Immunobiochemical Analysis of Paraoxonase1 (Anti-Oxidant), Xanthine Oxidase (Oxidant) Enzymes and Lipid Profile of Cardiac Disease Patients in Lahore Metropolitan, Pakistan

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Cardiac diseases are the major cause of death. Paraoxonase1 (PON1) is known as free radicals scavenger/anti-atherosclerosis, whereas xanthine oxidase (XO) is a free radicals generator. This study was undertaken to determine and compare the Paraoxonase and arylesterase activities of PON1 enzyme and activity of XO enzyme. The concentration of XO and PON1 enzymes along with lipid profile, lipid peroxides, and thiol level in plasma of cardiac patients ($n=200$) and healthy persons ($n=200$) of Lahore metropolitan, Pakistan was also determined. Anti-PON1 and anti-XO antibodies were developed, purified, and used to measure the concentration of PON1 and XO by competitive ELISA. It is observed that low paraoxonase ($P=0.0073$)/arylesterase activity ($P=0.0038$)

of PON1 enzyme and its low concentration ($P=0.0049$) were observed in cardiac patients, whereas elevated level of XO activity ($P=0.0129$) and its concentration ($P=0.0097$) was observed in cardiac patients as compared with healthy persons. Low levels of HDL ($P=0.0013$), thiol ($P=0.0014$) and high level of cholesterol ($P=0.0025$), triglycerides ($P=0.0018$), LPO ($P=0.0014$), and LDL level ($P=0.05$) were observed in cardiac patients admitted in intensive care unit as compared with hypertensive patients and control subjects. It is concluded that overall low PON1 and high XO activities do cause imbalance of free radical system which ultimately leads to or enhance the cardiac pathological conditions. *J. Clin. Lab. Anal.* 24:348–356, 2010. © 2010 Wiley-Liss, Inc.

Key words: paraoxonase1; xanthine oxidase; free radicals; antibodies; competitive ELISA

INTRODUCTION

Paraoxonase 1 (PON1, EC. 3.1.8.1) enzyme is a calcium-dependent enzyme synthesized in liver and is associated with high-density lipoproteins (HDL). It hydrolyzes paraoxon, diazoxon, arylesters, insecticides and nerve gases (1). PON1 inhibits the lipid peroxidation of low-density lipoprotein (LDL) (2). The biochemical activity of PON1 and concentration of HDL is decreased in cardiac patients as compared with normal healthy persons. It is observed that low activity of PON1 may lead to progression of coronary artery disease (CAD) and atherosclerosis (3). The PON1 also detoxify the oxygen derived free radicals (ODRs) and hydrogen peroxide which is produced during oxidative stress, but the exact mechanism of detoxification is not known (4).

In mammals, PON1 belongs to multigene family of PON enzymes such as PON1, PON2, and PON3. PON1 and PON3 are liver enzymes, whereas PON2 is expressed in other tissues (1). The genetic polymorphism of PON1 is due to glutamine (Q-allele) or arginine (R-allele) amino acid at position 192 and leucine or methionine amino acid at position 55 (5). The ratio of

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PON1 activity of Q-allele (Gln-192) isoform is less than that of R-allele (Arg-192) isoform. The QQ allele isoform is more effective to protect from lipid peroxidation (6–10). The detail informations of Q and R alleles of PON1 are important for epidemiological and clinical studies to understand its role in lipid metabolism linked to coronary heart diseases (CHD) or myocardial infarction (MI).

Xanthine oxidase (XO) enzyme is involved in the production of ODR ($O_2^{\bullet}$, $OH^{\bullet}$ and $RCOO^{\bullet}$) under oxidative stress or ischemic conditions (11). ODRs are involved in the lipid peroxidation which may be the major cause of myocardial dysfunction. In humans, XO is normally present in liver, milk, and heart as dehydrogenase form (D-form) and utilizes the nicotinamide adeninedinucleotide (NAD) as electron acceptor. Under physiological malfunctions, postischemic reperfusion injury, or during liver and heart damage xanthine dehydrogenase form is converted into oxidase form (O-form) and it utilizes oxygen as electron acceptor (12,13). The conversion of leakage of XO from injured tissues may damage the vascular endothelium, epithelium, and the near sites (14). The level of XO activity is low in healthy persons but activity is increased during pathological conditions such as ischemia/reperfusion and calcium paradox conditions of heart (13,15). The ODR are also generated by the biological activity of neutrophils and mitochondrial respiratory chain (16). At cellular level, the elevated levels of ODR cause deterioration in cardiomyocytes function and damage the structural integrity of ion channels via lipid peroxidation that leads to apoptosis (17,18).

