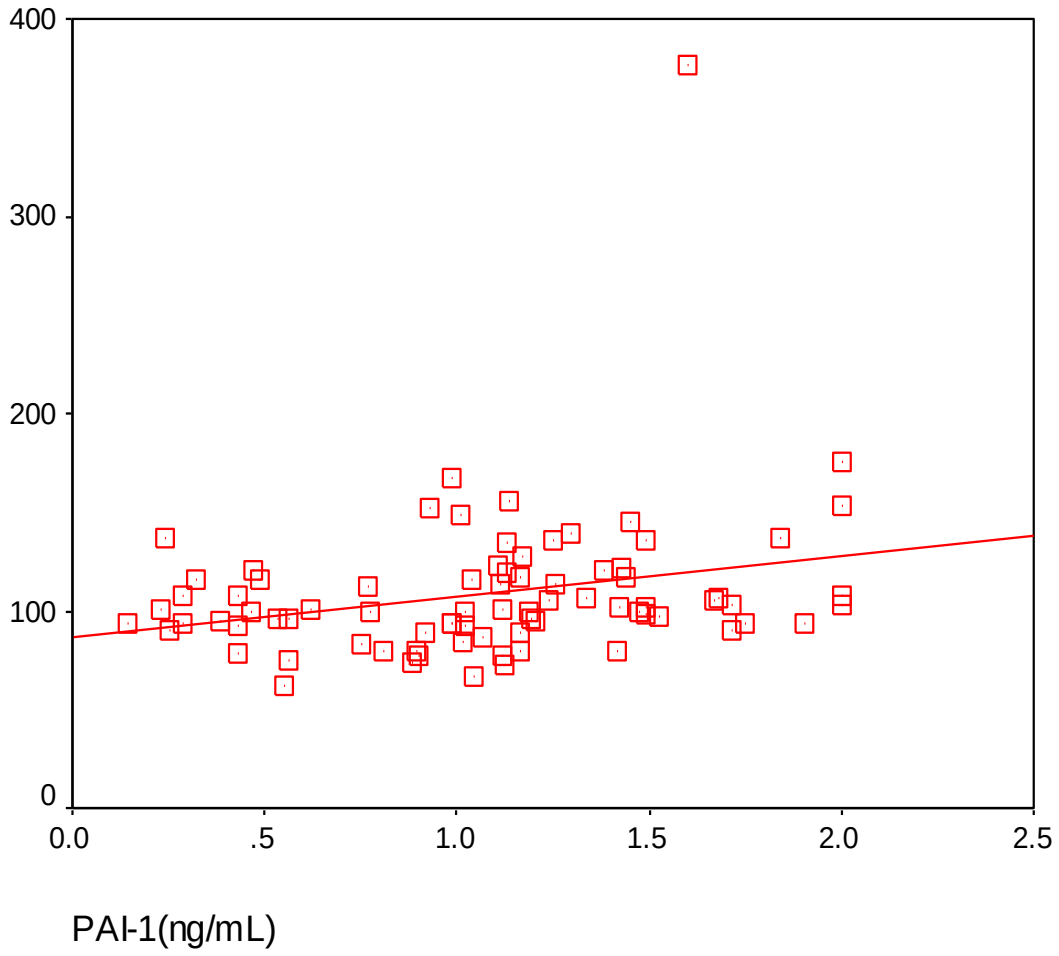


Fig 27. Graph showing correlation between PAI-1 and blood glucose 2 hr

Table 13: Correlation of Plasminogen Activator Inhibitor-1 (PAI-1) with different lipid parameters procoagulant and inflammatory markers.

Parameters	r	p
Total cholesterol (mg/dl)	0.056	0.621
Serum Triglycerides (mg/dl)	0.334**	0.002
HDL (mg/dl)	-0.138	0.220
LDL (mg/dl)	0.099	0.379
Factor VIII	0.013	0.907
TNF-α (pg/ml)	-0.143	0.202
MCP-1 (pg/ml)	0.126	0.261
s-ICAM-1 (ng/ml)	0.090	0.422

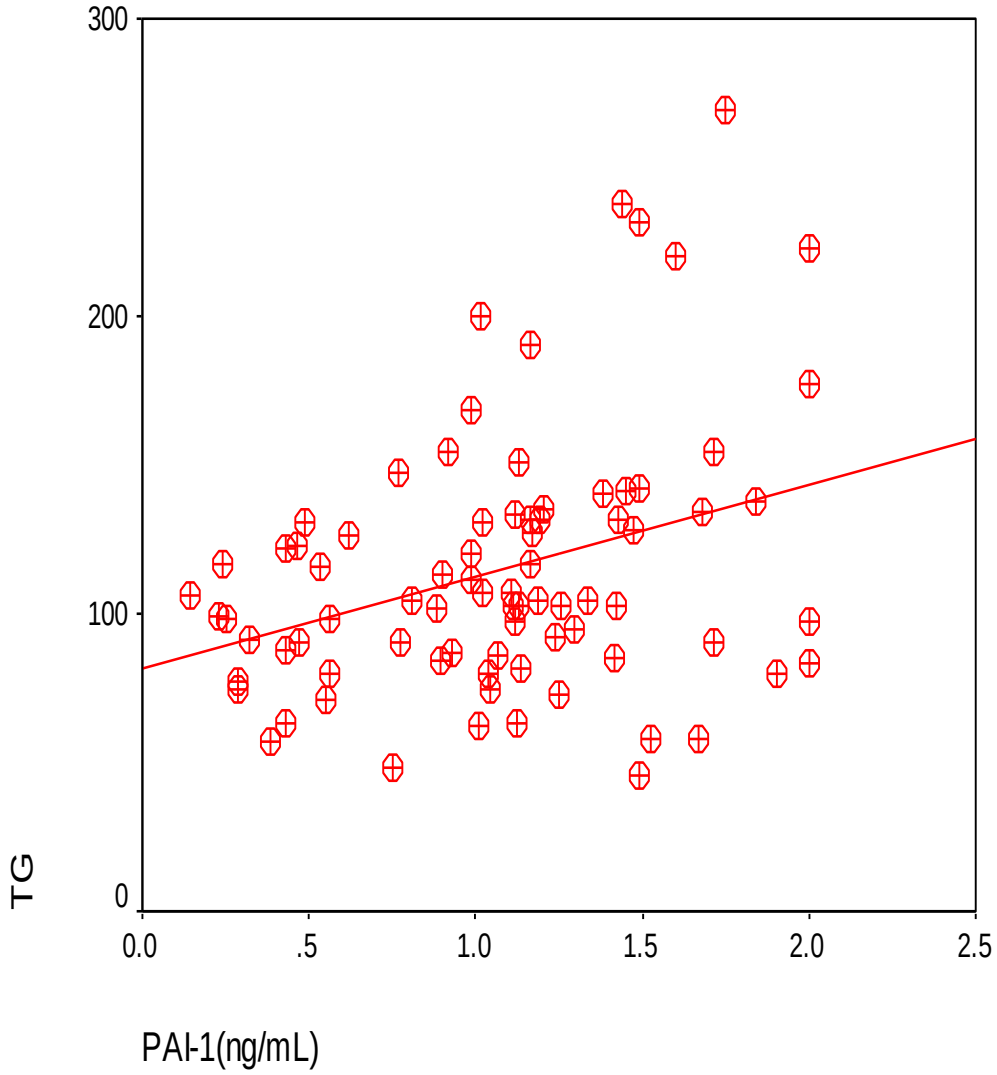


Fig 28.Graph showing correlation between PAI-1 and Triglyceride

TNF-α showed statistically significant but negative correlation with FSH levels (r=-0.255, p=0.022), Insulin one hour (r=-0.308, p=0.005)(fig 29) but statistically significant and positive correlation with sICAM-1 (r=0.292, p=0.008) shown in table 14-16.

