Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 2907–2915

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Assessment of PON1 activity and circulating TF levels in relation to BMI, testosterone, HOMA-IR, HDL-C, LDL-C, CHO, SOD activity and TAC in women with PCOS: An observational study

Humira Jeelani [a, b], Mohd Ashraf Ganie [c], Akbar Masood [b], Shajrul Amin [a, b], Iram Ashaq Kawa [a, b], Qudsia Fatima [a, b], Saika Manzoor [a, b], Tabasum Parvez [d], Niyaz Ahmad Naikoo [e], Fouzia Rashid [a, b, *]

[a] Department of Biochemistry, University of Kashmir, Srinagar, Jammu and Kashmir, India
[b] Department of Clinical Biochemistry, University of Kashmir, Srinagar, Jammu and Kashmir, India
[c] Department of Endocrinology, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India
[d] Department of Obstetrics and Gynecology, Trust Hospital, Sher-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India
[e] Department of Biotechnology, Govt College for Women, M A Road, Srinagar, Jammu and Kashmir, India

ARTICLE INFO

Article history:
Received 22 July 2019
Accepted 1 August 2019

Keywords:
Polycystic ovary syndrome
Inflammation
Paraoxonase1
Tissue factor
Oxidative stress

ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is the most common female endocrinopathy among premenopausal women associated with hyperandrogenism, obesity, dyslipidemia, insulin resistance and inflammation. Oxidative stress is an important component of cardio-metabolic risk seen in PCOS.
Material and methods: A total of 95 women with PCOS and 95 healthy controls were included in this observational study. Serum PON1 activity and stress markers were measured by spectrophotometric methods. Circulating TF level was measured by ELISA.
Results: We found decreased PON1 activity and increased TF levels in women with PCOS compared to healthy controls. Fasting insulin, HOMA-IR, testosterone, LDL-C, MDA, PC and SOD activity were significantly increased whereas FGIR, QUICKI, HDLC, CAT and TAC were significantly decreased in PCOS women than controls. We observed a positive association of PON1 activity with FGIR, QUICKI, HDL-C and TAC, and its negative association was observed with LH, testosterone, fasting insulin and HOMA-IR in PCOS women. We further observed a positive association of TF with waist, waist to hip ratio, BMI, glucose 1hr, cholesterol, LDL-C, SGPT, uric acid and SOD activity in PCOS women.
Conclusions: Decreased PON1 activity and raised circulating TF levels are respective indicators of pro-inflammatory and procoagulant status in PCOS women. The imbalanced oxidant/antioxidant status further supports the evidences that PCOS is an oxidant state. Further, the association of PON1 activity and TF levels with the clinical, laboratory findings and stress marker levels suggest that these factors taken together are involved in aggravating the pro-inflammatory status in PCOS women.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common heterogeneous female endocrine and metabolic disorders affecting 18% of women of reproductive age [1]. This disorder is characterized by hyperandrogenism, chronic oligo- or anovulation and involves a broad spectrum of metabolic alterations such as glucose intolerance, insulin resistance, dyslipidemia, obesity, systemic low-grade inflammation, clinical and subclinical atherosclerosis, impaired fibrinolysis and increased oxidative stress [2–4]. There is increasingly growing research on the involvement of inflammatory mechanisms and coagulatory disturbances in the development of insulin resistance and hyperandrogenism in PCOS [5].

Paraoxonase1 (PON1) is a calcium dependent HDL-associated antioxidant/anti-atherogenic enzyme. It is mainly synthesized by the liver and is widely distributed in many tissues including liver, brain, lungs, heart, kidneys, small intestines and aorta [6]. It has aryl-esterase and lactonase activity. The enzyme hydrolyses various

* Corresponding author. Department of Clinical Biochemistry, University of Kashmir, Srinagar, Jammu and Kashmir, India.
E-mail address: rashid.fouzia@gmail.com (F. Rashid).

https://doi.org/10.1016/j.dsx.2019.08.001
1871-4021/

organophosphorus insecticides and plays an important role in protection from xenobiotic toxicity. It also hydrolyses lipid peroxides, lactones, homocysteine thiolactones, inhibits LDL oxidation and also increases macrophage associated cholesterol efflux capacity which are considered as an important cardio-protective functions of PON1 enzyme. Therefore PON1 contributes to the antioxidant, anti-inflammatory and anti-atherogenic properties of HDL particle [7]. Earlier studies have reported that reduced PON1activity is associated with increased oxidative stress and insulin resistance in human serum. Epidemological studies have reported decreased PON1 activity to be an independent risk factor for atherosclerosis. Decreased serum PON1 activity has also been reported to be associated with many inflammatory diseases including rheumatoid arthritis, psoriasis, muscular degeneration, systemic lupus erythromatosis, renal and hepatic diseases, renal failure, Parkinson's disease and also with cancers including breast cancer [8].

In addition to PON1, coagulation and fibrinolysis disturbances are the risk factors of inflammation and atherosclerosis [9]. Several studies have reported that women with PCOS have dysregulated haemostatic and coagulatory mechanisms which points towards prothrombotic state, including hypofibrinolysis, hypercoagulability, and endothelial and platelet dysfunction. Women with PCOS have increased fibrinolytic activity and a higher prevalence of coronary artery calcification reflecting an increased coagulation tendency. There is evidence that plasma levels of haemostatic factors are modulated by factors such as hyperglycemia, insulin resistance, obesity, proinflammatory agents and dyslipidemia all of which are typical features of PCOS [10]. In one of our previous studies we also observed significantly deranged haemostatic parameters in PCOS women compared to healthy controls [11]. Insulin resistance one of the key features of PCOS is associated with accelerated capacity of proinflammatory and coagulatory markers [12]. The mechanisms of potential disturbances of the haemostatic system in women with PCOS are unknown and recent studies are primarily focused on the components of the coagulation and fibrinolytic pathways. Tissue factor (TF) one of the principal components of coagulation cascade is the receptor for coagulation factor VII, and is the principal initiator of blood coagulation which induces thrombin generation to promote fibrin formation and platelet activation. TF is a procoagulant glycoprotein that resides in the cellular membranes of monocytes, macrophages, and endothelial cells, and is also produced by foamy macrophages and activated vascular smooth muscle cells within atherosclerotic plaque. It is known that spontaneous plaque rupture exposes TF to circulating blood which in turn triggers activation of the coagulation cascade, platelet aggregation, clot formation, and ultimately results in blood vessel occlusion and generation of atheromatous plaques [13]. Inflammatory stimuli such as TNF-α, oxidized low density lipoprotein (ox-LDL), hyperglycemia, hyperinsulinemia, advanced glycation end products- AGE and reactive oxygen species (ROS) are capable of stimulating increased production of TF. Increased TF levels have been found in patients with hypertension, insulin resistance, diabetes mellitus, inflammation, atherogenesis and in various cancers [14–16]. Thus it was considered imperative to evaluate TF levels along with PON1 activity in PCOS women vs. healthy controls to find out their association with the clinical and laboratory findings of PCOS women.