Both PON1 and XO enzymes are important to understand the pathophysiological conditions related to heart diseases. It is reported that the concentration and activity of PON1 is closely related to cardiac disease then genotype (10). Dietary habits, environmental factors, and uses of pesticides may also play important role to disturb the PON1 and XO activities. Imbalanced diet and excessive use of fat contents in diet of our local population may imbalance the oxidative and anti-oxidative activities of XO and PON1 enzymes.

The population of Lahore metropolitan is more than 10 million and it is observed that 1.6–1.7% patients suffering from heart diseases visit the outdoor patient department, whereas 0.17–0.2% is admitted for angiography and angioplasty per year. 0.05 and 0.17 percent patients are admitted for stents and cardiac surgery, respectively, per year (personal communication). Owing to availability of basic data of cardiac functions in Lahore metropolitan there is need to check the other reasons then the existed one by more precise method. There is need to develop the quantitative immunochemical analysis of PON1 and XO in normal healthy

persons and cardiac disease patients for correlating with diet, environmental pollution, and lipid profile.

In this study, the level of XO, PON1 enzymes, and lipid profile in normal healthy persons and cardiac patients were determined by biochemical and immunochemical assays in local population of Lahore. This study will help to understand the involvement of these enzymes and/or ODRs in CADs.

MATERIALS AND METHODS

Paraoxon, phenyl acetate, XO, glutathione, 5, 5' dithiobis-2-nitro benzoic acid, protein A, and other chemicals required for routine analysis were purchased from Sigma-Aldrich (St. Louis, MO). Randox kits (Antrim, UK) were used for the determination of lipid parameters. Cyanogen-bromide-activated sepharose-4B was purchased from Pharmacia.

Collection and Preparation of Samples

Two-hundred blood samples of normal healthy persons (Group-1A, $n = 60$ and Group-1B, $n = 140$) 25–55 years old who had never diabetes mellitus, CAD, or blood pressure were collected as control.

Two-hundred blood samples of age-matched cardiac patients Group-II ($n = 60$) and Group-III ($n = 140$) 25–56 years old were collected from Punjab Institute of Cardiology, Lahore; Jinnah Hospital, Lahore and from different medical clinics. Sixty patients suffering from cardiac disease included in this study were admitted in intensive coronary care unit (ICU) of hospital and were under treatment, whereas other 140 patients were hypertensive and not admitted in the hospital. Hypertensive patients taking only those medicines that are used to keep the blood pressure in normal range. The systolic blood pressure greater than 150 mmHg and diastolic blood pressure 90 mmHg or above were considered abnormal for hypertensive patients. The age, sex, occupation, and clinical histories of patients were also noted.

Blood (5–7 ml) was drawn in EDTA tubes and centrifuged at 4,000 rpm for 5 min. Plasma was separated and stored at -80°C till further use. All blood samples were collected with the consent of patients/guardians and normal healthy persons by following the Helsinki Declaration of 1975.

Estimation of Paraoxonase and Arylesterase Activities of PON1 Enzyme

Paraoxonase activity of PON1 enzyme was estimated by using synthetic paraoxon as substrates as described by Eckerson et al. (6) and Samra et al., Submitted with little modification. Five microliter of human plasma was

incubated with 2 mM paraoxon in the presence of 1.0 mM CaCl_2 , 1.0 M NaCl in 50 mM glycine-NaOH buffer, pH 10.5. The generation of *p*-nitrophenol was monitored at 412 nm at 37°C for 5 min. The enzyme activity was determined by using molar extinction coefficients 18,290 M/cm of *p*-nitrophenol. The paraoxonase activity was described as nmol/ml/min under the assay conditions.

Arylesterase activity of PON1 enzyme was measured by using synthetic phenyl acetate as described by Gan et al. (19) with little modification. Five microlitre of human plasma was incubated with 2 mM phenyl acetate in the presence of 1.0 mM CaCl_2 in 50 mM Tris-Cl buffer, pH 8.0. The generation of phenol was monitored at 270 nm at 37°C for 5 min. The enzyme activity was determined by using molar extinction coefficients 1,310 M/cm of phenol. The arylesterase activity was described as $\mu\text{mol/ml/min}$ under the assay conditions. Enzyme assays were done in triplicate. One unit is defined as the formation of phenol or para-nitrophenol/min under the assay conditions.