Table 14: Correlation of plasma Tumor Necrosis Factor-α (TNF-α) levels with different clinical and anthropometric parameters

Parameters	**r**	**p**
Age	-0.040	0.722
Menarche	0.142	0.206
Cycles/year	-0.064	0.571
FG score	-0.105	0.350
Wt kg	-0.035	0.756
BMI kg/m^2	-0.005	0.962
Waist (cm)	-0.077	0.497
Waist hip ratio	0.030	0.791
SBP mm of Hg	-0.40	0.726
DBP mm of Hg	-0.093	0.411

Table 15: Correlation of plasma Tumor Necrosis Factor-α (TNF-α) levels with different hormonal, biochemical and insulin sensitivity parameters.

Parameters	r	p
LH (IU/L)	-0.135	0.229
FSH (IU/L)	-0.255*	0.022
Serum total testosterone (ng/ml)	0.012	0.914
Blood glucose fasting (mg/ml)	-0.038	0.739
Blood glucose -1 hr(mg/dl)	0.093	0.408
Blood glucose -2 hr (mg/dl)	-0.049	0.665
Plasma insulin fasting (µIU/ml)	0.076	0.501
Plasma insulin -1 hr (µIU/ml)	-0.308**	0.005
Plasma insulin -2hr (µIU/ml)	-0.162	0.149
HOMA-IR	0.059	0.603
FGIR	0.069	0.542
QUICKI	0.054	0.631

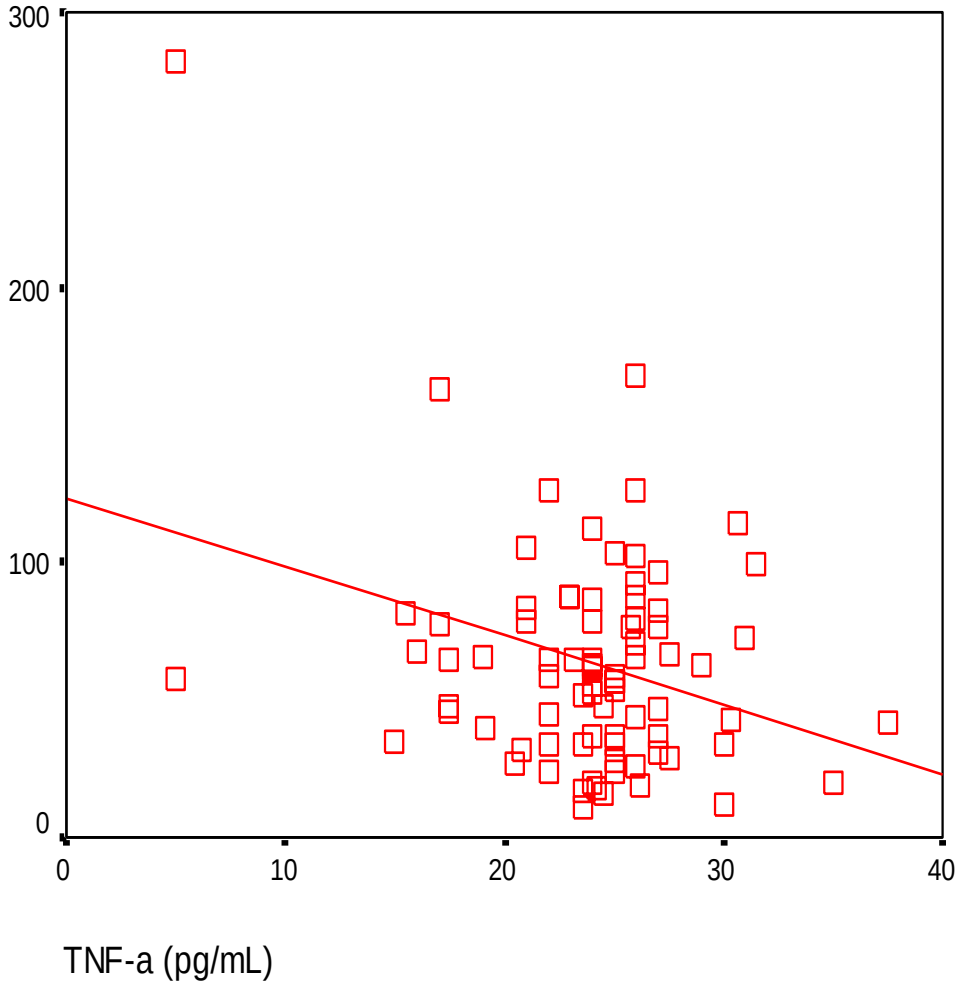


Fig 29.Graph showing correlation between and TNF-α and insulin 1hr

Table 16: Correlation of plasma Tumor Necrosis Factor-α (TNF-α) levels with different lipid parameters, procoagulant and inflammatory markers.

Parameters	**r**	**p**
CHOL (mg/dl)	0.120	0.286
TG (mg/dl)	0.172	0.124
HDL (mg/dl)	0.082	0.466
LDL (mg/dl)	0.078	0.488
Factor VIII	-0.158	0.158
TNF-α (pg/ml)	-0.143	0.202
MCP-1(pg/ml)	0.111	0.322
s-ICAM-1 (ng/ml)	0.292**	0.008

MCP-1 showed statistically significant and positive correlation with fasting blood glucose levels (r=0.296, p=0.007)(Fig 30).statistically significant but inverse correlation with QUICKI (r=-0.274, p=0.013) (Fig 31)shown in table 17-19.

Table 17: Correlation of Monocyte chemo attractant protein-1 (MCP-1) with different clinical and anthropometric parameters

Parameters	**r**	**p**
Age	-0.115	0.306
Menarche	0.051	0.651
Cycles/year	-0.138	0.218
FG score	0.013	0.909
Wt	-0.014	0.903
BMI kg/m^2	0.098	0.384
Waist (cm)	-0.009	0.934
hip (cm)	0.040	0.725
Waist hip ratio	-0.069	0.5420
SBP mm of Hg	-0.124	0.268
DBP mm of Hg	-0.067	0.553

Table 18: Correlation of plasma Monocyte chemoattractant protein-1 (MCP-1) levels with different hormonal ,biochemical and insulin sensitivity parameters.

