

Association of Oxidative Stress Markers with Cholelithiasis

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Abstract

Oxidative stress markers are of important diagnostic parameters for many disorders including cholelithiasis. This present study has aimed to assess the state of oxidative stress in symptomatic radiographically confirmed (Cholelithiasis) patients by measuring two parameters used as oxidative stress parameters which are serum myeloperoxidase (MPO) and superoxide dismutase (SOD). This study was carried out on 100 patient diagnosed as (Cholelithiasis) patients with 30 age and sex matched healthy controls by measuring serum (MPO) and (SOD) by ELIZA technique. Results showed significantly decrease in antioxidant enzyme(SOD) and increase in serum level of (MPO) comparing with controls.

Keywords: Cholelithiasis, Oxidative stress, MPO, SOD.

مصاحبة عوامل الاجهاد التأكسدي مع حصوة المرارة
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الخلاصة

عوامل الاجهاد التأكسدي هي مؤشرات قوية للعديد من الحالات المرضية بما في ذلك (حصوة المرارة). البيانات الموجودة على العلاقة بين حصوة المرارة وعلامات الاجهاد التأكسدي محفزة ولكن محدودة. لذلك هدفت الدراسة الحالية الى تقييم حالة الاجهاد التأكسدي لدى مرضى حصوة المرارة المشخصين بالاشعة السينية من خلال عاملين من عوامل الاجهاد التأكسدي (MPO & SOD). شملت الدراسة 100 مريض مشخصين بحصوة المرارة مع 30 من الاصحاء كمجموعة سيطرة مطابقة لمجموعة المرضى في العمر والجنس، وظهرت النتائج انخفاض معنوي بمستوى انزيم SOD مع ارتفاع معنوي بمستوى MPO بالمقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: حصوة المرارة، الاجهاد التأكسدي، MPO، SOD.

Introduction

Cholelithiasis is a worldwide disease and it remains to be one of the most common health problems leading to surgical intervention ^(1,2). Cholelithiasis is common with the incidence ranging from (10-20)% of the world population ⁽³⁾, the prevalence of cholelithiasis varies considerably within population; the highest known prevalence is among American Indians with up to (60-70) % in females ⁽⁴⁾ and (10-15) % in white adults of developed countries ⁽⁵⁾. Cholelithiasis is classified as either cholesterol stones (Cs), pigment stones (Ps), or mixed stones (Ms) ⁽⁶⁾. These categories of gallstones can be identified according to their predominant chemical composition ⁽⁷⁾. Two thirds of individuals with cholelithiasis is asymptomatic ⁽⁸⁾. In these patients, the reasons why symptoms develop are unknown ⁽⁹⁾. Of these, symptoms will develop in 1% to 4% per year ^(10, 11). 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

defense 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 ⁽¹²⁾. Increased levels of an accelerated generation of reactive oxygen species and toxic degradation products of lipid peroxidation have been reported in the serum of individuals with cholelithiasis ⁽¹³⁾. 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 ^(14, 15). Superoxide dismutase or SOD is an enzyme that in humans is encoded by the SOD1 gene, located on chromosome 21. SOD is one of three human superoxide dismutase ^(16, 17). Superoxide dismutase (SOD) is an enzyme that in humans is encoded by the SOD1 gene, located on chromosome 21. SOD is one of three human superoxide dismutase ^(16, 17).

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Myeloperoxidase (MPO) is a peroxidase enzyme that in humans is encoded by the MPO gene⁽¹⁸⁾. Myeloperoxidase is most abundantly expressed in neutrophil granulocytes (a subtype of white blood cells). It is a lysosomal protein stored in azurophilic granules of the neutrophil. MPO has a heme pigment, which causes its green color in secretions rich in neutrophils, such as pus and some forms of mucus⁽¹⁹⁾. Gallstones and kidney stones are diseases with high prevalence⁽²⁰⁾. There has been a marked increase in the prevalence and incidence of these stones within the last 22 years⁽²¹⁾. In spite of different composition and different locations of their formation both types of stones may be associated⁽²²⁾. Obesity, insulin resistance and women with postmenopausal hormone use are at risk factors for renal stones as well as gallstones^(23, 24).