Estimation of XO Activity

Xanthine oxidase was obtained from Sigma-Aldrich (St. Louis, MO) and dialyzed against sodium phosphate buffer pH 7.5. The activity was determined spectrophotometrically by using 1.0 mM hypoxanthine as substrate in 50 mM sodium phosphate buffer pH 7.5. The production of uric acid was estimated by measuring the increase in absorbance at 290 nm at 25°C. The enzyme units were calculated by using molar extinction coefficients 12,200 M/cm of uric acid. One unit is defined as the formation of 1.0 μmol uric acid/min. The enzyme activity was described as units/ml under the assay conditions. Enzyme assay was done in triplicate.

Estimation of Lipids, Lipid Peroxides

The triglycerides, total cholesterol, HDL, and LDL in plasma were determined spectrophotometrically by using kit (RANDOX). The LDL concentration was determined by using Friedwald equation (20). The results are described as mg/dl. The iodometric assay for lipid peroxides was determined spectrophotometrically at 365 nm and results are described in nmol/ml (21). The assays were done in triplicate.

Estimation of Thiol Level

The thiol level in plasma was determined spectrophotometrically by using 5, 5' dithiobisnitrobenzoic acid (DTNB) (Elmen's reagent) as described (22). Plasma (0.05 ml) was mixed with 2.5 ml of 0.1 M Tris-EDTA buffer pH 8.0 and 0.05 ml of Elmen's reagent (4.0 mg of

DTNB in 0.1 M Tris-EDTA buffer pH 8.0 buffer). The mixture was incubated at 37°C for 25 minutes and the optical density was measured at 412 nm against the reagent blank. The production of 2-nitro-5-thiobenzoate (TNB) was calculated by using molar extinction coefficient 14,150 M/cm. Reagent blank was prepared by using methanol instead of DTNB. Glutathione (GSH) was used as standard. Thiol levels were expressed in $\mu\text{mol/l}$. The reaction is stichiometric and 1.0 M thiol release 1.0 M TNB. The assay was done in triplicate.

Production, Purification and Conjugation of Antibodies with Alkaline Phosphatase

Polyclonal antibodies against XO and PON1 were developed in rabbits separately following the guidelines for care of laboratory animals. Purified human PON1 enzyme (Samra et al., Submitted) and bovine milk XO (Sigma) were used as antigens to develop the antibodies. Total antibodies were purified from rabbit serum on protein A-sepharose chromatography and anti-XO and anti-PON1 antibodies were purified further on CNBr-activated Sepharose-4B coupled with XO and PON1 proteins as described (23). The purified anti-XO and anti-PON1 antibodies were coupled with alkaline phosphatase (AP) by glutaraldehyde method (24). The optimum dilutions of AP-conjugated anti-XO and anti-PON1 antibodies were determined direct ELISA by using 1.0 $\mu\text{g/well}$ purified XO or PON1 proteins.

Western Blot Analysis

The monospecificity of antibodies was checked by western blot. Purified XO and PON1 were separated on slab 10% SDS-PAGE as described by Laemmli (25) and electrophoretically transferred onto nitrocellulose membrane as described by Towbin et al. (26). After blocking the nonspecific binding sites on nitrocellulose membrane with blocking buffer (3% BSA in Tris-buffered saline-Tween 20), blots were incubated separately with AP-conjugated rabbit anti-PON1 or rabbit anti-XO antibodies (1:3,000 dilution) each for 45 min. After washing the blots in Tris-buffered saline-Tween 20, color reaction was developed using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates (23).

Immunoquantification of XO and Paraoxonase I

An antigen competitive ELISA was used to estimate the concentration of XO and PON1 enzyme in human plasma with little modification (27). 0.05 ml of XO and PON1 enzyme (0.01 mg/ml) in 0.05 M carbonate buffer pH 9.5 was coated on flat bottom microtitre plates separately and kept overnight at 4°C. The nonspecific

binding sites were blocked with 0.2 ml of blocking buffer (3% BSA in Tris-buffered saline-Tween 20) for 1 hr at 25°C. After washing, the wells were incubated with AP-conjugated rabbit anti-XO and rabbit anti-PON1 antibodies (1:1,600 dilutions) for 2 hr. After washing the color reaction was developed with 0.1 ml of p-nitrophenyl phosphate substrate (1.0 mg/ml in 0.1 M diethanolamine buffer pH 9.5). The reaction was blocked with 0.1 ml of 0.02 M EDTA solution pH 8.0 and optical density was measured at 405 nm. The concentration of XO and PON1 in plasma of patients was estimated by mixing 0.05 ml of human plasma with diluted anti-XO and anti-PON1 in above described assay for 50% inhibition. A standard curve was made in the presence of 0.05 ml of normal human plasma. The concentration was calculated and expressed in $\mu\text{g/ml}$.