Besides, there are ongoing efforts to study the involvement of oxidative stress (OS) as a baseline parameter in aggravating proinflammatory status. OS is highly associated with cumulative damage done in the body by free radicals and it adversely affects cell survival through the oxidative damage of lipids and proteins [17]. Lipid peroxidation products such as thiobarbituric acid reactive substances (TBARS) and products of protein oxidation such as protein carbonyls (PC) are considered as markers of oxidative damage [18,19]. Furthermore, oxidative damage is aggravated by the decrease in body's antioxidant defense mechanisms such as superoxide dismutase (SOD) and catalase (CAT) activities which acts as free radical scavengers in conditions associated with oxidative stress. Total antioxidant capacity (TAC) is sensitive to changes in plasma antioxidant levels and degree of insulin resistance. There is a growing interest in the protective effects of antioxidants and an imbalance in the antioxidant protective mechanism is being identified as a common factor in inflammatory diseases [20]. Therefore, along with PON1 activity and circulating TF levels, this study also aims to evaluate the oxidant/antioxidant status in PCOS women vs. controls to shed light on the role of OS as a co-mediator of inflammation and coagulation tendencies.

2. Materials and methods

The study was approved by the Institutional Board of Research Studies (BORS) and written informed consent was obtained at the study entry from all subjects. All the PCOS women belonged to Department of Endocrinology/Obstetrics and Gynecology, Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Kashmir. Controls were recruited from various medical screening camps organized at various colleges and at university of Kashmir.

2.1. Subjects

A total of 95 women with PCOS (mean age 25.3 years) and 95 sex-matched healthy control subjects (mean age 24.8 years) were enrolled in the study. The diagnosis of PCOS was based as proposed at the Rotterdam Consensus meeting [1]. Exclusion criteria included: hypertension, smoking, pregnancy, hyperprolactinemia, thyroid and adrenal dysfunction, diabetes mellitus, Cushing's syndrome, coronary artery disease, vascular disease, inflammatory diseases and drugs such as oral contraceptive pills and anti-diabetic agents. The healthy controls represented apparently normal women having regular menstrual cycles, displaying no evidence of clinical/biochemical hyperandrogenism, and having normal ovarian morphology on trans-abdominal ultrasonography.

2.2. Anthropometric and systemic examination

A full physical examination was performed including assessment of weight, height, waist and hip circumferences, and detailed systemic examination. Weight was measured in light clothing without wearing shoes and height was measured using a Stadiometer. BMI was calculated by the formula: weight/(height)^2 (kg/m^2). Waist circumference (WC) was measured while the patient was made to stand at a point midway between the lower costal margin and iliac crest in the mid-axillary. Blood pressure was measured manually or with a digital sphygmomanometer. Hirsutism assessment was evaluated using modified Ferriman-Gallwey score by counting hair growth at nine specified body areas. A score of >8 out of a total of 36 was taken as significant. All subjects underwent physical examination and were asked to complete a general questionnaire and gave informed consent before the onset of study.

2.3. Chemicals

All chemicals used were from Sigma-Aldrich and were of analytical grade or the highest grade available.

2.4. Sampling and biochemical analysis

Venous blood samples were collected from an antecubital vein, between 08:00 and 09:00 a.m., after an overnight fast and the samples were centrifuged, aliquoted and then immediately frozen at −80 °C until further analysis. Glucose concentrations were assayed immediately. The levels of glucose, cholesterol (CHO), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), aspartate transaminase (SGOT), alanine transaminase (SGPT), urea, creatinine and uric acid were measured using commercially available diagnostic kits and assays were performed on semi-automated analyzer (ERBA CHEM 7, Manheim, Germany).

Fasting insulin levels were measured by using ELISA kits and insulin resistance was assessed by means of homeostatic model assessment insulin resistance index (HOMA-IR), fasting glucose to insulin ratio (FGIR) and quantitative insulin sensitivity check index (QUICKI). The HOMA index was calculated as [fasting insulin (μIU/mL) x fasting glucose (mg/dL)]/405. The QUICKI was calculated as 1/[log fasting insulin (μIU/mL) + log fasting glucose (mg/dL)]. High HOMA-IR, low QUICKI and low FGIR scores denote insulin resistance (low insulin sensitivity).

2.5. Hormonal analysis

Serum LH, FSH, Testosterone, TSH, T4, Prolactin (PRL), 17-OHP and cortisol were determined by chemiluminescent immune assay. TSH was done to rule out the presence of hypothyroidism, T4 - to rule out hyperthyroidism and PRL-to rule out prolactinemia. 17 OHP- was done to rule out non classical congenital adrenal hyperplasia and cortisol to rule out cushing's syndrome. The sampling was arranged in such a way so that the sample for LH, FSH, 17-OHP and testosterone was collected on 2nd to 7th day of the follicular phase of either spontaneous or progesterone induced menstrual cycle.

2.6. Estimation of PON1 activity, circulating TF levels and oxidant/antioxidant markers

PON 1 activity in serum was measured according to a method described elsewhere [21]. The rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm and at 25 °C. The basal assay mixture contained 5ul of serum added to 1.0 mM paraoxon and 1.0 mM $CaCl_2$, in 0.05 M glycine buffer (pH 10.5). PON1 activity was defined as 1 nmol of p-nitrophenol formed per minute, and its activity was expressed as U/l of serum.

Enzyme linked immunosorbent assay (ELISA) kits (Ray Bio) were used to measure plasma concentrations of Tissue factor and results were expressed as pg/ml.

Malondialdehyde, an indirect index of lipid peroxidation, was assayed using TBARS method. Results were expressed as nmol/ml of serum [22]. Protein oxidation status was determined with a colorimetric assay that measures protein carbonyl (PC) content after reacting serum with dinitrophenylhydrazine. Results were expressed as nmol/L of serum [23]. SOD activity was determined by the method of Beauchamp and Fridovich. SOD activity was given in U/ml of serum [24]. Catalase activity was estimated by the method of Claiborne. One enzyme unit of catalase was defined as the amount of enzyme which catalyzed the degradation of 1 nmole H_2O_2 per mg protein per minute under assay conditions [25]. Total protein was estimated using Bradford assay for protein estimation. The total antioxidant capacity of serum was assessed by trolox equivalent antioxidant capacity (TEAC) assay. Results were expressed as mmol of Trolox per litre [26].

2.7. Statistical analysis

Statistical evaluation was carried out using SPSS software. Data obtained was expressed as Mean ± SD. The continuous data was analysed by using Student's *t*-test. Correlation analysis between variables was done by using Pearson's test. Regression analysis was also performed and the results were expressed at 5% level of significance i.e $P < 0.05$.

3. Results

The baseline anthropometric and clinical data of PCOS women and healthy controls are given in (Table 1). There was no significant difference in age but other parameters including weight, height, waist circumference, hip circumference, waist to hip ratio, BMI, systolic and diastolic blood pressure, and Ferriman-Gallwey score were found to be increased in women with PCOS, and these differences achieved statistical significance ($P < 0.05$).

