

Oxidative Stress and Some Liver Functions Parameters in Patients with Symptomatic Cholelithiasis

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Summary:

Background: Gallstone disease is one of a common surgical problem and one of the most common gastrointestinal diseases throughout the world but its pathogenesis remains unclear. Many theories have been put forward to explain the mechanism of stone formation. The living organism has enzymatic and non-enzymatic antioxidant systems neutralizing the harmful effects of the endogenous oxygen free radicals products. Under certain conditions, the oxidative or anti-oxidative balance shifts towards the oxidative status as a result of increase in oxygen free radicals and/or impairment in antioxidant mechanism.

Objective: To evaluate the oxidative stress markers (total antioxidants capacity and malondialdehyde) and some liver functions parameters (aspartate aminotransferase, alanin aminotransferase, alkaline phosphatase, total serum bilirubin) in serum of patients with gallstone disease and in control group.

Patients & Methods: The study group included 75 patient with gallstone disease and 33 age- and sex-matched healthy volunteers as control group. Antioxidative status of serum was evaluated by measuring total antioxidant capacity, serum: malondialdehyde, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin, were all measured in serum of patients with gallstones and controls.

Results : There was a significant increase ($P < 0.05$) in serum: malondialdehyde, alkaline phosphatase, alanin aminotransferase, aspartate aminotransferase, total serum bilirubin levels in patients with symptomatic gallstones compared to healthy controls. The study also showed that there was a significant decrease in serum total antioxidant capacity ($P < 0.05$) in the patients compared to control group.

Conclusion: The present study results indicated that patients with gallstones may have been exposed to oxidative stress. Change in liver functions parameters was also seen in serum of patients compared to controls. These findings may provide insights and help in understanding the various biochemical alterations undergoing in the gallbladder responsible for stone formation and would be helpful in developing strategies for the treatment and prevention of gallstones.

Keywords: Gallstones, Total antioxidants, Malondialdehyde, Oxidative stress, Liver functions parameters.

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Introduction:

Cholelithiasis (the presence of one or more gallstones in the bladder) is a very common gastrointestinal disorder that causes a significant burden for healthcare systems worldwide [1]. Most of gallstones are asymptomatic and only 20–30% of gallstones are symptomatic [2]. Symptoms of gallstone diseases are pain in the right upper abdomen (biliary colic), abdominal fullness, nausea and vomiting [1]. Despite its common occurrence and profound impact, the pathogenesis of gallstones remains incompletely understood [3]. Many theories have been put forward to explain the mechanism of stone formation [4]. The reported risk factors are obesity, female gender, childbearing, family history, age, hypertriglyceridemia, various medications (such as estrogens, clofibrate and ceftriaxone), terminal ileal resection, and gallbladder hypomotility as seen in post-vagotomy and total parenteral nutrition.

New risk factors continue to be identified [3]. Oxidative stress (OS) is a disturbance in the oxidant-antioxidant balance leading to potential cellular damage. Most cells can tolerate a mild degree of oxidative stress, because they have sufficient antioxidant defence capacity and repair systems, which recognize and remove molecules damaged by oxidation. The imbalance can result from a lack of antioxidant capacity caused by disturbances in production and distribution, or by an overabundance of reactive oxygen species (ROS) from other factors [5]. Antioxidants constitute a highly heterogeneous group. They include low molecular weight substances either water-soluble (e.g. ascorbic acid) or lipid-soluble (e.g. Vitamin E), incorporated in the body through nutrition. In addition, a number of endogenous metabolites (uric acid, lipids, albumin, bilirubin) possess antioxidant activities. The sum of endogenous and food-derived antioxidants represents the total antioxidant capacity (TAC) of extracellular fluids [6]. Increased levels of an accelerated generation of reactive oxygen species (ROS) and toxic degradation products of lipid peroxidation have been

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reported in the plasma of individuals with gallstones [7]. It is well known that, in chronic diseases such as cholelithiasis, the active inflammatory response is induced with neutrophilic infiltration. These neutrophils, macrophages and/or monocytes produce ROS which may cause DNA damage to the adjacent cells[8,9]. Some liver functions parameters change between gallstones patients and controls have been reported[10].

