Original Article

Evaluation of Paraoxonase, Malondialdehyde, and Lipoprotein Levels in Patients with Asymptomatic Cholelithiasis

Aytac Atamer, Ayse O. Kurdas-Ovunc, Atakan Yesil, Yildiz Atamer[1]

Departments of Internal Medicine, Division of Gastroenterology, Republic of Turkey, Ministry of Health Haydarpaşa Numune Training and Research Hospital, Istanbul, [1]Clinical Biochemistry, Dicle University Medical Faculty, Diyarbakır, Turkey

Address for correspondence:
Ass. Prof. Aytaç Atamer, Division of Gastroenterology, Department of Internal Medicine, Republic of Turkey, Ministry of Health Haydarpaşa Numune Training and Research Hospital, Tıbbiye Caddesi No: 40, Üsküdar, 34668, Istanbul, Turkey.
E-mail: aytacatamer1@gmail.com

ABSTRACT

Background/Aim: To compare lipoprotein and malondialdehyde levels and paraoxonase-1 activity between subjects with asymptomatic cholelithiasis and controls. **Patients and Methods:** Eighty subjects with asymptomatic cholelithiasis (55 women, 25 men, mean age: 51, SD 14 years) and 40 control subjects without cholelithiasis (25 women, 25 men, mean age: 51, SD 12 years) were enrolled to the study. Serum paraoxonase activity, lipoproteins, and malondialdehyde were measured. **Results:** In the cholelithiasis group, serum total cholesterol, low-density lipoprotein cholesterol, and malondialdehyde were significantly higher and high-density lipoprotein cholesterol (HDL-C) and paraoxonase-1 were significantly lower than the controls. In cholelithiasis patients with serum glucose level > 100 mg/dL, body mass index, serum total cholesterol, triglyceride (TG), and malondialdehyde levels were significantly higher than cholelithiasis patients with serum glucose level < 100 mg/dL. Paraoxonase-1 activity was significantly lower in patients with serum glucose level > 100 mg/dL. In cholelithiasis patients with TG > 150 mg/dL, mean age, body mass index, glucose, total cholesterol, and malondialdehyde were significantly higher than in cholelithiasis patients with TG < 150 mg/dL. In cholelithiasis subgroup with TG > 150 mg/dL, HDL-C level and paraoxonase-1 activity were lower than in the cholelithiasis subgroup with TG < 150 mg/dL. All of the above comparisons were statistically significant ($P < 0.05$). **Conclusions:** Patients with asymptomatic cholelithiasis have evidence of increased lipid peroxidation and decreased antioxidant capacity. Patients with asymptomatic cholelithiasis with components of the metabolic syndrome have more lipid peroxidation and less antioxidant capacity than patients with asymptomatic cholelithiasis but without the components of the metabolic syndrome.

Key Words: Cholelithiasis, lipoproteins, malondialdehyde, paraoxonase

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Factors increasing hepatic secretion of biliary cholesterol (obesity, aging, medications, oral contraceptives, oestrogen, and progesterone), oversaturation of bile with cholesterol, impaired gallbladder motility, and an increase in nucleating factors contribute to the formation of cholesterol gallstones.[1,2] Before the development of gallstones or cholesterol monohydrate crystals, inflammation of the gallbladder mucosa occurs.[3,4] The changes in gallbladder mucosa are characterized by an acute inflammatory reaction that contains granulocyte infiltration, edema, mucus hypersecretion, accumulation of mucus gel, glandular hyperplasia, and cell proliferation.[4] The mucin gel is thought to enhance gallstone formation by binding to biliary lipids and promoting cholesterol crystal precipitation and aggregation.[5] The gallbladder mucosal inflammation consists of infiltration with phagocytes that generate reactive oxygen species. The hydroxyl radical is an important oxidant that can abstract hydrogen atoms from polyunsaturated fatty acids, a reaction that can start lipid peroxidation. Lipid peroxidation in turn generates proinflammatory agents such as free fatty acids, lipid peroxides, or aldehydes, such as malondialdehyde and 4-hydroxynonenal.[6] Hydroxyl radicals stimulate the release of glycoproteins, such as mucin from gallbladder epithelium,[1] resulting in the formation of cholesterol crystal nucleation.

Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species

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that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products, in analogy to advanced glycation end-products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism.[7] Malondialdehyde is a potent stimulator of mucin secretion by cultured dog gallbladder epithelial cells.[1,5] Jüngst *et al*.,[5] showed that malondialdehyde stimulates mucin secretion of cultured gallbladder epithelial cells in a concentration-dependent manner. Lipid peroxidation promotes hypersecretion of mucin glycoprotein and causes nucleation and leads to the rapid formation of cholesterol crystals in bile.[3]

Paraoxonase (PON) hydrolyzes aromatic carboxylic acid esters and organophosphorus compounds, like paraoxon and nerve gas.[8] PON is anchored to high-density lipoprotein cholesterol (HDL-C) by connections between its hydrophobic N-terminal and Apo A-1.[9] PON multigene family comprises *PON-1*, *PON-2*, and *PON-3* genes located on chromosome 7q21-22.[9] In humans, both *PON-1* and *PON-3* genes are primarily expressed in the liver and their protein products are found in plasma associated with HDL-C.[10,11] *PON-2* gene is ubiquitously expressed as an intracellular enzyme and is not found in the circulation.[12] PON prevents low-density protein cholesterol (LDL-C) oxidation, cellular lipid oxidation, and protects against LDL-induced cytotoxicity.[13,14] In macrophage cell culture experiments, PON-1 has been found to decrease the ability of macrophages to oxidise LDL-C, decrease cholesterol influx and cholesterol synthesis, increase cholesterol efflux from macrophages[15] and suppress macrophage proinflammatory responses.[16] PON-1 also inhibits monocyte chemoattractant protein-1 production by arterial cells[17] and reduces lipid hydroperoxide concentrations.[14,15] PON-1 has homocystein-thiolactonase activity, which protects proteins from *N*-homocysteinylation.[18] *N*-homocysteinylation causes damage to proteins, resistance to fibrinolysis, and enhances atherosclerosis.[19] PON-1 was suggested as the major determinant of clopidogrel antiplatelet efficiency[20] but this was not confirmed by a later study.[21] A number of clinical studies have shown reduced PON-1 activity in patients with cardiovascular disease[22-24] or in patients with cardiovascular risk factors, such as obesity, diabetes mellitus, and dyslipidemia.[25] Cardiovascular risk associated with low PON-1 activity seems to be independent of HDL-C.[14]

Geetha[26] studied oxidant and antioxidant levels in mucosal scrapings of 30 subjects with gallstones who underwent cholecystectomy and found that lipid hydroperoxides, conjugated dienes, and oxidized lipids were increased in gallbladder mucosa of subjects with cholelithiasis, whereas glutathione, catalase, and superoxide dismutase were decreased when compared with the mucosa of gallstone-free gallbladders.[26] PON activity was not evaluated in that study.

We think that gallbladder inflammation and increased lipid peroxidation in gallbladder may be a manifestation of a systemic subclinical inflammatory status rather than a local process limited to the gallbladder. Therefore, we undertook a study that compared malondialdehyde and lipid levels and the activity of an antioxidant enzyme, PON-1 in subjects with cholelithiasis and healthy controls. We also evaluated the changes in these parameters in cholelithiasis subjects when stratified according to metabolic syndrome component cut-offs.

PATIENTS AND METHODS

Patients visiting our Gastroenterology Clinic between November 2008 and December 2010 were enrolled to the study. Medical history was obtained and clinical, biochemical, and abdominal ultrasound examinations were performed. The same physician obtained all measurements related to physical examination. All patients signed informed consent. The Ethical Committee of our hospital approved the study. The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Inclusion criteria

Patients who were diagnosed with asymptomatic cholelithiasis with normal C-reactive protein level (to rule out subclinical inflammation) and normal serum bilirubin, alkaline phosphatase, and gamma-glutamyltransferase (GGT) and patients without biliary obstruction on abdominal ultrasonography were included.

Exclusion criteria

Patients with hepatic, renal, endocrine or autoimmune disease, acute cholecystitis, biliary colic, acute pancreatitis, chronic inflammatory bowel disease, uncontrolled hypertension, known gastrointestinal disease, acute blood loss, history of excessive alcohol and cigarette use, cognitive impairment, and patients who are on restrictive diets were excluded. Subjects using hypolipidemic drugs or oestrogen-containing products were also excluded from the study.