Laboratory results of Biochemical parameters, lipid profile and Hormonal parameters are documented in (Table 2) and provide evidence of the spectrum of disease. There was no significant difference between fasting glucose in women with PCOS and healthy controls ($P>0.05$). Compared with healthy controls, women with PCOS had statistically significantly increased 1hr post glucose (Glu), 2hr post glucose, cholesterol (CHO), triglycerides (TG) and LDL-C levels ($P = 0.000$). Fasting insulin (FI) and HOMA-IR index were also found to be significantly increased in PCOS women whereas HDL-C, FGIR and QUICKI were found to be significantly decreased in PCOS women compared to healthy controls ($P = 0.000$). Other metabolic parameters SGPT and uric acid were also found to be increased in PCOS women compared to healthy controls ($P = 0.000$). SGOT, urea and creatinine were found to be slightly increased in PCOS women than healthy controls but these differences did not achieve statistical significance ($P > 0.05$). As anticipated in the PCOS women LH, LH/FSH ratio and testosterone were significantly increased compared with healthy controls ($P = 0.000$). Also, FSH was lower in patients with PCOS compared to the control group but decrease in FSH was not found to be statistically significant ($P > 0.05$).

PON1 activity, TF levels and stress markers are given in table (Table 2). PON1 activity was found to be significantly lower in PCOS women compared to healthy control group ($P = 0.000$). The levels of TF was found to be significantly increased in PCOS women compared to healthy controls ($P = 0.000$). Women with PCOS had significantly higher concentration of MDA (index of lipid peroxidation) and PC (index of protein oxidation status) compared to healthy controls ($P = 0.000$). SOD activity was found to significantly increased in PCOS women whereas CAT activity and TAC was found to be significantly decreased compared to healthy controls ($P = 0.000$).

Correlation of PON1, TF, MDA, PC, SOD, CAT and TAC with the anthropometric, hormonal and biochemical parameters in women with PCOS was also evaluated and is shown in (Table 3). A positive correlation of PON1 activity was observed with FGIR ($r = 0.664$ $P = 0.000$), QUICKI ($r = 0.597$ $P = 0.000$), HDL-C ($r = 0.739$ $P = 0.000$) and TAC ($r = 0.8361$ $P = 0.000$) whereas a negative correlation of PON1 activity was observed with LH ($r = -0.220$ $P = 0.032$), testosterone ($r = -0.814$ $P = 0.000$), FI ($r = -0.688$ $P = 0.000$) and HOMA-IR ($r = -0.617$ $P = 0.000$) (Figs. 1 and 3). TF levels were found to be positively correlated with waist ($r = 0.746$ $P = 0.000$), waist to hip ratio ($r = 0.630$ $P = 0.000$), BMI ($r = 0.284$ $P = 0.000$), glucose 1hr ($r = 0.750$ $P = 0.000$), cholesterol ($r = 0.818$ $P = 0.000$), LDL-C ($r = 0.697$ $P = 0.000$), SGPT ($r = 0.769$ $P = 0.000$), uric acid ($r = 0.684$ $P = 0.000$) and SOD activity ($r = 0.7324$ $P = 0.000$) (Figs. 2 and 3). A positive correlation of

Table 1
Baseline anthropometric and clinical characteristics of PCOS women vs. controls.

Parameters	PCOS women (N = 95)	Healthy controls (N = 95)	P-Value
Age (years)	25.30 ± 4.31	24.8 ± 3.19	0.360
Weight (kg)	61.64 ± 10.27	54.04 ± 8.02	0.000
Waist circumference (cm)	83.35 ± 10.21	76.73 ± 7.16	0.000
Waist/hip ratio	0.93 ± 0.11	0.90 ± 0.07	0.037
BMI (kg/m^2)	25.58 ± 3.99	21.42 ± 3.73	0.000
SBP (mmHg)	124.6 ± 10.09	115.93 ± 10.20	0.000
DBP (mmHg)	77.34 ± 7.82	74.48 ± 6.69	0.008
FG-Score	13.13 ± 2.57	5.04 ± 1.45	0.000

Data was expressed as Mean± SD. The continuous data was analysed by using student's t-test. Abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, Ferriman Gallwey score.

Table 2
Laboratory findings, PON1 activity, TF level and stress markers of PCOS women vs. controls.

Parameters	PCOS women (N = 95)	Healthy controls (N = 95)	P-Value
Blood Glu Fasting (mg/dl)	88.54 ± 10.92	87.24 ± 10.95	0.415
Blood Glu 1 h (mg/dl)	138.40 ± 20.03	128.48 ± 17.98	0.000
Blood Glu 2 h (mg/dl)	108.67 ± 15.70	96.68 ± 13.07	0.000
Insulin Fasting (μIU/ml)	20.87 ± 5.38	9.82 ± 2.73	0.000
HOMA-IR	4.56 ± 1.29	2.11 ± 0.62	0.000
FGIR	4.49 ± 1.16	9.77 ± 3.56	0.000
QUICKI	0.31 ± 0.01	0.34 ± 0.02	0.000
Cholesterol (mg/dl)	185.62 ± 24.52	155.72 ± 19.77	0.000
Triglycerides (mg/dl)	157.90 ± 22.59	109.12 ± 18.32	0.000
HDL-C (mg/dl)	43.87 ± 9.74	48.85 ± 13.37	0.004
LDL-C (mg/dl)	121.03 ± 19.33	92.48 ± 13.73	0.000
SGOT (U/L)	21.46 ± 6.72	20.79 ± 5.72	0.462
SGPT (U/L)	23.34 ± 5.90	15.97 ± 3.53	0.000
Urea (mg/dl)	25.20 ± 6.97	24.16 ± 4.94	0.236
Creatinine (mg/dl)	0.77 ± 0.19	0.76 ± 0.17	0.747
Uric acid (mg/dl)	4.48 ± 1.08	3.16 ± 0.82	0.000
LH (IU/L)	10.39 ± 3.62	5.58 ± 2.06	0.000
FSH (IU/L)	6.072 ± 1.42	6.42 ± 1.79	0.144
LH/FSH	1.80 ± 0.77	0.96 ± 0.53	0.000
Testosterone (ng/dl)	51.95 ± 15.25	30.44 ± 9.15	0.000
Paraoxonase1 (U/L)	159.19 ± 25.80	198.98 ± 22.55	0.000
Tissue Factor (pg/ml)	101.77 ± 23.13	83.12 ± 14.34	0.000
MDA	5.80 ± 1.38	2.43 ± 0.92	0.000
PC	19.16 ± 3.12	14.24 ± 1.70	0.000
SOD	1.93 ± 0.084	1.53 ± 0.082	0.000
CAT	332.16 ± 37.60	365.84 ± 35.51	0.000
TAC	1.30 ± 0.43	1.79 ± 0.57	0.000

Data was expressed as Mean ± SD. The continuous data was analysed by using student's t-test. Abbreviations: PCOS, polycystic ovary syndrome; HOMA-IR, homeostasis model assessment; FGIR, fasting glucose to insulin ratio; QUICKI, quantitative insulin sensitivity check index; HDL-C, HDL-Cholesterol; LDL-C, LDL-Cholesterol; SGOT, aspartate transaminase; SGPT, alanine transaminase; LH, luteinizing hormone; FSH, follicle stimulating hormone; MDA, malondialdehyde; PC, protein carbonyl; SOD, superoxide dismutase; CAT, catalase; TAC, total antioxidant capacity. Results were expressed at 5% level of significance i.e $P < 0.05$.