Materials and Methods

Subjects

This study was conducted at Tikrit Teaching Hospital, Tikrit, Iraq, and the laboratory investigations were done at Tikrit University, Department of Central Laboratories. It is carried from December 2012 to June 2013. A total of 100 patients (group A) were included in the study, among which 68 were females patients and 32 were males in the age group of (20-80) years and mean age (48.38 ± 1.17), to compare the results of 30 (age and sex matched) healthy controls (Group B) (18 females, 12 males) with mean age (47.16 ± 1.5) years were also included.

Patient's including criteria:

All patients were scanned for presence of gallstone by an abdominal ultrasonography (the standard diagnostic test for gallstone), and to confirm the absence of kidney stone.

Patient's exclusion criteria:

1. Detection complicated gallstone diseases like (acute cholecystitis, pancreatitis).
2. Patients with liver cirrhosis, viral hepatitis, renal failure and thyroid diseases and any type of cancer.
3. Patients with a history of alcohol consumption and smoking.
4. Subjects suffered from diseases like asthma.

Questionnaire

Subjects were asked to complete a questionnaire that asked for information on name, sex, age, marital state, number of children, pregnancy (for females only), family history of GS, chronic diseases, urinary tract complications, weight, height, occupation, residence, smoking habits, alcohol consumption, and drugs used.

Ultrasound for patients with cholelithiasis and nephrolithiasis were checked to confirm the

presence of calculi, number of stones and their average diameter.

All the laboratory investigations were done in Tikrit University, Central Laboratories Department.

Sample collection

After 12 hours of fasting, venous blood samples, about 10 ml were collected from patients before laparoscopic cholecystectomy and from healthy volunteers in plain tubes. After allowing the blood to clot at room temperature for about 15-30 min, blood samples were centrifuged at 3000 rpm for about 15-30 min to obtain serum. The serum were separated in multiple epiendroff tubes (0.5 ml) to prevent thawing of the serum frequently, The serum was stored and frozen at -20°C until analysis was performed.

Clinical and laboratory evaluation

1. Determination of Myeloperoxidase (MPO):

The test is based on the immobilisation of highly purified MPO to the solid phase of microtiter strips and subsequent binding of anti-MPO antibodies from patient serum by using ELISA technique. The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG. After addition of substrate solution, a color appears which intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow. The concentration of MPO in the samples is then determined by comparing the optical density of the samples to the standard curve.⁽²⁵⁾

2. Determination of human superoxide dismutase [Cu-Zn] (SOD1):

This assay employs the quantitative sandwich enzyme immunoassay technique (ELISA). Antibody specific for SOD1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SOD1 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for SOD1 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SOD1 bound in the initial step. The color development is stopped and the intensity of the color is measured⁽²⁶⁾.

Statistical analysis

The results were expressed as mean \pm 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).

Table (1): Demographic presentation of Cholelithiasis patients and control.

| Parameter | Control N =30 | Patient N = 100 | P- value |
|------------------------|---------------------|--------------------|-------------|
| Age (mean \pm SD) | 48.38 \pm 1.17 | 47.16 \pm 1.5 | 0.724 |
| F : M | 18:12 (60%) | 32:68 (68%) | 0.300 |
| Pregnancy | 2 (6.6%) | 4 (5.8%) | 0.485 |
| Family History | 10 (34%) | 55 (55%) | 0.092 |
| H.T | 4 (4%) | 14 (14%) | 0.748 |
| D.M | 3 (10%) | 5 (5%) | 0.748 |

Continuous variables presented as Mean \pm SD, and discrete variables as numbers and frequencies; F:M= female and male ratio; n= Number ; H.T= hyper tension; D.M= Diabetes mellitus; (*) = significant difference P < 0.05

Results

Demographic presentation of 100 cholelithiasis patients and 30 controls were elucidated in (table 1). Frequency matching was successful with Patient and control groups having similar age distributions (mean \pm SD) ages.

Table 2 showed the result of the parameters for both controls and cholelithiasis patients.

