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Physiological Evaluation Of Someoxidative Stressbiomarkers And Enzymatic Endogenous Antioxidantamong Acute Cholecystitis Patients

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Abstract:The current study was conducted to estimate the physiological role of enzymatic endogenous antioxidants and some cellular oxidiative stress biomarkers in acute cholecystitis patients (AC). The sample of present study consist of (150 participants) male and female including four ages groups (≤ 30, 31-45, 46-60, and ≥ 60 years) and divided into two groups: the first was the control groups of the healthy (50 participants) without acute cholecystitis and other diseases and the second was the groups of (AC) patients (100 participants) who recommended by specialist physician for laparoscopic cholecystectomy.The biochemical tests were performed for allserum blood samples taken from both study participants, including the biochemical tests for estimation of oxidiative stress biomarkers (malondialdehyde (MDA) and total oxidant status (TOS)), and some enzymatic endogenous antioxidant (catalase, glutathione peroxidase1 (GPX1), and superoxide dismutase1 (SOD1)). The serum biochemical assay for (AC) patients indicated for a clearly significant increased in theconcentrations of oxidiative stress biomarkers (MDA and TOS) in compression to control subjects. In contrast, biochemical analysis for (AC) patientsrecorded significantly decreased in the concentrations of serum enzymatic endogenous antioxidant (Catalase, SOD1, GPX1) as compared with control volunteers.It was concluded from current study data, that the oxidative stress disturbances was one of the most common causes of acute cholecystitis infection represented by highly production of reactive oxygen species (ROS), in term of functional cellular response for serum enzymatic endogenous antioxidants.

1. INTRODUCTION

Acute cholecystitis is one of the commonest biliary tract emergency. Early diagnosis and prompt treatment is essential to reduce the morbidity and mortality associated with the disease. So, assessment of the severity of the disease is essential to develop a safe therapeutic plan for the patients (Vagholkar, 2020). The risk of (AC) increased in patients with larger gallstones that are more likely to be trapped within the gallbladder. Gangrenous cholecystitis and perforation of the gallbladder are serious complications of acute cholecystitis (NHS, 2012). Pathologically, cholecystitis occurs as a result of both cystic duct obstruction and damage to the gallbladder mucosa. Gallstones may become impacted in the neck of the

gallbladder of the cystic duct and cause mechanical mural irritation as the result of the gallbladder contracting against the stone. Damage to the mucosa leads to the release of phospholipases from the epithelial cells lining the gallbladder lumen (Kenneth Vera, 2018). Gallstones are present in about 10% to 15% of the adult western population. Between 1% and 4% of these adults become symptomatic in a year (the majority due to biliary colic but a significant proportion due to acute cholecystitis). Acute cholecystitis is secondary to gallstones in 90-95% of cases (Gurusamy*et al*., 2015). Mostly, an impacted gallstone in the gallbladder infundibulum or in the cystic duct is the cause for the inflammatory process. The continuing mucin production from the gallbladder's epithelium in combination with the impacted gallstone results in gallbladder distension and edema with acute inflammation. This can eventually result in micro-and macro-circulatory perfusion deficits with subsequent ischemia, necrosis and may be complicated by secondary infection which occurs in acute cholecystitis patients (Werner and Büchler-Markus, 2014).

Antioxidant is a molecule which has the ability to prevent or slow the oxidation of macromolecules. The role of antioxidants is removing of free radicals or inhibiting other oxidation reactions by being oxidized themselves. So, antioxidants are often reducing agents such as polyphenols or thiols(Duarte and Lunec, 2005). Although oxidation reactions are vital for cells, they have damaging effects; hence, plants and animals contain various antioxidants, such as vitamins C and E and glutathione, as well as different enzymatic systems which catalyze the antioxidants reactions as catalase, superoxide dismutase (SOD) and peroxidases. The defects in or inhibition of these antioxidant enzymes will lead to oxidative stress and may damage and lyse the cells(Valko*et al*., 2007).

It is well known that, in chronic diseases such as cholelithiasis, the active inflammatory responseisinducedwithneutrophilicinfiltration.Theseneutrophils, macrophages and monocytes produce (ROS) which may cause DNA damage to the adjacent cells. Oxidative stress provoked by (ROS) plays an important role in the pathogenesis of many diseases such as hepatitis, cholecystitis, gallstones, gastroduodenal mucosal inflammation, peptic ulcer disease, and gastric cancer(Galli *et al*., 2005). Many markers are currently used to assess oxidant and antioxidant status such as total oxidant capacity (TOC), total antioxidant capacity (TAC), oxidative stress index (OSI) (Faienza*et al*., 2012). An imbalance between the formation of (ROS) in cells and antioxidant defense causes oxidative stress, which is responsible for oxidative damage to lipids, proteins, and nucleic acids, and modifies their structure as well as functioning (Martin-Gallan*et al*., 2007).

2. MATERIALS AND METHODS

The cases for this study were collected from the patients with a diagnosis of acute cholecystitis before admitted for surgical treatment (laparoscopic cholecystectomy) at the department of general surgery in AL- Karama and AL-Helal hospital of Wasit health directorate in AL-Kut city, Wasit province, Iraq; during the period from December 1, 2018 to December, 1, 2019.