Protein Determination

The protein concentration was determined by Bradford reagent assay using human immunoglobulins as standard (28).

Data Analysis

The Student's *t*-test was applied to compare and present the values as standard error of means (SEM). Statistical analysis was further completed by using SSPS 11.version (computer program of statistical package for social sciences). The data of study Groups was compared by using Mann-whitney *U* test. *P*-value less than 0.05 was considered as a significant value.

RESULTS

Polyclonal antibodies against XO and PON1 proteins were developed, purified, and conjugated with AP for antigen competitive ELISA. The cross reactivity of purified anti-XO antibodies toward PON1 enzyme and anti-PON1 antibodies toward XO enzyme was also checked and we did not find any cross reactivity. The monospecificity of antibodies was checked by western blot (Fig. 1). The antigen competitive ELISA was used to estimate the concentrations of PON1 and XO in normal healthy individuals (Group-IA and Group-IB) and cardiac patients (Group II & III) (Table 2). A standard ELISA curve was obtained by using XO (0.1–50 μg) and PON1 (0.1–50 μg) proteins, whereas competition ELISA curve was drawn by using 50% normal human plasma under the standard assay conditions. The levels of XO and PON1 enzymes in plasma determined by antigen competitive ELISA in normal healthy persons and patients suffering from cardiac diseases are shown in Table 2. The correlation coefficient of XO and PON1 between normal persons

Fig. 1. SDS-PAGE (10%) and western blot analysis of XO and PON1 enzymes. (A) (Lane 1), standard molecular weight protein marker, (Lane 2), Purified xanthine oxidase, (Lane 3), purified paraoxonase1 enzyme gel was stained with coomassie brilliant blue R-250. Single protein bands of XO and PON1 enzymes with molecular weight of 155 kDa and 45 kDa are seen. (B) (Lane 1), a single immunoreactive protein band of 155 kDa of XO indicated the monospecificity of purified anti-XO antibodies. (Lane 2), Immunoreaction was not observed with PON1 enzyme. (C) (Lane 2), a single immunoreactive protein band of 45 kDa of PON1 enzyme indicated the monospecificity of purified anti-PON1 antibodies. (Lane 1), Immunoreaction was not observed with XO enzyme.

and cardiac patients is shown in Tables 5 and 6. The comparative level of XO was increased and PON1 level was decreased in cardiac patients as compared with normal healthy individuals.

The level of cholesterol, HDL, LDL, triglycerides, thiol, and lipid peroxide as well as biochemical activity of PON1 and XO enzymes was determined in human plasma of cardiac patients and normal healthy individuals (Tables 1 and 2). The mean age of cardiac patients admitted in intensive care unit and hypertensive patients were 42.50 ± 10.86 and 40.50 ± 4.2 , respectively. The normal healthy persons were divided into two groups, Group-IA and Group-IB for age matched to cardiac patients in ICU and hypertensive patients, respectively. The mean ages of Group-IA and Group-IB are 42.50 ± 13.22 and 40.23 ± 5.12 , respectively.

Table 1 shows that increased level of cholesterol (190.50 ± 20.50), triglycerides (148 ± 11.62), LDL (124 ± 18.67), lipid peroxides (60.91 ± 5.15), and decreased level of HDL (31.20 ± 11.25) was observed in cardiac patients admitted in intensive care unit (Group II) as compared with control healthy persons (Group-IA and Group-IB) and hypertensive patients (Group-III). Slightly increased level of cholesterol and LDL was observed in hypertensive patients (Group III) as compared with normal healthy persons (Group-IA and Group-IB). The comparative values of HDL ($<40 \text{ mg/dl}$) was low in patients admitted in ICU (Group-II) as compared with normal healthy persons (Group-IA and Group-IB). The difference in lipid