MDA was observed with testosterone (r = 0.586 P = 0.000), FI (r = 0.600 P = 0.000) and HOMA-IR (r = 0.561 P = 0.000) whereas MDA levels were found to be negatively correlated with FGIR (r = -0.470 P = 0.000), QUICKI (r = -0.470 P = 0.000) and HDL-C (r = -0.535 P = 0.000). A positive correlation of SOD activity was observed with waist (r = 0.638 P = 0.000), waist to hip ratio (r = 0.513 P = 0.000), glucose 1hr (r = 0.614 P = 0.000), cholesterol (r = 0.632 P = 0.000), LDL-C, (r = 0.631 P = 0.000), SGPT (r = 0.628 P = 0.000) and uric acid (r = 0.616 P = 0.000). TAC were found to be positively correlated with FGIR (r = 0.611 P = 0.000), QUICKI (r = 0.547 P = 0.000) and HDL-C (r = 0.616 P = 0.000) whereas as negative correlation of TAC was observed with testosterone (r = -0.673 P = 0.000), FI (r = -0.631 P = 0.000), HOMA-IR (r = -0.558 P = 0.000) and TG (r = -0.316 P = 0.002).

The results were further emphasized by using regression analysis. Regression analysis was carried out with weight, waist, waist/hip, BMI, FG score, LH, LH/FSH, testosterone, insulin, HOMA, FGIR, QUICKI, Glu 1hr, Glu 2hr, CHO, TG, HDL-C, LDL-C, SGPT and uric acid as independent variables, and PON1, TF, MDA, PC, SOD, CAT, TAC as dependent variables in PCOS women. PON1 activity was found to be significantly associated with testosterone (Beta = -0.470 P = 0.000), FI (Beta = 0.665 P = 0.033), FGIR (Beta = 0.630 P = 0.000), HDL-C (Beta = 0.321 P = 0.000) and LDL-C (Beta = -0.161 P = 0.020). TF was found to be significantly associated with waist (Beta = 0.213 P = 0.007), Glu 1hr (Beta = 0.194 P = 0.002), CHO (Beta = 0.224 P = 0.002), LDL-C (Beta = 0.139 P = 0.019), SGPT (Beta = 0.197 P = 0.005) and uric acid (Beta = 0.167 P = 0.005). MDA was found to be significantly associated with HDL-C (Beta = -0.273 P = 0.023). PC was observed to be significantly associated with glucose 2hr in women with PCOS (Beta = 0.250 P = 0.048). SOD activity in PCOS women showed significant association with waist (Beta = 0.391 P = 0.006), Glu 1hr (Beta = 0.262 P = 0.016), Glu 2hr (Beta = 0.193 P = 0.017), LDL-C (Beta = 0.238 P = 0.025) and uric acid (Beta = 0.259 P = 0.013). Catalase showed significant association with triglycerides in women with PCOS (Beta = -0.268 P = 0.031). TAC was found to be significantly associated with testosterone (Beta = -0.290 P = 0.005), FGIR (Beta = 0.485 P = 0.038), Glu 1hr (Beta = 0.242 P = 0.020), TG (Beta = -0.161 P = 0.031), HDL-C (Beta = 0.228 P = 0.014) and LDL-C (Beta = -0.210 P = 0.038).

Table 3
Correlation of PON1, TF, MDA, PC, SOD, CAT and TAC with the anthropometric and laboratory findings in women with PCOS.

	PON1	TF	MDA	PC	SOD	CAT	TAC
Weight							
r							
P							
Waist							
r		0.746			0.638		
P		0.000			0.000		
Waist/hip							
r		0.630			0.513		
P		0.000			0.000		
BMI							
r		0.284		0.250			
P		0.005		0.015			
LH							
r	−0.220						
P	0.032						
Testo							
r	−0.814		0.586			−0.221	−0.673
P	0.000		0.000			0.031	0.000
GLU (1hr)							
r		0.750			0.614		
P		0.000			0.000		
GLU (2hr)							
r				0.207			
P				0.045			
Insulin (F)							
r	−0.688		0.600				−0.631
P	0.000		0.000				0.000
HOMA-IR							
r	−0.617		0.561				−0.558
P	0.000		0.000				0.000
FGIR							
r	0.664		−0.470				0.611
P	0.000		0.000				0.000
QUICKI							
r	0.597		−0.470				0.547
P	0.000		0.000				0.000
CHO							
r		0.818			0.632		
P		0.000			0.000		
TG							
r	−0.257					−0.306	−0.316
P	0.012					0.003	0.002
HDL-C							
r	0.732		−0.535				0.616
P	0.000		0.000				0.000
LDL-C							
r		0.697			0.631		
P		0.000			0.000		
Uric acid							
r		0.684			0.616		
P		0.000			0.000		
SGPT							
r		0.769			0.628		
P		0.000			0.000		

PCOS: polycystic ovary syndrome; BMI: body mass index; HOMA-IR: homeostasis model assessment; FGIR: fasting glucose to insulin ratio; QUICKI: quantitative insulin sensitivity check index; HDL-C: HDL-Cholesterol; LDL-C: LDL-Cholesterol; SGPT; alanine transaminase; LH: luteinizing hormone; FSH: follicle stimulating hormone; MDA: malondialdehyde; PC: protein carbonyl; SOD: superoxide dismutase; CAT: catalase; TAC: total antioxidant capacity. Results were expressed at 5% level of significance i.e * $P < 0.05$.

4. Discussion

PCOS being part of metabolic syndrome has been reported to be associated with dyslipidemia [27]. LDL oxidation is considered to be an important factor in the dyslipidemia and development of atherogenic state. The mechanisms that prevent the oxidation of LDL have received increasing attention in recent years, and one such mechanism is the prevention of LDL oxidation by PON1. There are increasing evidences which suggest that PON1 associated with HDL particle prevents the oxidative modification of LDL particle and preserves the antioxidant and anti-atherogenic integrity of HDL particles [28]. In previous studies, reduced serum PON1 activity has been reported to be associated with insulin resistance, OS, atherosclerosis, neuropathy, retinopathy and other complications in diabetic populations compared with healthy controls [29]. In one study PON1 was removed from the mouse models and more than 50% increase in the amount of fatty streaks was observed, which are the features of initial stage of atherosclerosis [30]. PON1 inactivates the toxic products resulting from the oxidation of LDL and prevents the accumulation of oxidized lipids. Also, PON1 was reported to hydrolyse platelet activating factor (PAF) which is well known pro-inflammatory mediator that causes the transformation of monocytes to macrophages and is thus involved in premature attenuation of atherosclerosis [31]. In the present study we found decreased serum PON1 activity in women with PCOS in comparison to healthy controls. A positive correlation of PON1 activity was observed with FGIR, QUICKI, HDL-C and TAC, and a negative association was observed with testosterone, FI, HOMA-IR, LDL-C and TG in PCOS women (Table 3). Multiple regression analysis showed PON1 activity to be significantly associated with testosterone, insulin, FGIR, HDL-C and LDL-C in PCOS women. These findings suggest that decreased PON1 activity in PCOS may contribute to increased OS and development of hyperandrogenism and insulin resistance. Besides the association of PON1 as observed with HDL-C and LDL-C in this study reinforces its importance in the development of dyslipidemic state in PCOS women. The exact mechanism of the observed decrease in serum PON1 activity in PCOS women remains largely unclear. This decrease in PON1 activity may be because of enhanced ROS mediated lipid peroxidation, which is reported to inhibit PON1 activity [32]. Another possible mechanism that could explain the decreased PON1 activity may be because of genetic origin. Serum PON1 activity is under the control of genetic and environmental factors, and it appears to vary between individuals and populations [33].