Parameters	r	p
LH (IU/L)	0.161	0.151
FSH (IU/L)	0.101	0.371
TESTO (ng/ml)	0.019	0.866
BG OA(ng/ml)	0.296**	0.007
BG IA (mg/dl)	0.184	0.101
BG 2A(mg/dl)	0.127	0.257
IN OA (µIU/ml)	0.016	0.886
IN IH (µIU/ml)	0.074	0.509
IN 2H (µIU/ml)	-0.030	0.791
HOMA-IR	0.052	0.642
FGIR	0.035	0.759
QUICKI	-0.274*	0.013

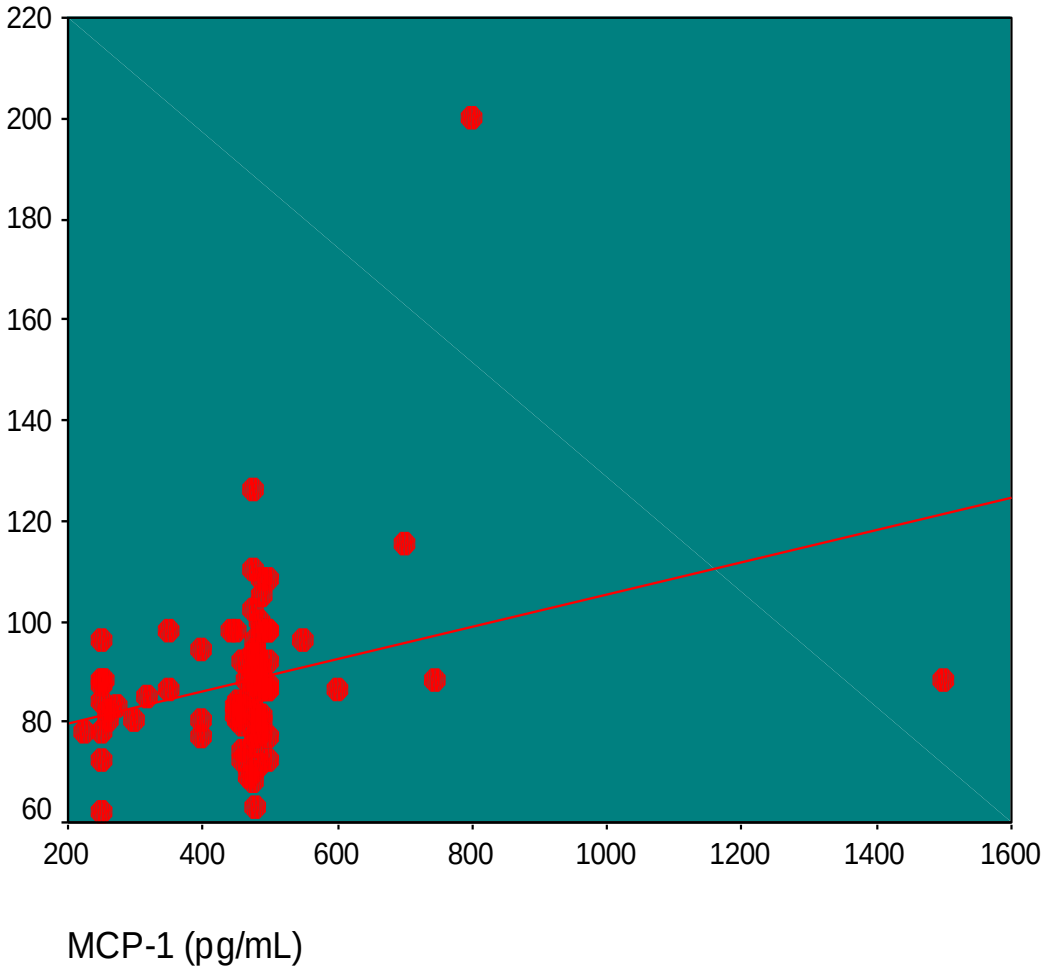


Fig 30 Graph showing correlation between MCP-1 and fasting blood glucose

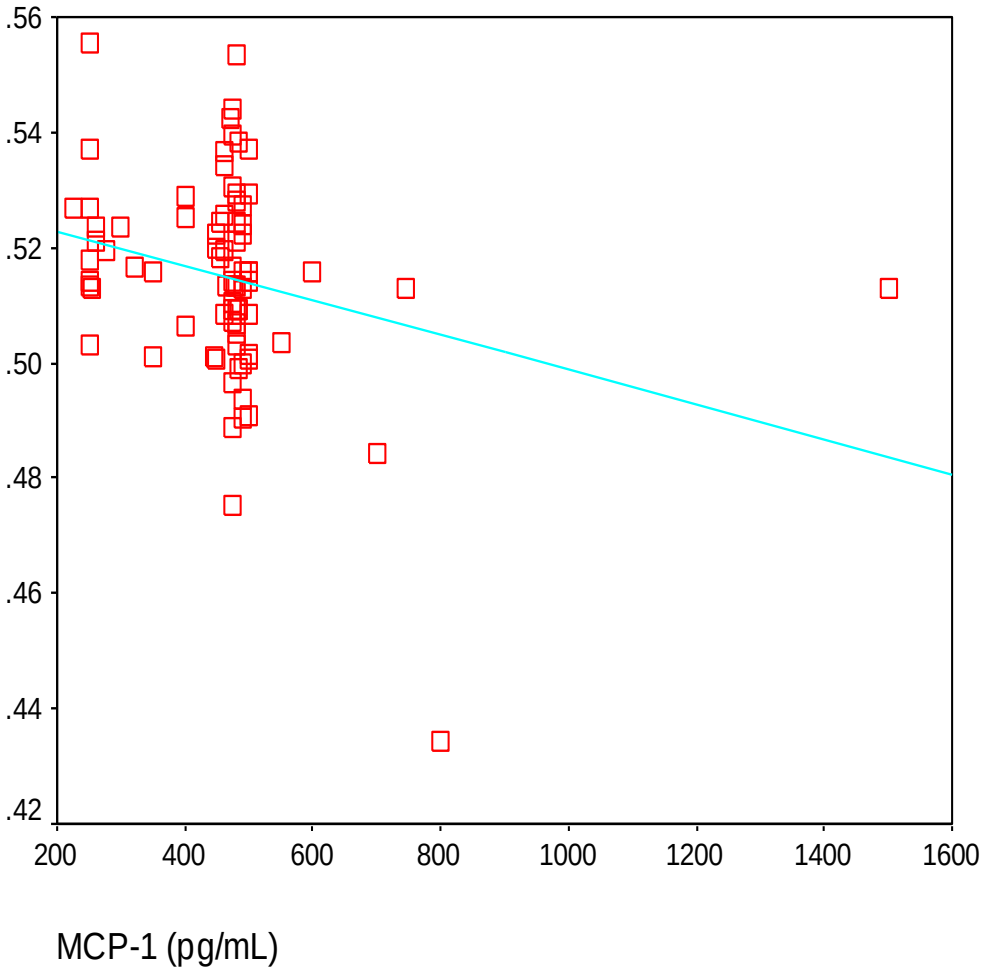


Fig 31. Graph showing correlation between MCP-1 and QUICKI

Table19: Correlation of plasma Monocyte chemoattractant protein-1 (MCP-1) levels with different lipid parameters, procoagulant and inflammatory markers.