TABLE 1. Clinical Parameters of Normal Healthy Persons and Cardiac Patients

Subjects	Biochemical parameters						
	Age (years)	CHO (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)	LPO (nmol/ml)	Thiol (μmol/l)
<i>Normal persons (n = 200)</i>							
(Group I-A) (n = 60)	42.50 ± 13.22	145.50 ± 18	42.47 ± 10.09	90.57 ± 29.83	106.5 ± 18.06	42.38 ± 5.12	263.50 ± 19
(Group I-B) (n = 140)	40.23 ± 05.12	151.29 ± 25	45.12 ± 10.20	92.36 ± 26.18	110.0 ± 22.06	44.39 ± 3.12	261.40 ± 36
<i>Cardiac patients (n = 200)</i>							
Admitted in ICU							
(Group II) (n = 60)	42.50 ± 10.86	190.5 ± 20.5	31.20 ± 11.25	124.5 ± 18.67	148 ± 11.62	60.91 ± 5.15	251 ± 25.50
Hypertensive							
(Group III) (n = 140)	40.50 ± 4.2	159 ± 17.43	43.82 ± 10.05	97.50 ± 13.68	105 ± 28.53	43.73 ± 2.64	257.5 ± 6.24

Values are presented as mean ± SD. CHO (Cholesterol), HDL (High-density lipoprotein), LDL (Low-density lipoprotein), TG (Triglycerides), LPO (Lipid peroxide).

TABLE 2. Paraoxonase1 and XO Activities and Their Concentration in Human Plasma of Normal Healthy Persons and Cardiac Patients

Subjects	Enzyme activity and concentration					
	Age (years)	PON activity (nmol/ml/min)	ARY activity (nmol/ml/min)	XO activity (U/ml)	PON1 conc (μg/ml)	XO conc. (μg/ml)
<i>Normal persons (n = 200)</i>						
(Group I-A) (n = 60)	42.50 ± 13.22	89 ± 39.16	51 ± 10.87	0.015 ± 0.07	79.5 ± 1.55	0.62 ± 0.29
(Group I-B) (n = 140)	40.23 ± 05.12	91 ± 38.32	53 ± 11.07	0.013 ± 0.09	81.5 ± 2.36	0.60 ± 0.19
<i>Cardiac patients (n = 200)</i>						
Admitted in ICU (n = 60)						
(Group II)	42.50 ± 10.86	71 ± 8.14	38.40 ± 3.36	0.11 ± 0.04	57 ± 1.7	1.82 ± 0.20 ^a
Hypertensive (n = 140)						
(Group III)	40.50 ± 4.20	83 ± 38.08	49.50 ± 9.54	0.081 ± 0.05	64 ± 1.25	0.92 ± 0.14 ^a

Values are presented as mean ± SD. PON (Paraoxonase), ARY (Arylesterase), XO (Xanthine oxidase), PON1 (Paraoxonase1).

^aElevated levels of activity and concentration of XO enzyme and low levels of activity and concentration of PON1 enzyme were observed.

TABLE 3. Corelation Coefficients Between Biochemical Parameters of Normal Healthy Persons and Cardiac Patients Admitted in ICU

Parameters	Control Group I-A (n = 60)	Cardiac patients (Admitted in ICU) Group II (n = 60)	Significant value (P)
Age (years)	42.50 ± 13.22	42.50 ± 10.86	—
CHO (mg/dl)	145.50 ± 18.00	190.50 ± 20.50	0.0025
HDL (mg/dl)	42.47 ± 10.09	31.20 ± 11.25	0.0012
LDL (mg/dl)	90.57 ± 29.83	124.50 ± 18.67	0.070
TG (mg/dl)	106.50 ± 18.06	148.00 ± 11.62	0.0018
LPO (nmol/ml)	42.38 ± 5.12	60.91 ± 5.15	0.0014
Thiol (μmol/l)	263.50 ± 19.00	251.00 ± 25.50	0.0014

Values are presented as mean ± SD. CHO (Cholesterol), HDL (High-density lipoprotein), LDL (Low-density lipoprotein), TG (Triglycerides), LPO (Lipid peroxide).

profiles between control healthy groups was not significant (Table 1).