As already stated that PCOS is a hypercoagulable state that contributes to coagulation disturbances and vascular complications and TF is one of the main coagulation factors which is the principal activator of extrinsic coagulation pathway. TF belongs to serine protease family and is pro-inflammatory and pro-atherogenic in nature. Its expression is elevated in response to several factors. Hyperglycemia and oxidative stress activators of mononuclear cells are known to stimulate adipocyte production of TNF-α, ROS and OX-LDL, which are the potent stimulators of TF expression from monocytes, macrophages and endothelial cells [14,15]. Also, abdominal adiposity has been reported to be associated with raised TF levels, suggesting that the accumulation of abdominal fat is an important contributing factor in promoting coagulation disturbances and atherosclerosis [34]. In vitro and in vivo studies have shown HMG-CoA reductase inhibitors (statins) and fibric acid derivatives which are known for cholesterol lowering effect were found to decrease TF expression in monocytes and macrophages through the activation of peroxisome proliferator activated receptor γ (PPAR-γ) [35]. It has been reported that there is enhanced expression of TF in obese accompanied by several factors such as PAI-I, angiogenesis and cell adhesion all of which results in the development of hypercoagulability state [36]. In diabetes TF expression is upregulated either directly by increased glucose levels, hyperinsulinemia or indirectly via activation of NFκB through advanced glycation end-products-AGE or reactive oxygen species (ROS). Under hyperglycemic conditions glucose conjugates non-enzymatically with various plasma proteins to form AGE. AGE stimulates NF$\kappa\beta$ via their membrane Receptor for Advanced AGEs while ROS directly activate the transcription factor. AGEs via their

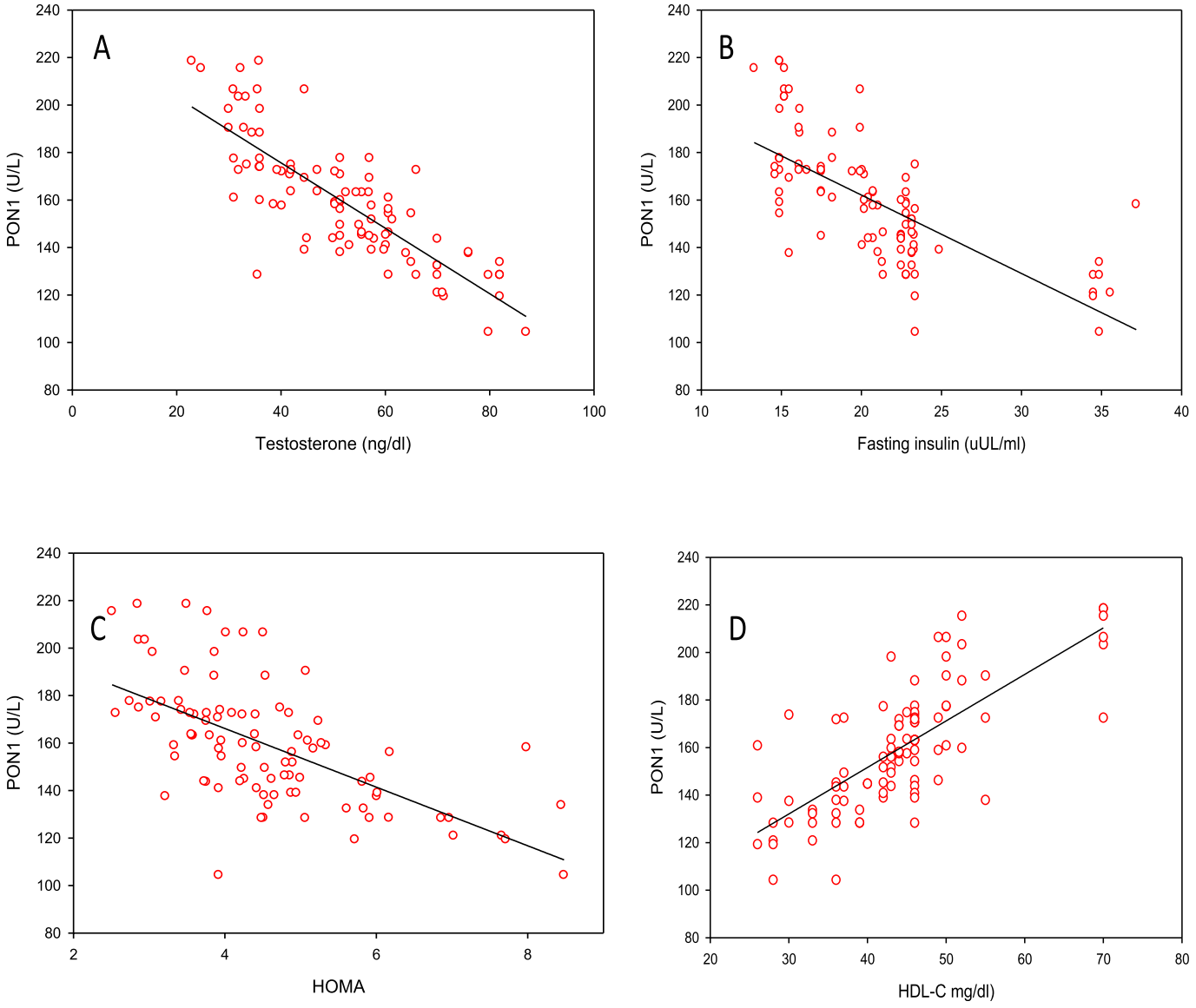

Fig. 1. Correlation of (A) Testosterone (r = -0.814, P = 0.000), (B) Fasting insulin (r = -0.688, P = 0.000), (C) HOMA, homeostatic model assessment insulin resistance (r = -0.617, P = 0.000) and (D) HDL-C, high density lipoprotein cholesterol (r = 0.739, P = 0.000) with serum paraoxonase1 (PON1) activity in women with PCOS.

receptors lead to biological damage such as renal failure and vascular complications. Hyperinsulinemia directly mediates damage through the activation of MAP kinase, protein kinase C, NF-kβ and intracellular adhesion molecule 1 (ICAM-1) in the vascular endothelial cells and mainly results in hemostatic alterations. It is also reported that TF signaling as mediated by coagulation-inflammation cascade could be responsible for the development of insulin resistance, where TNF-α blocks the downstream insulin mediated signaling cascade. TF has a two-fold effect on diabetes where it not only promotes diabetic hypercoagulable and thrombotic state but plays a pronounced role in augmenting the development of insulin resistance. Raised TF levels have also been reported in inflammation and endothelial dysfunction which are the underlying risk factors in the development of atherosclerosis [37–40]. Early in atherogenesis, TF mRNA and antigen are only detected in monocytes and plaque macrophages, while as endothelial cells, smooth muscle cells and foam cells also express TF in later stages. Studies have found that the collagen rich and fibrous plaques are less prone to rupture whereas atherosclerotic plaques with lipid rich core, extensive macrophage infiltration and with enhanced TF expression are much susceptible to rupture [41]. In the present study we observed raised circulating TF levels in PCOS women compared to healthy controls, suggesting PCOS is a pro-coagulant state. We also observed a positive correlation of TF levels with BMI, glucose 1hr, LDL-C, cholesterol, TG, SGPT, uric acid and SOD activity in PCOS women (Table 3). Multiple regression analysis showed TF levels to be significantly associated with waist, glucose 1hr, CHO, LDL-C, SGPT and uric acid in PCOS women. The association of TF with BMI, glucose 1hr, LDL-C, cholesterol, TG, SGPT, uric acid and SOD activity suggest these factors may be involved in elevating TF levels and in predisposing to increased coagulation and plaque formation tendencies in PCOS women. The association of TF with uric acid as observed here can be supported by the previous studies that have confirmed the relationship between high levels of uric acid and the severity of atherosclerosis [42].