Laboratory methods

After taking detailed medical history, all subjects underwent physical examination and height, weight, systolic and diastolic blood pressures were measured. Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2). Blood sampling and ultrasound studies were performed after 8-12 h of fasting. Blood samples were taken in the morning between 8 am and 9 am. Serums were divided into two parts. Routine biochemical parameters, fasting blood glucose, total cholesterol, triglycerides (TGs), HDL-C, LDL-C, highly sensitive C-reactive protein (hsCRP), alanine transaminase (ALT), aspartate transaminase, GGT, bilirubin, electrolytes, urea, creatinine, calcium, phosphate, and iron

were measured in the hospital laboratory immediately. Dimension Clinical Chemistry System (Dade Behring, Inc., Newark, DE, USA) was used to measure routine biochemical parameters. Serum was stored at −70°C for PON-1 activity and malondialdehyde assay.

Measurement of thiobarbituric acid reactive substances (TBARS) was used to reflect malondialdehyde levels.[27,28] The conversion of lipid hydroperoxides to malondialdehyde is thought to be one of the major contributors in the TBARS assay.[27] The samples were heated with thiobarbituric acid under acidic conditions. The adduct formed during the reaction was measured by absorbance.[27,28] The pink color formed at the thiobarbituric acid reaction was read at a spectrophotometer at 532 nm. Serum malondialdehyde values were calculated using the extinction coefficient of the malondialdehyde-thiobarbituric acid complex (532 nm = 1.56×10^5 mol/cm) and expressed as nmol/mL. PON-1 activity was measured as described by Furlong *et al.*[29] PON-1 activity is expressed as units per litre. GE Logic 200 (GE Medical Systems, Milwaukee, WI, USA) was used for abdominal ultrasonography. Intraluminal echogenicity with posterior acoustic shadowing was assessed as gallstones.

Statistical analysis

The data were evaluated for statistical analysis by using the Statistical Package for Social Sciences for Windows software, version 17.0 (SPSS Inc., Chicago, IL, USA). Distribution of the quantitative variables for normality was tested with the Kolmogorov–Smirnov test. Qualitative variables were presented as frequencies and percentages and tested with a Chi-square test. Quantitative variables with a normal distribution were tested with a Student's *t* test. Independent samples with an abnormal distribution were tested by a Mann–Whitney *U* test. Pearson product–moment correlation coefficient was estimated to explore the relationship between normally distributed quantitative variables. Spearman's rank correlation coefficient was estimated to explore the relationship among quantitative variables with a skewed distribution. Statistical significance was accepted if $P < 0.05$.

RESULTS

Cholelithiasis group included 55 women (68.8%) and 25 men (31.3%) with a mean age ± standard deviation (SD) of 50.56 ± 14.28 years. Twenty-five women (62.5%) and 15 men (37.5%) with a mean age ± SD of 50.93 ± 11.73 years were included in the control group. Clinical and biochemical parameters of cholelithiasis and control groups are shown in Table 1. In the cholelithiasis group, total cholesterol, LDL-C, and malondialdehyde levels were significantly higher than in the control group. In the cholelithiasis group, HDL-C and PON-1 were significantly lower than in the control group [Table 1]. In cholelithiasis patients with serum glucose level > 100 mg/dL, BMI, total cholesterol, TGs, and malondialdehyde [Figure 1] levels were significantly higher than in cholelithiasis patients with serum glucose level < 100 mg/dL [Table 2]. PON-1 activity was lower in cholelithiasis patients with serum glucose level > 100 mg/dL [Table 2 and Figure 1]. In cholelithiasis patients with serum TG level > 150 mg/dL, mean age, BMI, glucose, total cholesterol, and malondialdehyde [Figure 2] levels were significantly higher than in cholelithiasis patients with serum TG level < 150 mg/dL [Table 3]. HDL-C levels and PON-1 activity were lower in patients with serum TG level > 150 mg/dL [Table 3 and Figure 2]. In cholelithiasis patients with serum total cholesterol > 200 mg/dL, BMI, glucose, LDL-C, TGs, ALT, and malondialdehyde [Figure 3] levels were significantly higher than in cholelithiasis patients with serum total cholesterol < 200 mg/dL [Table 4]. PON-1 activity was lower in cholelithiasis patients with serum total cholesterol >200 mg/dL [Table 4 and Figure 3]. In the cholelithiasis group, a negative correlation was detected