Parameter	r	p
CHOL (mg/dl)	-0.031	0.782
TG (mg/dl)	0.064	0.569
HDL (mg/dl)	0.018	0.875
LDL (mg/dl)	-0.059	0.601
Factor VIII	-0.033	0.767
PAI (ng/ml)	0.126	0.261
TNF-α (pg/ml)	0.111	0.322
s-ICAM-1 (ng/ml)	0.103	0.362

s-ICAM-1 showed statistically significant but positive correlation with fasting blood glucose (r=0.427,p=0.000) blood glucose one hour (r=0.350,p=0.001), blood glucose two hour (r=0.287,p=0.009),fasting insulin levels (r=0.347,p=0.001)(fig 32), HOMA-IR (r=0.405,p=0.000)(fig 33), and triglyceride (r=0.344,p=0.002) and TNF-α (r=0.292,p=0.008).ICAM showed statistically significant but negative correlation with QUICKI (r=-0.437,p=0.000) (fig 34), FACTOR VIII (r=-0.239,p=0.032) and systolic blood pressure (r=-0.248,p=0.026) shown in table 20-22.

Table 20: Showing correlation of Intercellular adhesion molecule-1 (ICAM-1) with different clinical and anthropometric parameters

Parameters	**r**	**p**
Age	-0.055	0.626
Menarche	-0.044	0.695
Cycles/year	0.057	0.616
FG score	-0.077	0.496
Wt	0.060	0.593
BMI kg/m^2	0.063	0.579
Waist (cm)	0.067	0.551
hip (cm)	0.021	0.855
Waist hip ratio	0.085	0.449
SBP mm of Hg	-0.248*	0.026
DBP mm of Hg	-0.200	0.074

Table 21: Correlation of plasma Intercellular adhesion molecule-1 (ICAM-1) levels with different hormonal, biochemical and insulin sensitivity parameters.

Parameters	**r**	**p**
LH (IU/L)	0.054	0.634
FSH (IU/L)	-0.037	0.744
TESTO (ng/ml)	-0.204	0.067
BG OA(ng/ml)	0.427*	0.000
BG 1A (mg/dl)	0.350**	0.001
BG 2A(mg/dl)	0.287**	0.009
IN OA (µIU/ml)	0.347**	0.001
IN IH (µIU/ml)	-0.215	0.054
IN 2H (µIU/ml)	0.000	0.997
HOMA-IR	0.405**	0.000
FGIR	0.065	0.567
QUICKI	-0.437**	0.000