Besides PON1 and TF, OS is a base line parameter in induction of PCOS and its associated complications. OS not only impairs insulin

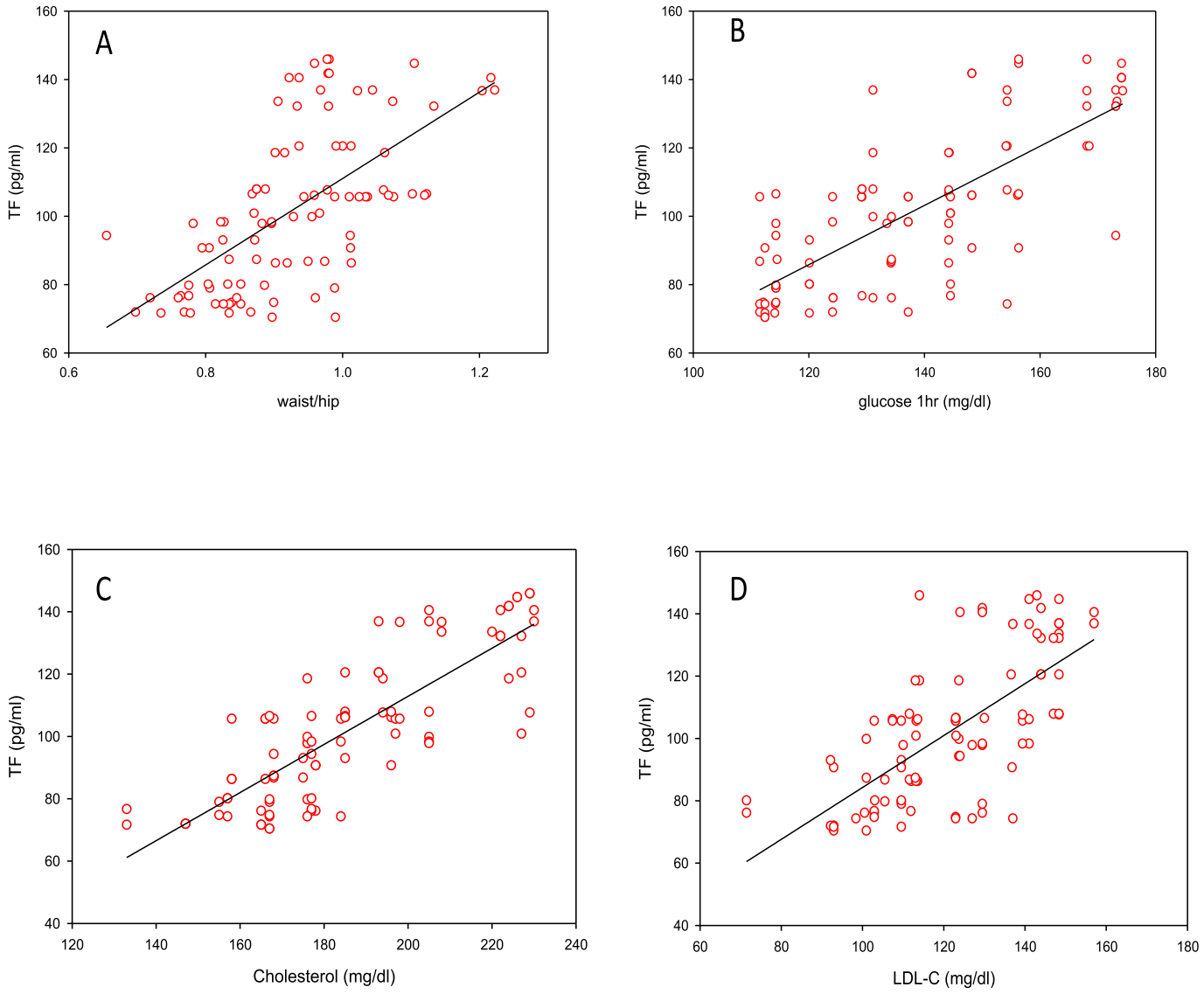

Fig. 2. Correlation of (A) Waist/hip (r = 0.630, P = 0.000), (B) Glucose 1hr (r = 0.750, P = 0.000), (C) Cholesterol (r = 0.818, P = 0.000) and (D) LDL-C, low density lipoprotein Cholesterol (r = 0.697, P = 0.000) with circulating tissue factor (TF) in women with PCOS.

action in women with PCOS but also promotes insulin resistance which is quite common to that seen in patients with type 2 diabetes mellitus. This is demonstrated and evidenced by the increased concentration of circulating protein carbonyls, lipid peroxidation and TNF-α, and decreased insulin signaling [43]. In various investigations OS and inflammatory markers were found to be positively correlated with the androgens such as testosterone and androstenedione levels in women with PCOS. In vitro studies reported that OS enhances the activities of ovarian steroidogenesis enzymes, while as antioxidant chemicals such as statins were found to suppress the activity of steroidogenic enzymes [44]. OS also contributes to various cardiovascular risk factors including obesity, dyslipidemia, and metabolic syndrome and is also known to independently contribute to endothelial dysfunction, inflammation and atherosclerosis. OS directly induces the expression of specific genes such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and also of those located in the upstream regions of cytokines and growth factors including monocyte chemoattractant protein-1 and platelet derived growth factor (PDGF), which are the markers of low-grade inflammation and a predictor of disease related to PCOS [45]. Previous investigations have also reported high concentrations of prooxidant molecules (MDA and ox-LDL), angiogenic factors such as interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) and proinflammatory cytokines interleukin-6 (IL-6), TNF-α, and interleukin-beta (IL-beta) in the peritoneal fluid of patients with endometriosis [46,47]. In the present study an imbalanced oxidant-antioxidant status was also observed in PCOS women. We observed raised MDA and PC levels in PCOS women compared to controls. We also observed increased SOD activity and decreased CAT and TAC levels in PCOS women compared to healthy controls. The increased SOD activity may be an adaptive response to increased OS. MDA levels showed a positive correlation with testosterone, FI and HOMA-IR and a negative correlation with FGIR, QUICKI and HDL-C in PCOS women. TAC showed a positive correlation with FGIR, QUICKI and HDL-C, and a negative correlation with LH, testosterone, FI, HOMA-IR and triglycerides in PCOS women. SOD activity showed a positive correlation with waist, waist to hip ratio, glucose 1hr, cholesterol, LDL-C, SGPT and uric acid in PCOS women. (Table 3). Multiple regression analysis also showed significant