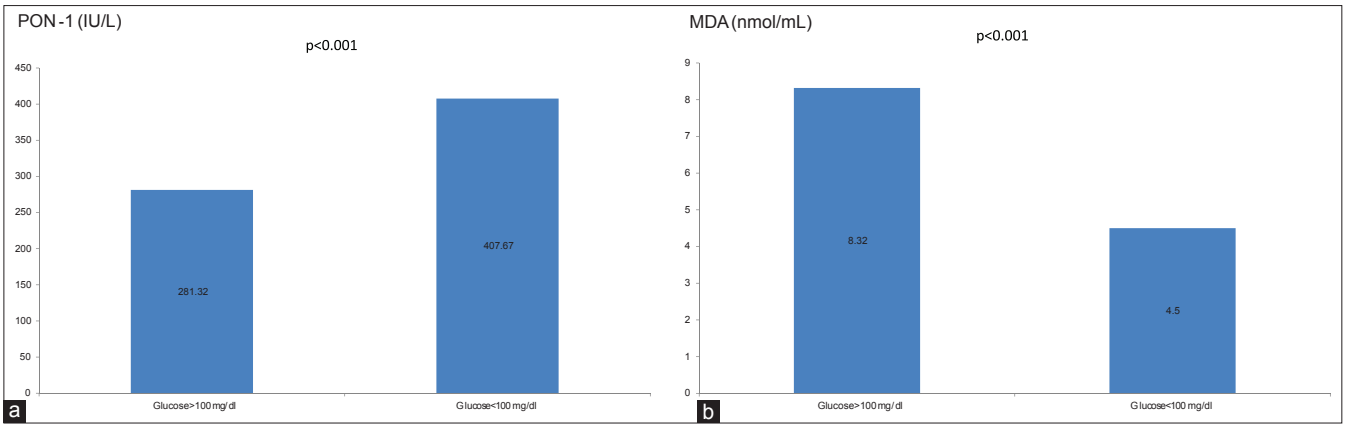

Figure 1: Comparison of PON-1 activity (a) and MDA (b) levels stratified by glucose level>100 mg/dL or<100 mg/dL in patients with cholelithiasis. PON-1, paraoxonase, MDA, malondialdehyde (Mann–Whitney *U* test)

between the PON-1 activity and malondialdehyde levels and a positive correlation was detected between the PON-1 activity and HDL-C level [Tables 5 and 6, Figure 4].

DISCUSSION

In summary, we found that malondialdehyde level, the end-product of lipid peroxidation was considerably higher and the antioxidant enzyme PON-1 activity was lower in subjects with cholelithiasis than in healthy controls. In cholelithiasis patients, malondialdehyde levels were correlated positively with fasting blood glucose, TG, and total cholesterol and negatively with HDL-C and PON-1 activity. No significant correlations were observed in the control group. Because PON-1 is an antioxidant enzyme, it may be considered that abnormal changes in lipid parameters that cause oxidation may decrease PON-1 activity. It was suggested that PON-1 is inactivated by the interaction between the oxidized lipids and the sulphydryl group of PON. Therefore, lower PON-1 activity may reflect increased oxidative stress.[30] PON-1 neutralizes the atherogenic effects of the lipid peroxides and protects cell membranes. We think

Table 1: Comparison of clinical and biochemical parameters between healthy control and cholelithiasis group

Variables	Healthy control (*n*=40)		Cholelithiasis (*n*=80)		*P*
	Mean	SD	Mean	SD	
Age (years)[a]	50.93	11.73	50.56	14.28	0.350
Glucose (mg/dL)[b]	95.70	10.00	101.70	21.37	0.218
T.Chol (mg/dL)[b]	179.45	20.05	200.15	39.91	0.001
HDL-C (mg/dL)[a]	49.35	6.74	41.18	14.18	0.001
LDL-C (mg/dL)[a]	102.84	23.96	124.26	31.62	0.049
Triglyceride (mg/dL)[b]	146.20	14.71	159.34	54.15	0.452
hsCRP (mg/dL)[b]	0.31	0.11	0.35	0.21	0.764
ALT (IU/L)[b]	33.60	6.61	34.15	19.05	0.335
AST (IU/L)[b]	26.00	6.75	27.48	12.50	0.350
GGT (IU/L)[b]	41.70	4.05	40.81	17.00	0.550
MDAb (nmol/mL)	3.62	1.44	5.64	1.89	0.000
PON-1[b] (IU/L)	441.20	47.02	346.07	109.83	0.001
Male/Female[c]	***n***	**%**	***n***	**%**	
Female	25	62.5	55	68.8	0.494
Male	15	37.5	25	31.3	

SD: Standard deviation, T.Chol: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hsCRP: Highly sensitive C-reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase, MDA: Malondialdehyde, PON-1: Paraoxonase. [a]Student's *t* test, [b]Mann-Whitney *U* test, [c]Chi-square test