Tables 2, 5, and 6 represents enzyme assays and antigen competitive ELISA of PON1 and XO enzymes

in plasma of healthy persons and cardiac patients. The paraoxonase activity ($71 \pm 8.14 \mu\text{mol/ml}$, $P = 0.0073$), arylesterase activity ($38.40 \pm 3.36 \mu\text{mol/ml}$, $P = 0.0038$), and concentration of PON1 enzyme ($57 \pm 17 \mu\text{g/ml}$,

TABLE 4. Corelation Coefficients of Clinical Parameters of Normal Healthy Persons and Cardiac Patients

Parameters	Control Group I-B (<i>n</i> = 140)	Cardiac patients (Hypertensive patients) Group III (<i>n</i> = 140)	Significant value (<i>P</i>)
Age (years)	40.23 ± 05.12	40.50 ± 4.20	
CHO (mg/dl)	151.29 ± 25.00	159.00 ± 17.43	0.017
HDL (mg/dl)	45.12 ± 10.20	43.82 ± 10.05	0.540
LDL (mg/dl)	92.36 ± 26.18	97.50 ± 13.68	0.631
TG (mg/dl)	110.0 ± 22.06	105.00 ± 28.53	0.429
LPO (nmol/ml)	44.39 ± 3.12	43.73 ± 2.64	0.642
Thiol (μmol/l)	261.40 ± 36.00	257.50 ± 6.24	0.0017

Values are presented as mean ± SD. CHO (Cholesterol), HDL (High-density lipoprotein), LDL (Low-density lipoprotein), TG (Triglycerides), LPO (Lipid peroxide).

TABLE 5. Corelation Coefficients Between PON1 and XO Enzyme Activities Between Normal Healthy Persons and Cardiac Patients Admitted in ICU

Parameters	Control (Group I-A) (<i>n</i> = 60)	Cardiac patients (Admitted in ICU) (Group II) (<i>n</i> = 60)	Significant value (<i>P</i>)
Age (years)	42.50 ± 13.22	42.50 ± 10.86	—
PON activity (nmol/ml/min)	89.00 ± 39.16	71.00 ± 8.14	0.0073
ARY activity (nmol/ml/min)	51.00 ± 10.87	38.40 ± 3.36	0.0038
XO activity (U/ml)	0.015 ± 0.07	0.14 ± 0.06	0.0122
PON1 conc. (μg/ml)	79.50 ± 1.55	57.00 ± 1.70	0.0049
XO conc. (μg/ml)	0.62 ± 0.29	1.82 ± 0.55 ^a	0.0097

PON (Paraoxonase), ARY (Arylesterase), XO (Xanthine oxidase), PON1 (Paraoxonase1).

^aHigh activity of XO enzyme.

TABLE 6. Corelation Coefficients Between PON1 and XO Enzyme Activities Between Normal Healthy Persons and Hypertensive Cardiac Patients

Parameters	Control (Group I-B) (<i>n</i> = 140)	Cardiac patients (Hypertensive patients) (Group III) (<i>n</i> = 140)	Significant value (<i>P</i>)
Age (years)	40.23 ± 15.12	42.50 ± 10.86	—
PON activity (nmol/ml/min)	91.00 ± 38.32	83.00 ± 38.08	0.0052
ARY activity (nmol/ml/min)	53.00 ± 11.07	49.50 ± 9.54	0.0013
XO activity (U/ml)	0.013 ± 0.09	0.081 ± 0.05	0.0016
PON1 conc (μg/ml)	81.50 ± 2.36	64.00 ± 1.25	0.0053
XO conc. (μg/ml)	0.60 ± 0.19	1.10 ± 0.19 ^a	0.0083

PON (Paraoxonase), ARY (Arylesterase), XO (Xanthine oxidase), PON1 (Paraoxonase1).

^aHigh activity of XO enzyme.

P = 0.0049) were decreased in cardiac diseases patients (Group II and III) as compared with normal healthy persons (Group-IA and Group-IB). The XO enzyme activity (0.14 ± 0.06 U/ml) and its concentration (1.8 ± 0.55 μg/ml) was high in plasma of cardiac patients (Group II) admitted in intensive care unit as compared with XO enzyme activity (0.015 ± 0.07 U/ml, *P* = 0.008) and concentration of XO (0.62 ± 0.29 μg/ml, *P* = 0.0097) in healthy persons (Group-IA). The XO activity and concentration between Group-IA and Group-IB is relatively same but the *P*-value is dropped from 0.0122 to 0.0016 and 0.0097 to 0.0083 for XO activity and

concentration, respectively. The mean thiol level was also low in cardiac patients admitted in ICU (251 ± 25.50 μmol/l) than control (263.50 ± 19 μmol/l) and the *P*-value is 0.0014.