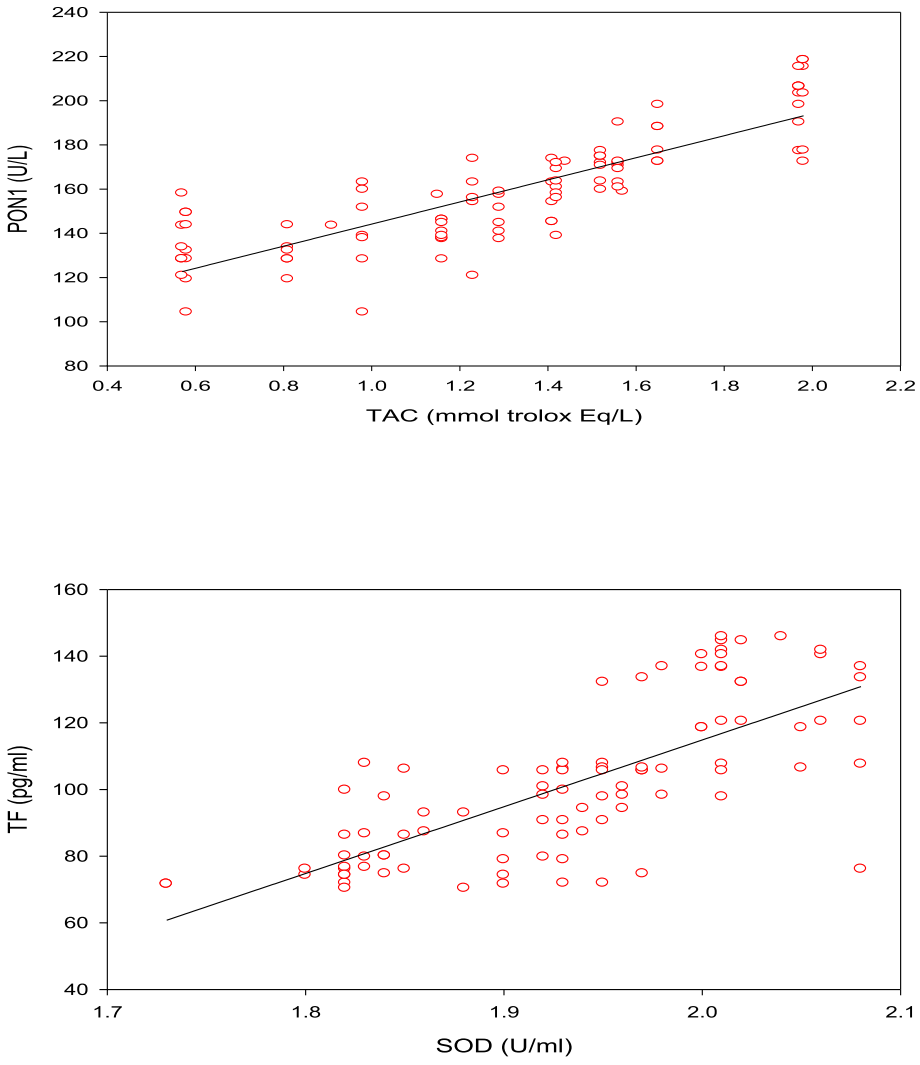

Fig. 3. Correlation of paraoxonase1 (PON1) with the total antioxidant capacity (TAC) (r = 0.8361, P = 0.000) in women with PCOS. Correlation of tissue factor (TF) with superoxide dismutase (SOD) (r = 0.7324, P = 0.000) in women with PCOS.

association of MDA with HDL-C, and a significant association of TAC was observed with testosterone, FGIR, glucose 1hr, triglycerides, HDL-C and LDL-C in PCOS women. A significant association of SOD was observed with waist, glucose 1hr, glucose 2hr, LDL-C and uric acid in PCOS women. These findings taken together support the evidences that PCOS is a pro-oxidant state and stress mediators are involved in generating and aggravating hyperandrogenic, insulin resistance and dyslipidemic tendencies, therefore supports the role of OS as a co-mediator in worsening the inflammatory milieu in PCOS women.

5. Conclusion

In conclusion, the findings of present study showed decreased PON1 activity and raised TF levels in PCOS women compared to controls. Decreased PON1 activity together with its association with testosterone, FI, HOMA-IR, FGIR, QUICK, triglycerides, HDL-C and TAC suggest that low PON1 activity may contribute to hyperandrogenism, insulin resistance and lipid peroxidation and vice-versa, thus increase the risk for atherogenesis. Raised TF levels in PCOS women suggests increased coagulation tendencies in PCOS women compared to controls. The association of TF with waist, waist to hip ratio, BMI, glucose 1hr, cholesterol, LDL-C, SGPT, uric acid and SOD activity strongly suggest that these factors may increase the pro-coagulant status of women having PCOS. This study provides an understanding of multifactorial risk factors associated with this syndrome and may help in identifying high risk PCOS women, therefore pave a way for making better management and preventive strategies. In addition excess oxidant markers and decreased antioxidant parameters and their association with insulin, hormonal and lipid parameters together with PON1 and TF suggest that antioxidant treatment in the initial stage of disease may be useful in the prevention of its progression to various pathologies involving inflammation-coagulation changes, atherogenesis, and other long term complications.

Conflicts of interest

The authors declare no conflict of interest related to this manuscript.

Acknowledgement

Authors would like to acknowledge UGC, India for providing the financial assistance under grant number (F.42-1051/2013(SR)).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.08.001.