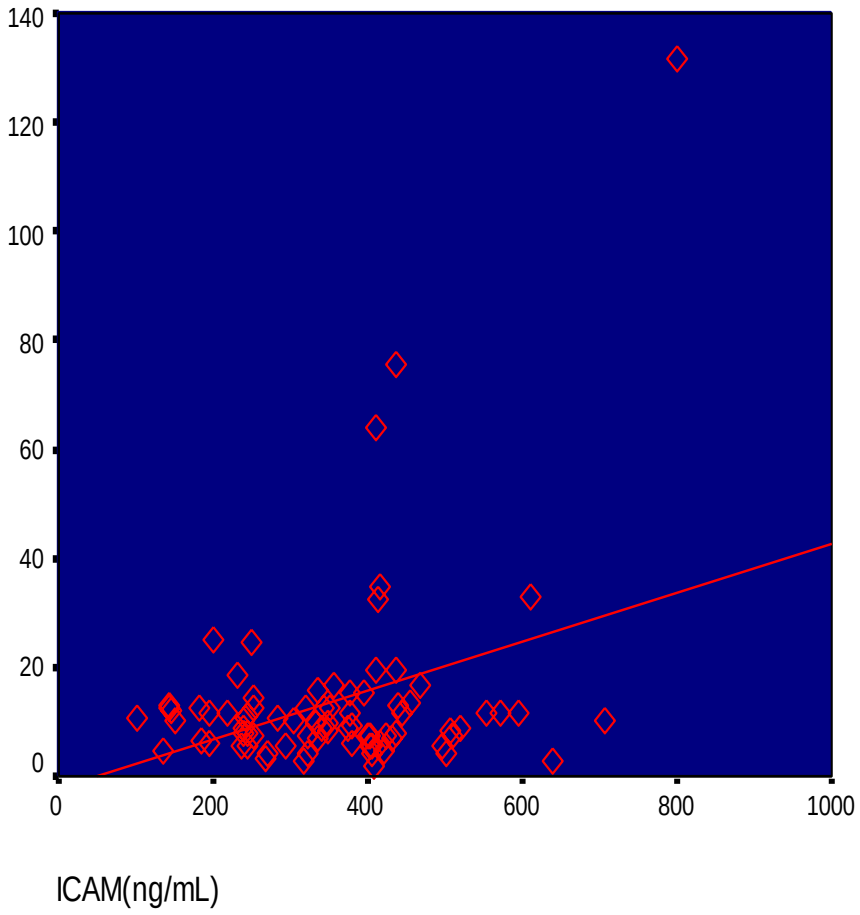


Fig 32. Graph showing correlation between ICAM-1 and fasting insulin

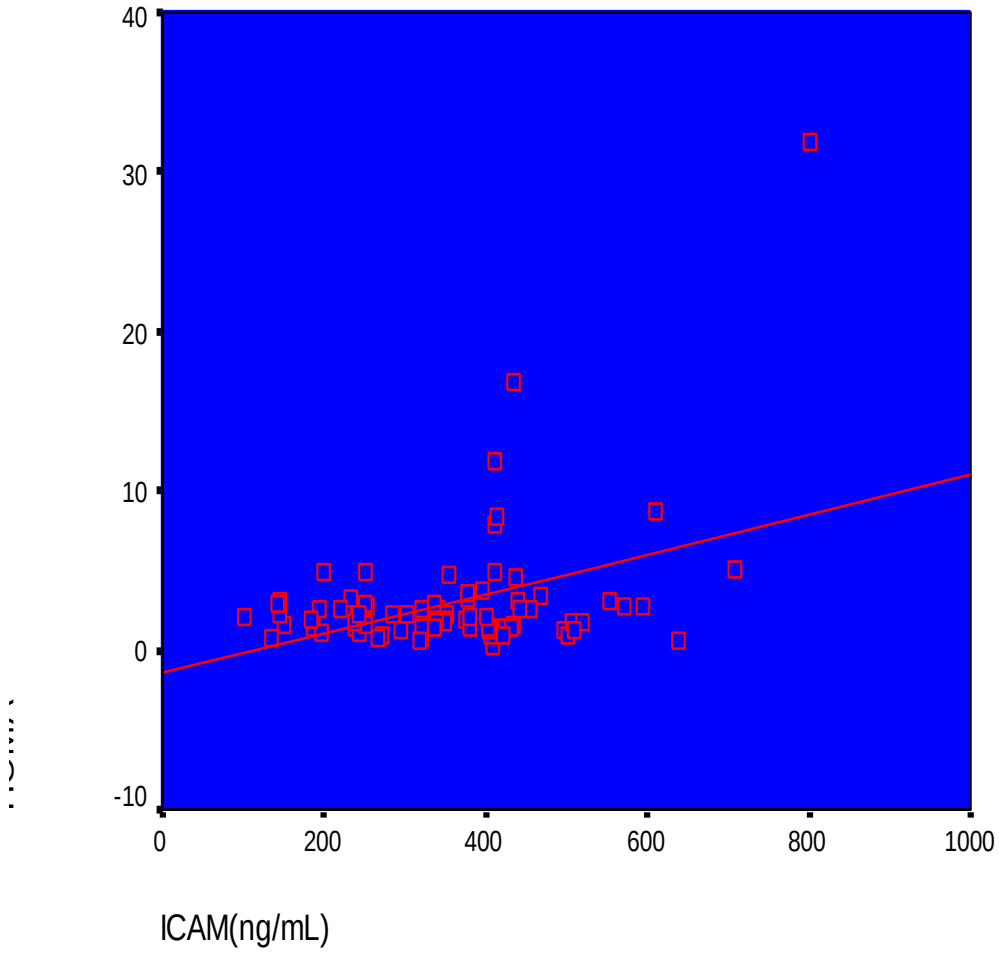


Fig 33. Graph showing correlation between ICAM-1 and HOMA-IR

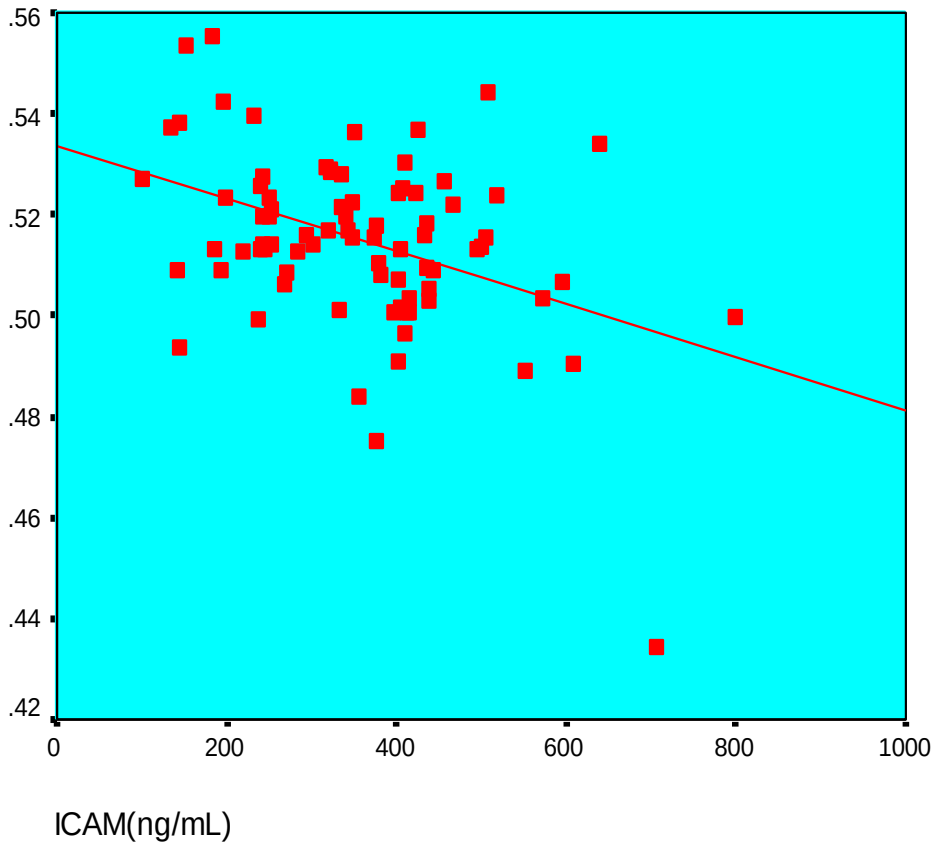


Fig 34. Graph showing correlation between sICAM-1 and QUICKI

Table22: Correlation of plasma Intercellular adhesion molecule-1 (ICAM-1) levels with different lipid parameters, procoagulant and inflammatory markers

Parameters	r	p
CHOL (mg/dl)	0.161	0.152
TG (mg/dl)	0.344**	0.002
HDL (mg/dl)	-0.166	0.138
LDL (mg/dl)	0.217	0.052
Factor VIII	-0.239*	0.032
PAI (ng/ml)	0.090	0.422
TNF-α (pg/ml)	o.292**	0.008
MCP-1 (pg/ml)	0.103	0.362

No statistically significant correlation was observed between markers (PAI-1 TNF-α, MCP-1, factor VIII and s-ICAM-1) and rest of anthropometric, hormonal and biochemical parameters as shown in tables above.

Chapter 6
DISCUSSION

PCOS is characterized by hyper androgenic manifestation like acne, hirsutism and chronic anovulation and is associated with much metabolic derangement such as hyperlipidemia, hyperinsulinemia, insulin resistance and type 2 diabetes. Inflammation has been implicated as an important etiological factor in the development of both insulin resistance and type 2 diabetes mellitus in PCOS. For most of the clinician's first line of treatment of PCOS is OCP's. Although OCP's seem to be an efficient mode of therapy for hyper androgenic symptoms associated with PCOS but their possible negative effects on insulin metabolism, glucose metabolism, lipid metabolism, blood coagulation, and inflammation should be taken into consideration. To know this our study aimed to evaluate the proinflammatory and procoagulant markers in drug naive and OCP treated women with PCOS. To help us to answer the question of safety of using OCP's as conventional treatment of PCOS and if monitoring is required to estimate the risk of CVD in PCOS women treated with OCP's.

Our results demonstrated that treatment with OCP's (E+P) is associated with significant improvement in FG score (androgenic hair growth), acne vulgaris and regularization of menstrual cycles. In agreement with our findings previous studies showed that OCP's induce predictable cyclic menses, reduce luteinizing hormone secretion and lower ovarian androgen production (154); the estrogen component increases SHBG, thus reducing free androgens (Ehrmann,2005) OCP's have been shown to reduce inflammatory acne counts by 30–60% with improvement in 50–90% of the subjects (James, 2005) (160). Besides the progestin component protects the endometrium from hyperplasia.

The present study showed statistically insignificant, small increase in anthropometric parameters like weight, BMI (Kg/m^2) and waist hip ratio in PCOS women in the OCP's arm as compared to drug naive PCOS arm. Few studies evaluating body composition during OCP treatment, showed no

significant change in body weight or body fat (214-216). Stachenfeld NS et al (1998) showed that OCP's can lead to significant body fluid retention (217). Our results suggest insignificant weigh gain in OCP users which shows negative effect of OCP's on body composition of PCOS women. We observed small elevation in systolic BP (mm Hg) in OCP treated PCOS group compared to drug naive patients which is in agreement with most of published studies in normotensive women (218). A review of 2 studies found an increase in systolic blood pressure by 7 to 8 mm Hg on average compared with systolic blood pressure in those not using OCP's (219,220). A study on 80 healthy women randomized into groups of 3 mg of drospirenone combined with a 30-, 20-, or 15-µg dose of equine estrogen (EE) found that systolic blood pressure at 6 months fell by a range of 1 to 4 mm Hg across the groups, compared with an elevation of blood pressure of 4 mm Hg in the control group of Levonorgestrel (LNG)/EEs (221). One of the study showed newer progestins such as drospirenone produce lower blood pressure (222). One study conducted by Oelkers W. K. H. et al, (1996) reported estrogen in very high doses, causes hypertension (223) which is in agreement with our study. Previous studies reported side effects of using OCP's, such as headache, nausea, breast tenderness, and weight gain (224-227) which were very minimal in our study.