The comparison study between male and female of control and cardiac patients for lipid profile, PON1 and XO enzymes activities was analyzed to understand the gender bias for biochemical parameters if any. Table 7 indicates that the average (±SD) of lipid profile is relatively high in male than female in control and cardiac patients. It is interesting to note that the PON1 enzyme activity is slightly higher in female

TABLE 7. Correlation Coefficients of Clinical Parameters, PON1 and XO Activities Between Male and Female of Cardiac Patients and Normal Healthy Persons

Parameters	Cardiac patients Male (n = 140)	Normal persons Male (n = 140)	(P) value	Cardiac patients Female (n = 60)	Normal persons Female (n = 60)	(P) value
Age (years)	42.53 ± 03.32	43.34 ± 11.22	–	40.50 ± 04.20	41.64 ± 08.20	–
CHO (mg/dl)	195.32 ± 26.00	152.64 ± 23.00	0.0141	185.02 ± 18.53	148.02 ± 17.53	0.0152
HDL (mg/dl)	40.12 ± 62.20	44.20 ± 09.18	0.069	39.72 ± 11.35	40.34 ± 10.08	0.072
LDL (mg/dl)	128.26 ± 26.28	93.36 ± 27.28	0.113	118.54 ± 10.78	91.24 ± 21.34	0.119
TG (mg/dl)	156.00 ± 12.06	109.34 ± 21.78	0.226	143.23 ± 12.53	103.24 ± 29.39	0.196
LPO (nmol/ml)	68.23 ± 6.12	55.23 ± 09.12	0.178	52.23 ± 05.23	49.76 ± 08.39	0.139
Thiol (μmol/l)	259.45 ± 36.00	264.10 ± 32.34	0.062	246.52 ± 10.50	260.39 ± 06.34	0.048
PON activity (nmol/ml/min)	64.23 ± 05.23	87.34 ± 36.48	0.0061	78.21 ± 12.34	93.64 ± 21.29	0.0052
ARY activity (nmol/ml/min)	36.00 ± 05.87	48.10 ± 10.98	0.0019	42.54 ± 03.38	52.98 ± 04.30	0.0012
XO activity (U/ml)	0.10 ± 0.062	0.016 ± 0.006	0.047	0.08 ± 0.02	0.013 ± 0.009	0.085
PON1 conc. (μg/ml)	62.60 ± 1.67	78.64 ± 02.56	0.00011	52.53 ± 1.69	83.38 ± 02.30	0.00003
XO conc. (μg/ml)	1.88 ± 10.72	0.78 ± 0.09	0.0020	1.56 ± 0.34	0.61 ± 0.01	0.0042

Values are presented as mean ± SD. PON (Paraoxonase), ARY (Arylesterase), XO (Xanthine oxidase), PON1 (Paraoxonase1).

(78.21 ± 12.34, $P = 0.0052$) than male (64.23 ± 05.23), whereas XO activity is high in male (0.10 ± 0.062, $P = 0.047$) than female (0.08 ± 0.02). The PON1 and XO activities is low and high, respectively, in cardiac patients, whereas PON1 and XO activities is high and low, respectively, in normal healthy persons.

A correlation was observed between PON1 activity and lipid profile among normal healthy persons and cardiac patients. The concentration of lipidperoxides was high in cardiac patients admitted in ICU (Group-II). The univariable analysis by using the age and sex, no significant difference was observed between XO and PON1 enzyme activity in plasma. The discriminate analysis did not show clear cut-off values for PON1 and XO activity which predisposes to cardiac diseases. Further correlation analysis showed that plasma lipid parameters, lipid peroxides, thiol level, XO and PON1 activity can be moderately correlated in all Groups. The P -values of lipid parameters, PON1 and XO activities between control healthy persons and cardiac patients are mentioned in Tables 3–6.

DISCUSSION

Paraoxonase1 and XO/dehydrogenase are important enzymes to maintain normal physiological functions (Table 7). PON1 is a calcium-dependent enzyme and is known as free radicals bioscavenger and anti-atherosclerosis. Among PON genes (PON1, PON2, and PON 3), the PON1 enzyme is conjugated with HDL and protects the tissues from lipid peroxidation as well as from free radicals that are associated with cellular dysfunction and oxidation of many biological compounds (12). XO is normally present as dehydrogenase form and during pathological conditions it is

converted into oxidase form which is known as ODR producing enzyme. The pathogenecity and etiology of heart diseases by ODRs is reported (12,13). The damage of vascular or endothelial tissue and repeated infection is involved to enhance the production of ODRs by the conversion of xanthine dehydrogenase to XO.