Table 2: Comparison of age, body mass index and laboratory parameters between subjects with cholelithiasis who have fasting blood glucose >100 mg/dL and <100 mg/dL

Variables	Glucose>100 (*n*=39)		Glucose<100 (*n*=41)		*P*
	Mean	SD	Mean	SD	
Age (year)[a]	52.08	11.47	49.12	16.53	0.421
BMI (kg/m^2)[b]	28.36	1.46	25.48	1.28	0.000
T.Chol (mg/dL)[b]	209.21	47.30	191.54	29.42	0.048
HDL-C (mg/dL)[a]	40.21	14.16	42.11	14.31	0.461
LDL-C (mg/dL)[a]	126.36	29.75	122.27	33.54	0.328
Triglyceride (mg/dL)[b]	167.49	59.43	151.59	48.06	0.024
hsCRP (mg/dL)[b]	0.33	0.21	0.38	0.22	0.279
ALT (U/L)[b]	32.80	15.15	35.44	22.25	0.893
AST (U/L)[b]	26.62	13.24	24.39	11.81	0.485
GGT (U/L)[a]	36.00	17.02	35.63	17.18	0.935
MDA[b]	8.32	9.27	4.50	1.51	0.000
PON-1[b]	281.32	96.44	407.67	83.74	0.000

SD: Standard deviation, BMI: Body mass index, T.Chol: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hsCRP: Highly sensitive C-reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase, MDA: Malondialdehyde, PON-1: Paraoxonase activity. [a]Student's *t* test, [b]Mann-Whitney *U* test

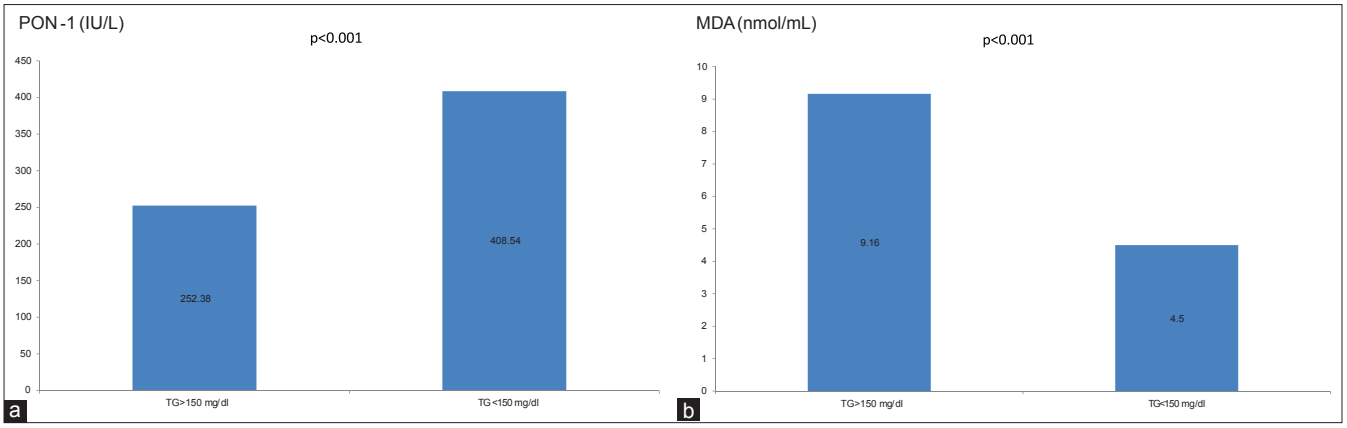

Figure 2: Comparison of PON-1 activity (a) and MDA level (b) stratified by triglycerides level > 150 mg/dL or < 150 mg/dL in patients with cholelithiasis. PON-1, paraoxonase, MDA, malondialdehyde (Mann–Whitney *U* test)