We observed increase in total cholesterol, LDL cholesterol, and insignificant increase in triglyceride & HDL cholesterol levels with OCP's when compared to the drug naive PCOS patients. Previous studies have shown increase in total cholesterol and TG following OCP treatment which is in agreement with our study (Hennekens CH, 1979) (228). Ibanez and de Zegher (2004) showed that abnormal adipocytokines, hypertriglyceridaemia and body adiposity became worse in a group of adolescents and young women given a drospirenone pill's (229). IIana J. Halperin (2010) also reported OCP use is

significantly associated with an increase in HDL-C and TG (230) as was shown by Costello et al on comparing OCP with metformin where significant increase in triglycerides in the OCP group occurred as compared to metformin in women with PCOS (231). George Mastorakos et al (2002) in a study reported combined oral contraceptives were associated with an increase of total cholesterol, LDL cholesterol, and HDL cholesterol levels and no change of the total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios (232). All these findings suggest OCP use is associated with elevation of Coronary risk as indicated by elevation of total and LDL cholesterol. However some authors showed that there were no detrimental effects of transdermal hormone replacement therapy (HRT) on lipid profile, glucose metabolism, CRP and urine protein levels in post menopausal women with type 2 diabetes and hypertension (233).While some studies have reported OCP's ameliorate the abnormal metabolic profile of women with PCOS (234). OGTT results showed insignificant increase in fasting plasma glucose, fasting insulin (µ IU/ml), insulin 2hr (µIU/ml), FGIR and HOMA-IR levels of PCOS patients treated with OCP's when compared to the drug naive PCOS patients. Previous studies reported that OCP's deteriorate glucose tolerance. One of the earliest prospective studies on the effect of OCP on carbohydrate metabolism in the general population was performed by Wynn and Doar (1969), they reported both oral and intravenous glucose tolerance area under the curve (AUC) deteriorated in 78 and 70% of the women respectively, and 13% developed chemical diabetes during OCP therapy. Significant elevations of plasma insulin after both oral and intravenous glucose were also observed which accelerate the rate of development of clinical diabetes and also of atherosclerosis (235). A prospective study by Rimm et al, in 1992 showed 10% greater risk of type 2 DM in past users of OCPs albeit with high-dose estrogen (236). Many authors (Korytkowski et al, 1995; Morin-Papunen et al, 2000; Cagnacci et al, 2003; Palep-Singh et al, 2004; Vrbikova et al, 2004)

demonstrated deterioration in carbohydrate metabolism on using OCP's (237-241). Development of frank diabetes in OCP users was also reported by Nader et al, 1997 (242). Some studies (Falsetti and Pasinetti, 1995; Armstrong et al., 2001; Cibula et al., 2002; Elter et al., 2002; Morin-Papunen et al., 2003) reported no change in carbohydrate metabolism after OCP use (243-247). Chasen-Taber et al, 1997 showed no significant increase in risk with low-dose pills (248) and Troisi et al, 2000 showed that past and present users did not differ from never users in glucose, insulin, C-peptide and haemoglobin A1C concentrations (249). Some studies (Pasquali et al, 1999; Escobar-Morreale et al, 2000; Cagnacci et al, 2003) have shown OCP use resulted in improvement in carbohydrate metabolism (250-252). Thus, the range of studies have shown conflicting results with OCP use but majority of data on insulin resistance and glucose intolerance favours our results suggesting a negative metabolic effect of OCP's.

As expected we observed decrease in total testosterone and serum LH levels in PCOS patients treated with OCP's compared to drug naive PCOS patients. Significant decrease in FSH was also observed in PCOS patients treated with OCP's compared to drug naive PCOS patients. In agreement with our observations Ehrmann DA et al (2005) showed that estrogen component (ethinyl estradiol) of OCP's has a role in suppression of FSH, stabilization of endometrium, potentiating of progestin action, suppression of dominant follicle formation, increase in sex hormone binding globulin and decrease in free androgen (154). Balen A et al 2001 suggested that progestin component has a role in suppression of LH, inhibition of LH surge, unreceptive endometrium, hostile cervical mucus, decrease in ovarian androgen secretion and androgen blocking effect (155). Although the effect in our study was marginal, most of times statistically insignificant, a larger number of subjects could have made the results more robust.

The present study showed a significant increase in serum sICAM-1 levels in OCP treated PCOS patients compared to drug naive PCOS patients. The marker also showed a positive correlation with markers of metabolic dysfunction and insulin resistance in women with PCOS. Previous studies related to sICAM-1 in PCOS showed significantly higher levels of sICAM-1 in PCOS group than in healthy group (253). Another study demonstrated that endothelial dysfunction coexists and is influenced by presence of increased serum levels of inflammation and endothelial activation markers (ET-1, s-ICAM, s-VCAM, hs-CRP) in young women with PCOS (254). Although OCP group had higher concentration than non-OCP users, we didn't have a healthy control group to compare the results. Nasiek M et al (2004) showed higher concentrations of sICAM-1 in women with PCOS suggesting a higher risk for cardiovascular diseases in this group (255) the levels have been shown to correspond to higher BMI, waist hip ratio and serum testosterone levels as in our study (256). Gonzalez F et al (2009) demonstrated higher IL-6, sICAM-1, CRP, PAI-1, systolic and diastolic blood pressures, triglycerides, fasting insulin, and HOMA in women with PCOS compared with weight-matched controls, and the highest levels in the obese regardless of PCOS status (257). In our study we demonstrated that ICAM-1 levels were significantly higher in subjects with increased waist circumference indicating role of visceral obesity in chronic low grade inflammation in insulin resistance states like PCOS and metabolic syndrome. Our Study also revealed highly significant correlation between ICAM-1 and fasting plasma glucose, post glucose load plasma glucose and novel markers of insulin resistance (HOMA-IR) again pointing towards role of endothelial dysfunction in insulin resistance both at hepatic as well as at peripheral level. Our study showed positive correlation (statistically significant) with fasting insulin, TG which is in favor of J Vrbikova et al (67) who demonstrated positive correlation of sICAM-1 with fasting and stimulated insulin and TG. Our study showed positive and statistically

significant correlation of sICAM-1 with TNF-α. Our findings may suggest possible role of chronic low grade inflammation mediated by sICAM-1 and other inflammatory markers in defective pancreatic insulin secretion besides their established role in mediating peripheral insulin resistance. Our study also showed that the treatment of PCOS subjects with OCP's worsen the already elevated levels of sICAM-1 in these patients but no data is available till date studying the effect of OCP treatment on sICAM-1 level in women with PCOS. The elevated sICAM-1 levels in OCP group in our study indicate elevated inflammatory response.