Methods & Materials:

The study was carried out on patients with Symptomatic Gallstones (SGS) admitted to the surgery unit in Baghdad Medical City Hospital from October 2010 to May 2011. A total 75 patients (9 males and 66 females) were included in the study, in the age group of 20-64 years, to compare the results ,33(4 males and 29 females- age and sex matched) healthy controls were also included. The diagnosis of symptomatic gallstones depend on the presence of typical symptoms and the demonstration of stones on diagnostic imaging, small stones(<8mm in diameter) pass in to the common bile duct, producing of indigestion and biliary colic (pain located in the epigastrium and/or right upper quadrant), larger stones are more likely to obstruct bile flow and cause jaundice . In ultrasound a gallstone appears as an echogenic structure within the gallbladder lumen that casts a distal acoustic shadow. After a 12 hours fasting , blood samples were obtained from patients(group A) and controls (group B) . Serum was separated from the blood by centrifuging at 3,000 rpm for 10–15 min.The serum were seperated and stored at - 80 C° till further use. Fresh serum was used for the measurement of malondialdehyde (biomarker of lipid peroxidation) and total antioxidant capacity Exclusion criteria were acute cholecystitis, pancreatitis, hepatobiliary disease, cirrhosis, malignant tumor, and antioxidant usage, Subjects suffered from diseases (hypertension , asthma and diabetes mellitus) or a history of ceftriaxone or somatostatin intake that may interfere with the data obtained were also excluded.Total antioxidant capacity and Maiondialdehyde were estimated colorimetrically using Chayman Chemicals (USA) kits Total bilirubin using Spinreact (Spain)kit , The activities of AST and ALT were calculated colorimetrically by using Randox (UK) kits, Alkaline phosphatase by using kit (Biolabo Reagents ,France).BMI calculated according to equation, Gene rally, the healthy weight falls between BMI values of 18.5 and 24.9, with underweight below 18.5, overweight between (25-30) , and obese above 30.

Statistical Analysis: The results were expressed as mean ± standard error of mean (SEM). Student’s t-test was used to examine the degree of significance. P values less than 0.05 was considered significant. The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 17) 2010.

Results:

The results of the study were showed in table 1 and 2 and figures 1 and 2 . Females (88%) to males (12%) ratio in the study was

approximately 7/1. BMI of gallstones patients is significantly higher(P<0.05) Mean ±SEM (29.86±0.57) than that of controls (21.34±0.55).There was a significant increase(P<0.05) in serum malondialdehyde, serum alkaline phosphatase ,serum alanin aminotranseferase ,aspartate aminotranseferase ,Total serum bilirubin .The study also showed that there was a significant decrease (P<0.05) in serum total antioxidant capacity , in patients symptomatic gallstone disease compared to control group .

Table 1: Some demographic charctrestics of SGS-patients and healthy controls.

	Variables	SGS-patients (N)=75 Mean ± SEM	Healthy controls N=33 Mean ± SEM
1.	Gender Female Male Female/ Male	66(88%) 9 (12%) 7.25/1	29(88%) 4 (12%) 7.3/1
2.	Age (years)	44.53 ± 1.39	44.78 ±2.02
3.	BMI (Kg/M2) Normal weight* Over weight* Obese*	29.86±0.57 14(18.67%) 21(28%) 40(53.3%)	21.34±0.55 28(84.85%) 3(9%) 2(6%)

* Normal weight(18.5-24.9), over weight (25-29.9), obese (≥30).

Table 2 : Comparison of the concentration of each analyte between the SGS- patient group and the control group Mean±SEM and P value are shown

	Analyte	SGS-patients N=75 Mean ± SEM	Controls N=33 Mean ± SEM	P-value
1.	Total antioxidant capacity (µmol/L)	0.93±0.01	2.15±0.05	<0.05
2.	Malondialdehyde (µmol/L)	7.85±0.40	2.53±0.11	<0.05
3.	Alanine aminotranseferase (U/L)	39.1±1.28	13.03±1.08	<0.05
4.	Aspartate aminotranseferase (U/L)	51.4±1.63	17.6±1.94	<0.05
5.	Alkaline phosphatase (U/L)	125.6±4.01	62.3±2.2	<0.05
6.	Total serum bilirubin (Mg/dl)	0.88±0.06	0.47±0.01	<0.05

NS = Not Significant

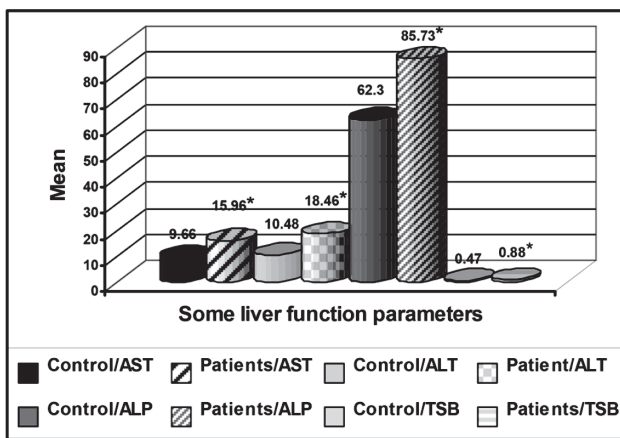


Figure (1): Mean of AST , ALT, ALP and TSB in SGS-Patients and controls.

