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

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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

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. Before the development of gallstones or cholesterol monohydrate crystals, inflammation of the gallbladder mucosa occurs. 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. The mucin gel is thought to enhance gallstone formation by binding to biliary lipids and promoting cholesterol crystal precipitation and aggregation. 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. Hydroxyl radicals stimulate the release of glycoproteins, such as mucin from gallbladder epithelium, 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.
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. Malondialdehyde is a potent stimulator of mucin secretion by cultured dog gallbladder epithelial cells. Jungst et al., 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.

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

Geetha 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. 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/m²). 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. Serum was 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 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.
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. The conversion of lipid hydroperoxides to malondialdehyde is thought to be one of the major contributors in the TBARS assay. The samples were heated with thiobarbituric acid under acidic conditions. The adduct formed during the reaction was measured by absorbance. 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. 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 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. 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...
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

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

| Variables               | Healthy control (n=40) | Cholelithiasis (n=80) | P    |
|-------------------------|------------------------|-----------------------|------|
| Age (years)²           | Mean 50.93             | Mean 50.56            | 0.350|
| Glucose (mg/dL)b        | Mean 95.70              | Mean 101.70           | 0.218|
| T.Chol (mg/dL)³         | Mean 179.45             | Mean 200.15           | 0.001|
| HDL-C (mg/dL)⁴         | Mean 49.35              | Mean 41.18            | 0.001|
| LDL-C (mg/dL)⁷         | Mean 102.84             | Mean 124.26           | 0.049|
| Triglyceride (mg/dL)⁴  | Mean 146.20             | Mean 159.34           | 0.042|
| hscRP (mg/dL)b         | Mean 0.31               | Mean 0.35             | 0.764|
| ALT (IU/L)⁴            | Mean 33.60              | Mean 34.15            | 0.335|
| AST (IU/L)⁵            | Mean 26.00              | Mean 27.48            | 0.350|
| GGT (IU/L)⁴            | Mean 41.70              | Mean 40.81            | 0.550|
| MDA(b) (nmol/mL)⁶      | Mean 3.62               | Mean 5.64             | 0.000|
| PON-1+b (IU/L)         | Mean 414.20             | Mean 346.07           | 0.001|

Male/Female¹           | n 25 62.5%               | n 55 68.8%            | 0.494|

Male                   | Mean 15.37              | Mean 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. ²Student’s t test, ³Mann-Whitney U test, ⁴Chi-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     |
|-------------------------|--------------------|--------------------|-------|
| Age (year)²             | Mean 52.08         | Mean 49.12         | 0.421|
| BMI (kg/m²)³            | Mean 28.36         | Mean 25.48         | 0.000|
| T.Chol (mg/dL)³         | Mean 209.21        | Mean 191.54        | 0.048|
| HDL-C (mg/dL)⁴         | Mean 40.21         | Mean 42.11         | 0.461|
| LDL-C (mg/dL)⁴         | Mean 126.36        | Mean 122.27        | 0.038|
| Triglyceride (mg/dL)⁴  | Mean 167.49        | Mean 151.59        | 0.024|
| hscRP (mg/dL)b         | Mean 0.33          | Mean 0.33          | 0.279|
| ALT (U/L)⁴             | Mean 32.80         | Mean 35.44         | 0.893|
| AST (U/L)⁵             | Mean 26.62         | Mean 24.39         | 0.818|
| GGT (U/L)⁴             | Mean 36.00         | Mean 35.63         | 0.935|
| MDA(b)                 | Mean 8.32          | Mean 4.50          | 0.000|
| PON-1+b                | Mean 281.32        | Mean 407.67        | 0.000|

Male/Female¹           | n 25 62.5%               | n 55 68.8%            | 0.494|

Male                   | Mean 15.37              | Mean 25 31.3%    |

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. ²Student’s t test, ³Mann-Whitney U test

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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)
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. [32] 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. [34] 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         | Control (n=40) | Cholelithiasis subjects (n=80) |
|-------------------|----------------|--------------------------------|
| Glucose           | 0.108 0.509    | 0.628 0.000                   |
| T.Chol            | −0.004 0.983   | 0.318 0.004                   |
| LDL-C             | −0.047 0.773   | −0.292 0.008                  |
| HDL-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                  |

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, *Student’s t test, †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         | Control (n=40) | Cholelithiasis subjects (n=80) |
|-------------------|----------------|--------------------------------|
| 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                   |

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, *Student’s t test, †Mann-Whitney U test

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. Lower mucin secretagogue activity of ursodeoxycholic acid-treated bile was thought to be secondary to lower malondialdehyde levels. 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 secretagogue activity of placebo-treated lithogenic bile to higher malondialdehyde concentrations. Lower mucin secretagogue activity of ursodeoxycholic acid-treated bile was thought to be secondary to lower malondialdehyde levels. 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.[13]

Worthington et al.,[31] 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 cholitis patients compared with controls.[13] 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 cholecystitis. These previous studies have not evaluated PON levels in subjects with cholecystitis. 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 cholecystitis have evidence of increased lipid peroxidation and decreased antioxidant capacity, pointing to oxidant/antioxidant imbalance. Patients with asymptomatic cholecystitis with components of the metabolic syndrome have more lipid peroxidation and less antioxidant capacity than subjects with asymptomatic cholecystitis but without the components of the metabolic syndrome.

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