References

[1] March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010;25(2):544–51.
[2] Jeelani H, Ganie MA, Amin S, Kawa IA, Fatima Q, Drabu Z, et al. Polycystic ovary syndrome and related disease. Presicion Med 2017a;2(1):23–9.
[3] Jeelani H, Ganie MA, Parvez T, Fatima Q, Kawa IA, Manzoor S, et al. Oxidative stress biomarkers in polycystic ovary syndrome (PCOS). Presicion Med 2017b;2(1):30–8.
[4] Fatima Q, Amin S, Kawa IA, Jeelani H, Manzoor S, Rizvi SM, et al. Evaluation of antioxidant defense markers in relation to hormonal and insulin parameters in women with polycystic ovary syndrome (PCOS): a case control study. Diabetes, Metab Syndrome: Clin Res Rev 2019:1957–61.
[5] Repaci A, Gambineri A, Pasquali R. The role of low-grade inflammation in the polycystic ovary syndrome. Mol Cell Endocrinol 2011;335:30–41.
[6] Precourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E. The three gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis 2011;214:20–36.
[7] Aviram M, Vaya J. Paraoxonase 1 activities, regulation, and interactions with atherosclerotic lesion. Curr Opin Lipidol 2013;24:339–44.
[8] Kotur-Stevuljevic J, Spasic S, Jelic-Ivanovic Z, Spasojevic- Kalimanovska V. PON1 status is influenced by oxidative stress and inflammation in coronary heart disease patients. Clin Biochem 2008;41:1067–73.
[9] Kopaei MR, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med 2014;5(8):927–46.
[10] Baldani DP, Skrgatic L, Ougouag R. Polycystic ovary syndrome: important underrecognised cardiometabolic risk factor in reproductive-age women. Internat J Endocrinol 2015;2015:786362.
[11] Kawa IA, Masood A, Ganie MA, Fatima Q, Jeelani H, Manzoor S, et al. Bisphenol A (BPA) acts as an endocrine disruptor in women with polycystic ovay syndrome: hormonal and metabolic evaluation. Obes Med 2019:10090.
[12] Rasool SUA, Ashraf S, Nabi M, Rashid F, Masoodi SR, Fazili KM, et al. Insulin gene VNTR class III allele is a risk factor for insulin resistance in Kashmiri women with polycystic ovary syndrome. Meta gene 2019:100597.
[13] Grover SP, Mackman N. Tissue factor an essential mediator of hemostasis and trigger of thrombosis. Arterioscler Thromb Vasc Biol 2018;38:709–25.
[14] Aljada A, Ghanim H, Mohanty P, Syed T, Bandyopadhyay A, Dandona P. Glucose intake induces an increase in activator protein 1 and early growth response 1 binding activities, in the expression of tissue factor and matrix metalloproteinase in mononuclear cells, and in plasma tissue factor and matrix metalloproteinase concentrations. Am J Clin Nutr 2004;80(1):51–7.
[15] Sambola A, Osende J, Hathcock J, Degen M, Nemerson Y, Fuster V, et al. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. Circulation 2003;107(7):973–7.
[16] Wang J, Ciaraldi Theodore P, Samad F. Tissue factor expression in obese type 2 diabetic subjects and its regulation by antidiabetic agents. J Obes 2015;291209:01–8.
[17] Mishra KP. Cell membrane oxidative damage induced by gamma radiation and apoptotic sensitivity. J Environ Pathol Toxicol Oncol 2004;23:61–6.
[18] Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotecticve enzymes in humans. Int J Obes 2002;26:1159–64.
[19] Uzun H, Konukoglu D, Gelisgen R, Zengin K, Taskin M. Plasma protein carbonyl and thiol stress before and after laparoscopic gastric banding in morbidly obese patients. Obes Surg 2007;17(10):1367–73.
[20] Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME, et al. Role of antioxidants and natural products in inflammation oxid. Med Cell Longev 2016;2016:5276130.
[21] Gan KN, Smolen A, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos 1991;19:100–6.
[22] Jain SK. Evidence for membrane lipid peroxidation during the in vivo aging of human erythrocytes. Biochim Biophys Acta 1988;937:205–10.
[23] Levine RL, Williams J, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1994;233:346–57.
[24] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gel. Anal Biochem 1971;44:276–87.
[25] Claiborne A. Catalase activity. In: Handbook of methods for oxygene radical. SCIRP; 1985. p. 2832–84.
[26] Mahfouz R, Sharma R, Sharma D, Sabanegh E, Agarwal A. Diagnostic value of the total antioxidant capacity (TAC) in human seminal plasma. Fertil Steril 2009;91(3):805–11.
[27] Macut D, Panidis D, Glisic B, Spanos N, Petakov M, Bjekic J, et al. Lipid and lipoprotein profile in women with polycystic ovary syndrome. Can J Physiol Pharmacol 2008;86:199–204.
[28] Girona J, Manzanares JM, Marimon F, Cabre A, Heras M, Guardiola M, et al. Oxidized to non-oxidized lipoprotein ratios are associated with arteriosclerosis and the metabolic syndrome in diabetic patients. Nutr Metab Cardiovasc Dis 2008;18:380–7.
[29] Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, et al. Serum paraoxonase (pon1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis 1998;139:341–9.
[30] Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free Radic Biol Med 2004;37(9):1304–16.
[31] Mackness MI, Mackness B, Durrington PN. Paraoxonase and coronary heart disease. Atherosclerosis Suppl 2002;3(4):49–55.
[32] Stefanovic A, Kotur-Stevuljevic J, Spasic S, Bujisic N. Association of oxidative stress and paraoxonase status with PROCAM risk score. Clin Biochem 2009;42:617–23.
[33] Jeelani H, Ganie MA, Amin S, Fatima Q, Kawa IA, Manzoor S, et al. Effect of Paraoxonase1 (PON1) gene polymorphism on PON1 activity, HDL, LDL and MDA levels in women with polycystic ovary syndrome (PCOS): a case-control study. Meta gene 2019;100552:01–8.
[34] Gonzalez F, Rote NS, Minium J, Kirwan JP. In vitro evidence that hyperglycemia stimulates tumor necrosis factor-a release in obese women with polycystic ovary syndrome. J Endocrinol 2006;188(3):521–9.
[35] Monetti M, Canavesi M, Camera M, Parente R, Paoletti R, Tremoli E, Corsini A, Bellosta S. Rosuvastatin displays anti-atherothrombotic and anti-inflammatory properties in apoE-deficient mice. Pharmacol Res 2007;55(5):441–9.
[36] Samad F, Pandey M, Loskutoff DJ. Regulation of tissue factor gene expression in obesity. Blood 2001;98(12):3353–8.
[37] Stegenga ME, Van der Crabben SN, Levi M, De Vos AF, Tanck MW, Sauerwein HP, Van Der Poll T. Hyperglycemia stimulates coagulation, whereas hyperinsulinemia impairs fibrinolysis in healthy humans. Diabetes 2006;55(6):1807–12.
[38] Herkert O, Djordjevic T, BelAiba RS, Gorlach A. Insights into the redox control of blood coagulation: role of vascular NADPH oxidase- derived reactive oxygen species in the thrombogenic cycle. Antioxidants Redox Signal 2004;6(4):765–76.
[39] Boden G, Vaidyula VR, Homko C, Cheung P, Rao AK. Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose. J Clin Endocrinol Metab 2007;92(11):4352–8.
[40] Kim JK. Inflammation and insulin resistance: an old story with new ideas. Korean Diabetes J 2010;34(3):137–45.
[41] Hatakeyama K, Asada Y, Marutsuk K, Sat Y, Kamikubo Y, Sumiyoshi A. Localization and activity of tissue factor in human aortic atherosclerotic lesions. Atherosclerosis 1997;133(2):213–9.
[42] Kaya EB, Yorgun H, Canpolat U, Hazırolan T, Sunman H, Ülgen A, et al. Serum uric acid levels predict the severity and morphology of coronary atherosclerosis detected by multidetector computed tomography. Atherosclerosis 2010;213:178–83.
[43] Takeda E, Arai H, Yamamoto H, Okumura H, Taketani Y. Control of oxidative stress and metabolic homeostasis by the suppression of postprandial hyperglycemia. J Med Investig 2005;52(Suppl):259–65.
[44] Piotrowski PC, Rzepczynska IJ, Kwintkiewicz J, Duleba AJ. Oxidative stress induces expression of CYP11A, CYP17, STAR and 3bHSD in rat theca-interstitial cells. J Soc Gynecol Investig 2005;12(2):319A.
[45] Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules; NFKappa B and cytokine inducible enhancers. FASEB 1995;9(10):899–909.
[46] Mier-Cabrera M, Jimenez-Zamudio L, Garcia-Latorre E, Cruz-Orozco O, Hernandez-Guerrero C. Quantitative and qualitative peritoneal immune profiles, T-cell apoptosis and oxidative stress-associated characteristics in women with minimal and mild endometriosis. Int J Obstetrics Gynaecol 2010;118(1):06–16.
[47] Rong R, Ramachandran S, Santanam N, Murphy AA, Parthasarathy S. Induction of monocyte chemotactic protein-1 in peritoneal mesothelial and endometrial cells by oxidized low-density lipoprotein and peritoneal fluid from women with endometriosis. Fertil Steril 2002;78(4):843–8.