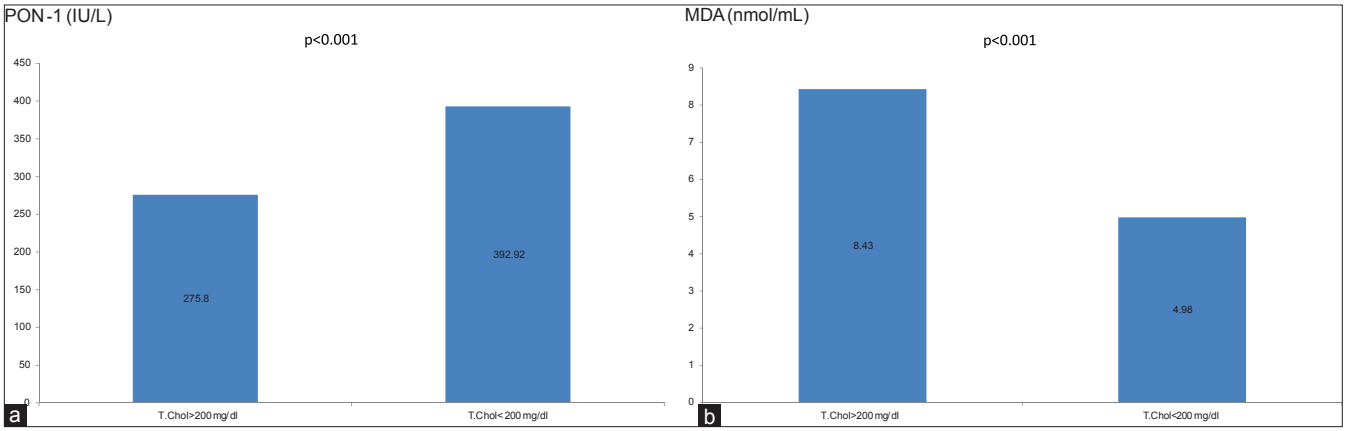

Figure 3: Comparison of PON-1 activity (a) and MDA level (b) stratified by total cholesterol >200 or <200 mg/dL in patients with cholelithiasis. PON-1, paraoxonase; MDA, malondialdehyde (Mann–Whitney *U* test).

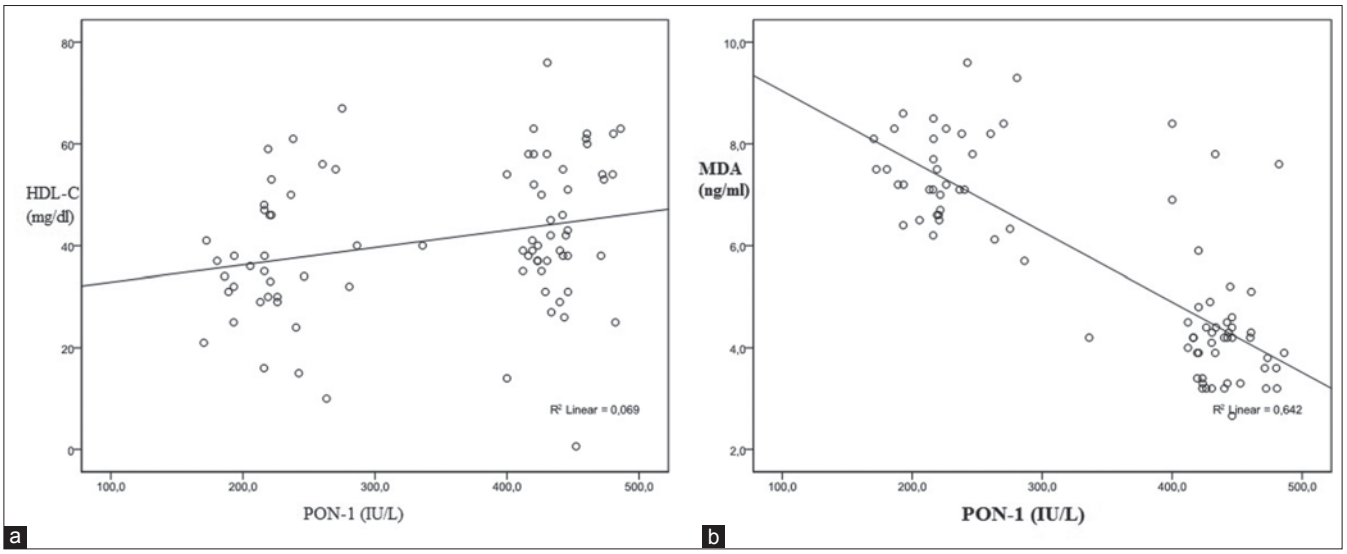

Figure 4: Correlation between HDL-C levels and PON-1 activity (a), and MDA level and PON-1 activity (b) in patients with cholelithiasis. HDL-C: High density lipoprotein cholesterol, PON-1: Paraoxonase, MDA: Malondialdehyde

that the relation between the PON-1 levels and cholelithiasis remains to be further elucidated in prospective studies.