The current study demonstrates a significant difference in SOD serum level between controls and patients P-value (0.026). so, there was a significant decrease in the serum levels of SOD indicates decreasing in antioxidant activity. While for MPO, current study shows also high significant difference between patient and control P-value (0.001).

Table (2):Parameters of the patients and controls

| Parameter s | Control N = 30 | Patients N = 100 | P- value |
|----------------|--------------------|---------------------|-------------|
| SOD | 0.67 \pm 0.51 | 0.42 \pm 0.29 | 0.026* |
| MPO | 7.01 \pm 1.55 | 9.27 \pm 2.66 | 0.001* |

Value were presented as mean \pm SD of mean; (*) = significance difference, (P<0.05)
N= no. of individuals in each group.

Discussion

Similar finding were shown by Correa P.⁽²⁷⁾ and ByungKyu Park *et al.*⁽²⁸⁾, they indicate significantly decrease in SOD serum levels in patient diagnosed with many gastrointestinal diseases like acute pancreatitis gallbladder cancer and Cholelithiasis. Also the current study supported by the results of Kaur T. and Kaur S.2010⁽²⁹⁾ found "significant decrease in antioxidant enzymes activities (superoxide dismutase) in patient with gallstone comparing with controls". Also RuhiDixit, *et al.*⁽³⁰⁾ and AlpaslanTerzet *al.*⁽³¹⁾ have the same findings. 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⁽³²⁾. The changes in bile composition can increase the biliary free radical formation⁽³³⁾. Increased levels of inducible nitric oxide (NO) synthesis activity was shown in inflamed gallbladders which has an effect on elevation of oxidative stress and on fluid transport as well⁽³⁴⁾. Moreover, inflammatory changes of the gallbladder mucosa are associated with granulocyte infiltration; the activated phagocytes can produce reactive oxygen metabolites, and lead to oxidative stress. Free radicals and other peroxides derivatives produced physiologically in the human body and increased in many pathological conditions, diffuse into the blood⁽³⁵⁾. Here, antioxidant components of plasma overwhelm them and they are consumed. Total antioxidants; therefore, is detected as being significantly lower in the cholelithiasis patients than in the controls. Many antioxidants and oxidants were studied to determine their effects. In particular, vitamin C, which is a strong external antioxidant, serum ascorbic acid level was inversely related to prevalence of gallbladder disease⁽³⁶⁾.

Bilirubin free radicals can damage the liver cells, which can induce the change of ingredients of the bile by decreasing the amount of bile acids. The abnormal metabolism in hepatocyte can lead to hydrolysis of the conjugated bilirubin, increase in the concentration of free bilirubin, which in turns make bilirubin supersaturated to the bile, and promote the formation of pigment gallstone, during the gallstone formation, bilirubin reacts with the active-oxygen species formed in vivo followed by its polymerization, aggregation, and calcification. It is speculated to be an important step in the formation of pigment gallstones⁽³⁷⁾.

For the MPO, similar findings of this study were found by (H.Ojima *et al.* 2005) who

consider serum level of MPO as diagnostic parameter for undifferentiated carcinoma and small cell carcinoma in gallbladder such as Extramedullary myeloid tumour (EMMT) ⁽³⁸⁾, also (Alfredo Menendez 2009) with (ShikoKuribaYashi, *et.al.* 2004) agree with this finding and used MPO and other oxidative stress markers as effective diagnostic parameters for many bladder dysfunction including cholelithiasis^(39,40).

Also in animal models, Rege RV, Prystowsky JB ⁽⁴¹⁾ found a significant elevation in both MPO and IL-1 in cholelithiatic animals, and (ÖzgürKasımayet *al*)2006 who found significant increase in MPO level in gastrointestinal diseases including Cholelithiasis⁽⁴²⁾.

Conclusion

Cholelithiasis patients showed low antioxidant activity, and high MPO serum levels as shown in this study; the oxidative stress markers are useful in the pathogenesis and diagnosis of Cholelithiasis.

Recommendation

1. Measuring more parameters that used in the diagnoses of cholelithiasis.
2. Measuring more oxidative stress markers and antioxidant enzymes in cholelithiasis patients.

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