It is also reported that the activated T-lymphocytes produce interleukins that protrude the conversion of endothelial dehydrogenase to oxidase form which is involved in the generation of ODR by the availability of the purine fuel for the degradation pathway. These ODR could be involved in the oxidation of LDL. It is also reported that PON1 is considered as an important candidate in the development of atherosclerosis (3,10). The variability of the PON1 in human plasma of heart patients in our local population may be associated with dietary habit or environmental conditions.

This study was undertaken to determine the level of oxidant (XO) and anti-oxidant (PON1) enzyme in cardiac patients and their comparison with normal healthy human persons in Lahore metropolitan area. Our study showed that the PON1 enzyme activity is low, whereas XO enzyme activity and its concentration are high in cardiac patients (Group-II and III) as compared with control healthy persons (Group-IA and IB). The PON1 and XO enzyme in local population is less than the values of PON1 and XO in plasma of western population (29,30). The level of cholesterol, LDL, TG, and lipidperoxides are high in cardiac patients (Group-II) admitted in intensive care unit, whereas no significant difference was observed in hypertensive cardiac patients (Group-III) in comparison to normal healthy persons. The overall mean value of thiol contents is low in heart patients as compared with normal healthy individuals.

In gender comparison study, it is observed that the activity of PON1 enzyme is high in female and XO activity is low in female, whereas in male it is reverse. Our results regarding PON1 (high) activity in female are corroborated with the results reported by Sumegova et al. (31). From this analysis, it is concluded that the male are more susceptible to cardiac diseases than female because of high level of lipid per-oxidation and due to high XO and low PON1 enzyme activities. The female is perhaps protected from atherosclerosis and cardiac diseases due to estrogen which is known as an antioxidant and vasodilator (32). The imbalance ratio of PON1 and XO activities is correlated to the habits of our local population for imbalance diet, contaminated drinking water, poor health, repeated infection status, and social problems. High fat diet and general socio-economic problems in our country may have an effect on health, lipid profile, and imbalance of free radical production and scavenging system.

The association of PON1 enzyme with HDL is an important stage to protect the oxidation of LDL. According to National Cholesterol Education Program Adult Treatment Panel III, the HDL serum level <40 mg/dl in humans is considered as below normal level. In our study, it was observed that range of HDL level was 29–38 mg/dl in cardiac patients (Group-II), whereas the HDL level in normal healthy persons and hypertensive patients is near to normal level. It may be concluded that level of HDL is low in Pakistani population as compared with western population and have low PON1 level. The polymorphism in PON1 and the genotype may be considered for the risk factor of CAD and in this study it is observed that the low HDL and PON1 level in plasma may be considered to lead the development of atherosclerosis stage.

It is reported that the plasma PON1 activity is decreased during MI and remained low (9,33). It is also reported that the XO level and its activity increased during cardiac ischemia/reperfusion (13). It is further concluded that during cardiac diseases the PON1 enzyme activity is low, whereas the XO enzyme activity is increased. The low activity of serum PON1 in MI patients can be related to polymorphism of PON1 gene (33). Many studies reported that the activity of PON1 enzyme is related to CAD and genotype. In CAD patients, it is observed that the inhibition of oxidation of LDL by HDL was low (33) and PON1 activity remained low during acute MI.

The history of the cardiac patients informed us that the medicine taking by the cardiac patients are not involved to raise the cholesterol level and the patients were not taking any medicines to control the cholesterol level. The presence of high activity of XO (oxidative enzyme), low activity of PON1 (anti-oxidative enzyme),

and low level of HDL may be the cause of ODRs. These ODRs are highly reactive and can damage the other cellular components. It is documented that the high level of free radicals, lipid peroxidation of LDL, and inactivity or low level of bioscavengers can lead to oncogenesis (34) and many heart diseases (25). The activity of XO is increased during ischemia, ischemia/reperfusion, and calcium paradox conditions of heart (13,15). The cytokines produced by leukocytes, macrophages, and neutrophils can convert the XDH into XO form of endothelial cells which may produce more free radicals in cardiovascular system (35). These free radicals may activate the lipid peroxidation which further inactivates the PON1 activity after reaction with –SH Groups or damaging the structure of PON1 enzyme (36). It may be concluded that free radicals are a major factor to reduce the bioscavenging activity of PON1 enzyme due to structural changes of proteins.

It is concluded that the elevated level of XO, low level of HDL, and PON1 enzyme may be involved in atherosclerosis and in the development of other pathological conditions. Experiments are underway to further examine the correlation of free radicals, PON1 and XO activities during pathological stages.

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