All major classes of biomolecules are affected by free radicals, but the most sensitive molecules are lipids.[31] Oxidative stress plays a role in the occurrence of several diseases. Oxidative stress requires either increased reactive oxygen species formation or decreased antioxidant defence mechanisms. Antioxidants not only prevent lipid peroxidation but also protect protein, nucleic acids, and carbohydrates.[31] LDL oxidation is an important step in the development of atherosclerotic lesions. PON-1 prevents the inflammatory response in arterial wall cells.[32] It has been emphasized that prolonged oxidative stress and changes in antioxidant capacity may also be associated with the occurrence of complications in chronic cholelithiasis.[1,33] Mediators released as a result of oxidative damage lead to an increase in free radicals, activate the coagulation cascade, and result in disturbances of microcirculation. Free radicals affect the formation of cholesterol crystals indirectly by stimulating the mucus hypersecretion.[3]

In vitro experiments have shown that lipid peroxidation induces cholesterol crystal formation in model bile.[6] *In vivo* studies have also shown that lipid peroxidation is increased in gallbladder bile of patients with cholesterol gallstones. Jüngst *et al*.,[34] confirmed the role of lipid peroxides in the formation of gallstones by showing that ursodeoxycholic acid treatment reduced malondialdehyde and hydrophobic bile acid (cholic, deoxycholic, and chenodeoxycholic acid) levels in the bile of subjects with cholelithiasis who underwent cholecystectomy. Ursodeoxycholic acid-treated

Table 3: Comparison of age, body mass index and laboratory parameters between subjects with cholelithiasis who have fasting triglyceride levels >150 mg/dL and <150 mg/dL

Variables	TG>150 (*n*=32)		TG<150 (*n*=48)		*P*
	Mean	SD	Mean	SD	
Age (year)[a]	55.34	11.57	47.38	15.11	0.013
BMI (kg/m^2)[b]	28.22	1.34	25.99	1.86	0.000
Glucose (mg/dL)[b]	112.28	15.98	94.65	21.72	0.000
T.Chol (mg/dL)[b]	219.44	46.56	187.29	28.74	0.000
HDL-C (mg/dL)[a]	36.16	15.00	44.53	12.69	0.009
LDL-C (mg/dL)[a]	127.75	26.87	121.94	34.50	0.424
hsCRP (mg/dL)[b]	0.37	0.25	0.34	0.19	0.949
ALT (U/L)[b]	34.69	14.78	33.79	21.58	0.401
AST (U/L)[b]	25.97	14.34	25.15	11.25	0.609
GGT (U/L)[a]	37.31	16.37	34.81	17.50	0.523
MDA[b]	9.16	10.06	4.50	1.40	0.000
PON-1[b]	252.38	82.08	408.54	76.62	0.000

TG: Triglyceride level, SD: Standard deviation, BMI: Body mass index, T.Chol: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hsCRP: Highly sensitive C-reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase, MDA: Malondialdehyde, PON-1: Paraoxonase activity, [a]Student's *t* test, [b]Mann-Whitney *U* test

Table 4: Comparison of age, body mass index and laboratory parameters between subjects with cholelithiasis who have total cholesterol >200 mg/dL and <200 mg/dL

Variables	T.Chol>200 (*n*=32)		T.Chol<200 (*n*=48)		*P*
	Mean	SD	Mean	SD	
Age (year)[a]	53.16	12.80	48.83	15.06	0.186
BMI (kg/m^2)[b]	27.97	1.47	26.16	1.97	0.000
Glucose (mg/dL)[b]	109.63	16.44	96.42	22.76	0.006
HDL-C (mg/dL)[a]	39.09	13.07	42.58	14.85	0.285
LDL-C (mg/dL)[a]	134.97	22.79	117.13	34.75	0.007
Triglyceride (mg/dL)[b]	193.13	55.86	136.81	39.69	0.000
hsCRP (mg/dL)[b]	0.35	0.23	0.36	0.20	0.655
ALT (U/L)[b]	37.03	14.49	32.23	21.50	0.048
AST (U/L)[b]	25.75	13.90	25.29	11.62	0.606
GGT (U/L)[a]	36.00	16.87	35.69	17.26	0.936
MDA[b]	8.43	10.27	4.98	1.80	0.000
PON-1[b]	275.80	103.33	392.92	87.35	0.000

T.Chol: Total cholesterol, SD: Standard deviation, BMI: Body mass index, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hsCRP: Highly sensitive C-reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase, MDA: Malondialdehyde, PON-1: Paraoxonase activity, [a]Student's *t* test, [b]Mann-Whitney *U* test