MCP-1 is an adipokine with insulin-resistance-inducing capacity that is related to increased adipose tissue mass in obesity and insulin resistance. It is thus a therapeutic target, and may represent an important factor linking adipose tissue inflammation, obesity and type 2 diabetes (258). In our study mild increase in MCP-1 levels in OCP treated PCOS women was observed although it did not reached statistical significance. MCP-1 showed significant and positive correlation with blood glucose fasting and negative correlation with QUICKI. No significant correlation was found between MCP-1 and other metabolic and insulin resistance markers in our study. Previous studies related to MCP-1 in PCOS by Glintborg D et al in 2009 showed that hirsute patients had significantly increased MCP-1 than controls of matched body composition. In PCOS, MCP-1 correlated positively with central fat mass (259). Recent data from Weihong Hu et al, 2011 in both obese and non-obese PCOS showed higher serum MCP-1 levels than controls and correlated positively with BMI, LH ,TG, Apo lipoprotein B and the ratio of Apo lipoprotein A/ Apo lipoprotein B (260). Serum CRP and MCP-1 levels were significantly higher in women with PCOS compared with controls (261). There is no data reported related to the effect of OCP's on MCP-1 in women with PCOS. Elevated MCP-1 levels in OCP group in our study indicated proinflammatory behavior of OCP's.

TNF-α an inflammatory cytokine (40) and is responsible for the expression of adhesion molecules such as VCAM-1 and ICAM-1 in the endothelium via NFκB (nuclear factor kappa B) pathway (41). In this study statistically insignificant increase in TNF-α levels in OCP treated PCOS women was found compared to the drug naive PCOS women. Previous studies showed increased levels of TNF-α in PCOS women as compared to controls. Ilhan Tarkun et al (2006) also studied TNF-α and IL-6 levels in women with PCOS and demonstrated that TNF-α and IL-6 concentrations were elevated in normal weight women with PCOS (262). Interestingly, lean PCOS women had higher TNF-α levels than normal lean women while the levels were similar in obese PCOS and obese controls (Gonzelez et al) (42). Contrarily Sayin NC et al (2003) found similar TNF-α levels in patients with PCOS and with PCO; however, there was no correlation between the TNF-α and insulin, glucose and androgen levels in the study (263). Again we didn't have a healthy control group to compare this part of observation. Consequently our results may suggest that the treatment of PCOS subjects with OCP's may worsen the already elevated levels of TNF-α indicating proinflammatory behavior and negative role of OCP's.

PAI-1 is mainly produced by the endothelium (cells lining blood vessels), but is also secreted by other tissue types, such as adipose tissue. PAI-1 inhibits the serine proteases tPA and uPA / urokinase, and hence is an inhibitor of fibrinolysis, the physiological process that degrades blood clots. In this study statistically insignificant increase in PAI levels in OCP treated PCOS women was found compared to the drug naive PCOS patients which suggests that OCP treatment elevates PAI levels in women with PCOS. In this study PAI showed statistically significant and positive correlation with blood glucose one hour, blood glucose two hour, insulin two hour and triglycerides. Previous studies related PAI levels in PCOS showed that plasma PAI-1 levels are elevated in overweight /obese women with PCOS but not in normal weight

women compared with BMI matched controls and that PAI-1 level are similar in the different phenotypes of the syndrome (264). In agreement with our findings higher PAI-1 levels in normal weight women with PCOS were reported and a correlation between PAI-1 levels and both serum insulin levels and the HOMA-IR index was demonstrated (265-267). Our study suggests that OCP's may have negative impact on the existing elevated procoagulant activity in PCOS women. However this effect seems mild in the present study. No work has been done on the effect of OCP's treatment on the PAI-1 levels in PCOS till date. In contrast many smaller studies did not identify any difference in circulating PAI-1 levels between normal weight women with PCOS and BMI-matched healthy controls (268-270).

In our study on comparison of factor VIII levels between the drug naive PCOS women and PCOS women treated with OCP's, a significant decrease was found in OCP users. Factor 8 showed significant, negative correlation with fasting insulin, HOMA-IR and ICAM. No significant correlation was obtained between factor VIII and other metabolic parameters in our study. This result is conflicting as it does not fit the paradigm of other coagulation markers like PAI-1 and inflammatory markers. Previous studies have shown that OCP's seem to have no effect on factor VIII levels (101,102). Robinson GE et al (1990) showed women taking the combined pill showed increases in fibrinogen and factor X and a reduction in anti thrombin III when compared with their control values. There were also small but significant increases in triglycerides and triglyceride-rich lipoproteins (188). Quehenberger P et al (1996) studied the effect of OCP treatment on selected factors involved in the activation i.e. circulating activated factor VII (cFVIIa), and in the inhibition of blood coagulation, i.e. plasma protein S activity and circulating thrombomodulin (cTM), these factors were measured for the first time in OCP users in a prospective study. They reported the increased level of activated factor VII and decreased plasma protein S activity and circulating

thrombomodulin during use of OCP's (192). Van Rooijen M et al (2002) compared the effects of two different combined OCP on levels of plasma lipoproteins and coagulation factor VII. A significant rise in plasma triglyceride levels was obtained with both preparations, although the increase was more pronounced with ethinyl estradiol/desogestrel. Plasma concentrations of factor VII and activated factor VII were increased significantly only with ethinyl estradiol/desogestrel (195).Theoretically speaking and from the existing data factor VIII levels should be elevated with use of OCP's as they are known to elevate procoagulant activity. At this moment we don't seem to have an explanation for this finding which needs to be evaluated in future studies.

In conclusion the present study was a pilot; it is the first attempt to investigate the role of OCP's (E+P) on the worsening of metabolic abnormalities, insulin resistance, pro-inflammatory and procoagulant factors. Although OCP's are commonly prescribed medications in women with PCOS and have significant benefit in clinical abnormalities, there is concern regarding their negative impact on the metabolic abnormalities. Our aim was address this concern. Our study findings imply that although OCP treatment helps in menstrual cyclicity and decreasing hyperandrogenism in women with PCOS, metabolic parameters such insulin resistance indices, glucose tolerance and lipid profile of PCOS subjects worsens with their use. In line with objectives of the present study we observed elevation of plasma concentrations of procoagulant (PAI-1) and proinflammatory (s-ICAM,MCP-1,TNF-α) markers in PCOS subjects treated with OCP's. Interestingly our data did not suggest elevation of factor VIII levels which looks intriguing. We don't have any apparent explanation for this contrary result at this moment and the finding needs to be looked at closely and in a systematic way to explain it. Although, we did not have a control group to see the pre-existing procoagulant and proinflammatory activity in PCOS women, our data suggests that OCP group had unfavorable

profile as compared to full blown PCOS women who were not treated. This indicates that E+P use in PCOS women can be viewed as unsafe and their routine use may be avoided. Larger controlled clinical trials with long term OCP use with graded doses with an arm of healthy control women on a larger cohort and more detailed parameters of coagulation and inflammation with longitudinal follow up will likely answer the question.

Chapter 7
CONCLUSION

We studied 81 PCOS women diagnosed on the basis of Rotterdam 2003 Criteria. Women who were not treated with any drug (controls, n=51) and PCOS women treated with OCP's (cases, n=30) were recruited from various gynecological clinics. The women who consented and qualified the above criteria were subjected to detailed clinical, anthropometric, hemodynamic, hormonal and metabolic evaluation. In addition to OGTT derived insulin indices plasma levels of s-ICAM, MCP-1, TNF-α, PAI-1 and were measured using ELISA kits and factor VIII levels were measured using Coagulometric method. The results are briefly presented as follows:

1) The mean age of controls and cases was (21.92±5.83 vs. 21.63±4.18 years) respectively and was comparable (p=0.813).