*refers to a significant differences between means (P < 0.05) , S = Serum

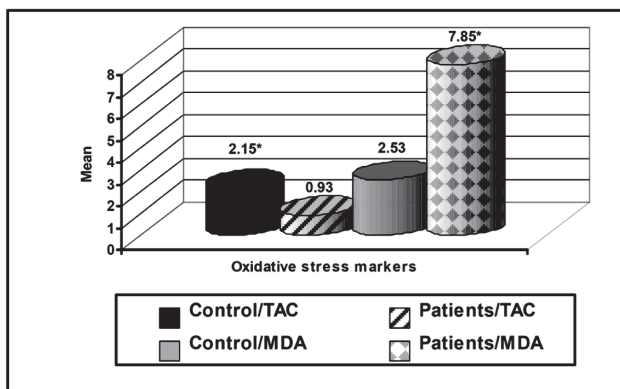


Figure (2):The mean of Serum MDA and Total antioxidants status in SGS-Patients and controls.

*refers to a significant differences between means (P < 0.05)

Discussion:

The present study in accordance with other studies[11,12] found that females are more susceptible to develop symptomatic gallstone than males, the female (88%) to male (12%) ratio in the study is 7 to 1 as shown in table 1. Henry Völzke, et al [11], found that Women had a two fold higher risk for cholelithiasis compared to men. Among persons younger than 40, the female/male prevalence ratio varied from 1.2 in Bergen, Norway, to 9.9 among Pima Indians. For ages 60 and older, the female/male ratio varied from 0.96 in Okinawa, Japan, to 2.9 among Mexican American in the south western USA [13]. The commonly perceived opinion that women are at greater risk of developing gallstone disease than men may largely be due to extraneous risk factors, such as pregnancy and sex hormones [12]. The results obtained from our study (as stated in table1) agree with other study which consider obesity as an important risk factor for cholelithiasis [14]. As stated in table1 that most of the patients in thw present study are obese (53%) and over

weight (28%) while only (6%) of the control group are obese. Obesity is recognized as a major gallstone risk factor, it has been associated with gallbladder dysmotility [15]. The study shows a non significance difference in mean age between SGS-patients and controls (P>0.05) the mean age is (44.53 ± 1.39) and (44.78 ±2.02) for the patients and controls respectively as stated in table 1 . The study also showed that increasing age increase the number of patients with gallstones in agreement with report which also showed that The greater incidence of gallstones is in the older persons[12] .Long-term exposure to many risk factors, as is true for the elderly, may increase the risk of gallstone disease. Table (2) and figure (2) showed that the mean of MDA was (7.85±0.40), for the patients and (2.53±0.11) for controls, the level of MDA in the patients was significantly higher (p<0.05) compared to healthy volunteers, the table also showed that the mean of TAC (0.93±0.17) of SGS-Patients was significantly lower(p<0.05), than that of controls (2.15±0.05). These results in agreement with other study [16] ,may suggest that SGS-patients were exposed to oxidative stress. Gallstones can induce inflammation in the gallbladder wall, and the composition of bile changes, at the same time the bilirubin metabolism, which is a potent antioxidant by radical scavenging and reducing activities, may be altered as cited by Sipos et al [17] .The changes in bile composition can increase the biliary free radical formation [18]. lipid peroxidation levels were found to be high in serum of gallstones patients as compared to patient without gallstones. Our results are supported by the recent findings that show increase in MDA concentration in the patients with Gallstones [19]. Total bilirubin concentration in sera showed a significant increase (P<0.05) in patients with Gallstones as compared to the patients without gallstones. Apart from the bilirubin values , AST, ALT, and alkaline phosphatase levels were compared between gallstone carriers and free subjects. Results showed a significant increase in the mean levels of serum AST, ALT, and alkaline phosphatase in the patients with gallstones as compared to controls in agree with other study [16], levels are within the normal range but the differerances between patients and controls in the levels of (AST, ALT, ALP, and TSB) are statistically signeficant. The results is in aggrement with Tranum et al[16] that show a significant increase in serum total bilirubin ,alanine aminotransferase , aspartate aminotransferase and alkaline phosphatase in Gallstone carrier compared to healthy controls . Gallstones diseases have been correlated with a variety of liver diseases and abnormality of liver functions enzymes [20,21].Bile stasis (one of the mechanisms that lead to gallstone formation) triggers release of enzymes like (AST,ALT,ALP) along with serum bilirubin level[22].

Conclusion:

In the light of these findings, we may conclude that the patients with gallstone are exposed to a potent oxidative stress, and elevation in some liver function enzymes and increased oxidative stress may play a role in the progression

of inflammatory changes in the gallbladder. Surgical removal of gallstones is the standard and the only effective treatment of symptomatic gallstones, supplementation of antioxidant-enriched diet to the therapy might shed light on the development of novel therapeutic and protection strategies for cholelithiasis, particularly for patients unfit for surgery. We need more studies that may develop the non-surgical treatment of gallstones.

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