Table 5: Correlation analysis among malondialdehyde level and laboratory parameters in subjects with cholelithiasis and controls

Variables	Control subjects (*n*=40) MDA		Cholelithiasis subjects (*n*=80) MDA	
	r	*P*	*r*	*P*
Glucose	0.108	0.509	0.628	0.000
T.Chol	−0.004	0.983	0.318	0.004
HDL-C	−0.047	0.773	−0.292	0.008
LDL-C	0.238	0.139	0.139	0.219
Triglyceride	−0.177	0.276	0.520	0.000
hsCRP	0.000	0.999	−0.026	0.820
ALT	0.096	0.554	0.091	0.421
AST	0.110	0.501	0.078	0.492
GGT	0.131	0.421	0.077	0.496
PON-1	0.166	0.307	−0.712	0.000

MDA: Malondialdehyde, *r*: Pearson product-moment correlation coefficient or Spearman's rank correlation coefficient as appropriate, T.Chol: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hsCRP: Highly sensitive C-reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase, PON-1: Paraoxonase activity

Table 6: Correlation analysis among paroxonase activity and laboratory parameters in subjects with cholelithiasis and controls

Variables	Control subjects (*n*=40) PON-1		Cholelithiasis subjects (*n*=80) PON-1	
	r	*P*	*r*	*P*
Glucose	0.247	0.125	−0.582	0.000
T.Chol	0.143	0.380	−0.360	0.001
HDL-C	−0.290	0.069	0.309	0.005
LDL-C	0.135	0.407	−0.205	0.069
Triglyceride	0.209	0.196	−0.556	0.000
hsCRP	0.075	0.647	0.089	0.431
ALT	0.037	0.820	−0.161	0.153
AST	−0.081	0.618	−0.033	0.768
GGT	−0.030	0.852	−0.174	0.123
MDA	0.166	0.307	−0.712	0.000

PON-1: Paraoxonase activity, *r*: Pearson product-moment correlation coefficient or Spearman's rank correlation coefficient as appropriate, T.Chol: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, hsCRP: Highly sensitive C-reactive protein, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase, MDA: Malondialdehyde

bile also showed decreased biliary cholesterol content, decreased mucin secretagogue activity, and less crystal observation time compared with placebo-treated bile. Both ursodeoxycholic acid- and placebo-treated bile were obtained in cholecystectomy. The authors attributed the higher mucin secretagogue activity of placebo-treated lithogenic bile to higher malondialdehyde concentrations.[34] Lower mucin secretagogue activity of ursodeoxycholic acid-treated bile was thought to be secondary to lower malondialdehyde levels.[34] The above-mentioned *in vitro* and *in vivo* experiments clearly show that gallbladder mucosal inflammation is caused by several synergistic factors including lipid peroxidation, a change in bile acid composition toward

more hydrophobic bile acids, a change in the amount and/or activity of inflammation-related enzymes, such as cyclooxygenase-2, more biliary cholesterol excretion and less biliary phospholipid excretion into the bile.[1] Carotti and coworkers showed higher number of CD68-positive monocytes/macrophages, granulocytes, mast cells, and inducible nitric oxide synthase positive cells and higher level of cyclooxygenase-2 enzyme in the muscle layer of gallstone patients compared with controls.[35]

Worthington *et al.*,[33] showed that dietary antioxidant deficiency contributes to the development of cholesterol gallstones. They reported that vitamin E to cholesterol ratio, β-carotene, vitamin C, glutathione, pyridoxyl-5-phosphate, and folate levels were lower in cholelithiasis subjects compared with controls.[33] Kaur *et al.*,[36] reported increased malondialdehyde level and glutathione disulfide to glutathione ratio significantly reduced total glutathione levels, and decreased activity of antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase in subjects with cholelithiasis. These previous studies have not evaluated PON levels in subjects with cholelithiasis. Inclusion of other elements of antioxidant system such as superoxide dismutase, catalase, glutathione peroxidase, vitamin B12, folic acid, or vitamin C level would have been more informative for our study, but we only measured two parameters of oxidant versus antioxidant status, malondialdehyde and PON-1, a limitation of our study.

We conclude that patients with asymptomatic cholelithiasis have evidence of increased lipid peroxidation and decreased antioxidant capacity, pointing to oxidant/antioxidant imbalance. Patients with asymptomatic cholelithiasis with components of the metabolic syndrome have more lipid peroxidation and less antioxidant capacity than subjects with asymptomatic cholelithiasis but without the components of the metabolic syndrome.

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