2) Similarly the mean age at menarche for controls and cases being (12.96±1.49 vs.13.13±1.27) years respectively and was comparable (p=0.597).

3) Mean number of menstrual cycles per year for controls and cases was similar (9.12±3.88 vs.9.90±3.30) respectively which was comparable (p=0.358) but cycles were regular in OCP group.

4) Mean FG score of the controls and cases was (12.27±4.71vs. 10.00±2.60) was statistically significant (p=0.017).

5) Mean weight of controls and cases was (58.57±8.52 vs. 59.07±6.34 kg) indicating an insignificant weight gain in OCP group (p=0.782). Mean BMI (Body mass index) of controls and cases was (23.66±3.43 vs. 24.07± 3.42 Kg/m^2) respectively (p=0.606) which was marginally high in OCP group. Mean waist circumference of controls and cases was (79.96±10.15 vs. 78.20±8.49 cm) respectively (p=0.427). Mean

waist hip ratio for controls and cases was (0.91±0.06 vs. 0.92±0.06) respectively which was comparable (p=0.544).

6) Mean of systolic blood pressure in controls and cases was (122.24±6.99 vs. 123.00±7.21 mm Hg) which was comparable (p=0.640). Mean diastolic blood pressure in controls and cases was (79.37±4.62 vs. 79.46±4.29 mm Hg) which was comparable (p=0.928) which showed an insignificant trend towards elevation in OCP group.

7) Mean fasting blood glucose in control and cases who participated in our study was (87.75±19.9 vs. 88.77±10.41 mg/dl) respectively which was comparable (p=0.795) which showed an insignificant trend towards elevation in OCP group.

8) Mean serum cholesterol level among controls and cases was (155.07 ±28.86 vs. 186.10±44.76 mg/dl) respectively which was statistically significant (p=0.000). Mean serum triglyceride levels in controls and cases was (113.00±46.47 vs. 118.17±41.14 mg/dl) respectively which was comparable (p=0.616). Mean HDL cholesterol in controls and cases was (45.35±9.99 vs. 48.90±11.90 mg/dl) which was comparable (p=0.155). Mean LDL value in controls and cases was (83.20±27.6 vs. 118.45±45.66 mg/dl) respectively which was statistically significant (p=0.000). So OCP use is associated with elevation of Coronary risk as indicated by elevation of total and LDL cholesterol.

9) Mean fasting insulin levels in controls and cases were slightly different (12.28±11.10 vs. 16.23±24.72 μIU/ml)) respectively which was comparable (p=0.326) indicating insulin resistance in the OCP group and thereby suggested a negative metabolic effect of OCP's. Insulin two hour in controls and cases was found to be (43.43±36.07 vs.

65.69±63.67µIU/ml) respectively (p=0.48) showing insignificant elevation in OCP group. FGIR (Fasting glucose to insulin ratio) in controls and cases was found to be (10.03±5.34 and 11.60±9.3) respectively which was comparable (p=0.336).

10) HOMA-IR in controls and cases was found to be (2.73±2.64 vs. 3.0±4.17) respectively which was comparable (p=0.335) indicating again impaired sensitivity. QUICKI (Quantitative Insulin Sensitivity Check Index) in controls and cases was found to be (0.516±0.01 vs. 0.513±0.013) respectively which was comparable (p=0.449). FGIR (Fasting glucose to insulin ratio) in controls and cases was (10.03±5.34 & 11.60±9.3) respectively which was comparable (p=0.336)

11) Mean LH value of controls and cases was found to be (8.17±6.14 vs. 6.75±6.26 IU/L) respectively which is comparable (p=0.322). Mean FSH value in controls and cases was found to be (6.17±2.21 vs. 4.88±2.17 IU/L) which is statistically significant (p=0.013) suggesting that OCP's may decrease gonadotrphins.

12) Mean testosterone levels of controls and cases were found to be (63.31±31.30 vs. 56.05±31.81 ng/dl) (p=0.320) respectively which suggests antiandrogenic effect of OCP's.

13) Mean sICAM-1 values in controls and cases were found to be (312.41±131.65 vs. 417.03±131.65 ng/ml) respectively and was statistically significant (p=0.001). The elevated ICAM-1 levels in OCP group indicate elevated inflammatory response.

14) Mean MCP-1value in controls and cases was found to be (456.78±187.2 vs. 464.82 ± 91.19 pg/ml) (p=0.827) which shows worsening of inflammation in the OCP group. Similarly TNF-α levels in controls and cases were found to be (22.85±5.19 vs. 25.60±4.24 pg/ml) which is

statistically significant (p=0.016) again indicating proinflammatory behavior of OCP's.

15) PAI-1 is a key regulator of fibrinolysis and inhibits the activation of plasminogen to plasmin by both the tissue-type and the urokinase-type plasminogen activators. In addition, PAI-1 is a marker of insulin resistance (IR) and plasma PAI-1 antigen levels and activity are frequently elevated in insulin resistant states, including abdominal obesity, the metabolic syndrome and T2DM. Several prospective studies in the general population suggested that elevated PAI-1 levels are associated with increased risk for T2DM.Given the increased prevalence of insulin resistance in women with PCOS and the association between circulating PAI-1levels and IR, several studies assessed plasma PAI-1 levels in PCOS. Most, but not all, reported elevated plasma PAI-1 levels in women with PCOS. Mean PAI -1 levels in controls and cases were found to be (1.05±0.40 vs. 1.10±0.59 ng/ml) which is comparable (p=0.682).This suggests that OCP's may have negative impact on the existing elevated procoagulant activity in PCOS women. However this effect seems mild in the present study.

16) Mean plasma factor VIII levels in controls and cases was found to be (0.685±0.32 and 0.350±0.33) which is statistically significant (p=0.00). Theoretically speaking and from the existing data factor VIII levels should be elevated with use of OCP's as they are known to elevate procoagulant activity. Interestingly the results from our study seem to be conflicting.

In conclusion the present study was a pilot; it is the first attempt to investigate the role of OCP'S (Estrogen + Progesterone) on the worsening of metabolic abnormalities, insulin resistance, proinflammatory and procoagulant factors. Although OCP's are commonly prescribed medications in women with PCOS and have significant benefit in clinical abnormalities, there is concern regarding

their negative impact on the metabolic abnormalities. Our aim was address this concern. Briefly short term use of OCP use in women with PCOS suggest worsening of already existing insulin resistance and glucose tolerance abnormalities although effect seems to be mild. Although, significant fall in serum testosterone translates into many clinical benefits such as regularization of menstrual cycles, improvement in acne and other androgenic features, some unwanted abnormalities surfaced. OCP use was associated with elevated coronary artery disease risk factors such as total and LDL cholesterol levels. Similarly the elevated anti-fibrinolytic factor such as PAI-1 portends a high coronary risk. The procoagulant effect as estimated by serum factor VIII levels showed a fall in OCP users which cannot be explained. The proinflammatory markers (TNF-α and MCP-1) showed elevated levels after 3-6 month use of OCP suggesting worsening of inflammatory state. Larger controlled clinical trials with long term OCP use with graded doses need to understand the impact of OCP use on various biochemical, inflammatory and procoagulant state in the PCOS women.

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