- One hundred patients were taken for this study, 24 males (24.00 %) with age (23-65year) and 76 females (76.00 %) with age (18-80year).

- fifty healthy individuals as control group, 20 males (40.00%) with age (25-65year) and 30 females (60.00%) with age (20-75year). Who were without acute cholecystitis symptoms, hyperlipidemia, diabetes mellitus, hypertension and other diseases.

The initial diagnosis of acute cholecystitis was made from the detailed history, clinical examination, and analysis of available laboratory data. Detailed history of the cases was taken such as (age, gender, fertility, weight, length, blood groups, habitation and clinical status) for both samples groups. Both patients and healthy individuals were divided into four groups according to their age ranges; The first age range (≤30year), second age range (31-45year), third age range (46-60year), and fourth age range (≥ 60year) and each group was divided into two subgroups according to their gender (male and female).

Samples collecting

Ten milliliters of venous blood were drawn from control subjects and (AC) patients by using disposable syringe of (10 ml) before admitted for laparoscopic cholecystectomy, the blood sample was put in disposablegel tubes, left at room temperature for (30) minutes forclot formation and then centrifuged for (10) minutes at (3000) run per minute the serum was distributed in to eppendorf tubes and frezone at (-20 °C). The serum biochemical assay were performed by using Huma reader(ELISA) method, according to kit assay Elabscience laboratories, China.

Statistical analysis

The results of current study was done by using statistical software package (SPSS 16) and analyzed by using ANOVA (one way analysis). The data were expressed as mean ± standard error of the mean (M±S.E.M.). LSD were used for comparisons between the acute cholecystitis patients and control group. ($P \leq 0.05$) was considered to be statistically significant and LSD was considered to be least significant difference.

3. RESULTS AND DISCUSSION

Statistical data of serum malondialdehyde (MDA)(ng/ml)level as oxidative stress biomarker in (AC) patients and control participants are listed in table (1). Results of current study indicated that the levels of serum (MDA)considerably significant increased ($p \leq 0.05$) in the second, third, and fourth age range (31-45, 46-60, and ≥ 60 years) for both gender of (AC) patients in compression to control volunteers. In contrast, no significant differences were noted in the first age range (≤ 30)year of (AC) patients as compered with control subjects. Even though, there was no significant differences within all age groups among control subjects. But, in patients group clearly significant increased were noted in the second, third, and fourth age range as compared with the first age range. Moreover, the serum (MDA)(ng/ml)levels showed no significantly differences between male and female for both study groups.

MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations. MDA is an end product of the radical initiated oxidative decomposition of poly unsaturated fatty acids (Giera*et al*., 2012).Thus,Jüngst*et al*., (2008) reported that lipid peroxidation such as malondialdehyde increased in gallbladder bile for cholesterol gallstone patients. Many studies are in harmony with our findings, such a study conducted by Kaur and

Kaur, (2011) who 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.

Table (1): The mean values of serum malondialdehyde (ng/ml) levelsof acute cholecystitis patients and control subjects according to age range for both genders.

Malondialdehyde (MDA) (ng/ml)				
Age range (Years)	**Control**		**(AC) Patients**	
	Male	**Female**	**Male**	**Female**
≤ 30	98.246+ 2.22 Aa	98.426+ 1.83 Aa	101.927+ 4.84 Aa	97.140+ 0.89 Aa
31-45	97.627+ 1.62 Aa	99.102+ 1.21 Aa	228.565+ 10.01 Bb	243.307+ 4.97 Bb
46-60	97.590+ 1.37 Aa	97.396+ 1.37 Aa	239.223+ 10.16 Bb	238.467+ 6.29 Bb
≥ 60	98.855+ 1.38 Aa	97.704+ 1.86 Aa	251.380+ 13.17 Bb	245.760+ 8.97 Bb
LSD= 12.080				

Data= Mean + S. E. M.
Different capital letters denote to significant differences between groups (P≤0.05).
Different small letters denote to significant differences within groups (P≤0.05).
Similar capital and small letters denote to non-significant differences (P>0.05).
LSD = Least significant difference.

In addition, table (2) illustrate the results of serum total oxidant status (TOS) (U/ml) as oxidative stress marker in (AC) patients and control subjects. Statistical data recorded highly significant increased (p≤0.05) in serum (TOS) levels for both gender of (AC) patients within all age groups (≤30, 31-45, 46-60, and ≥ 60 years) in comparison to control volunteers. While, the findings were showed non-significant differences within all age groups for both study participants. Additionally, the serum (TOS) (U/ml) also showed no significantly variations between male and female in healthy participants. While, significant increased (p≤0.05) was noted in female as compared with male in the first age range of (AC) patients.

Therefore, the total oxidant status (TOS) is usually used to estimate the overall oxidation state of the body. Similarly, the total antioxidant status (TAS) is used to measure the overall antioxidant status of the body. The oxidative stress index (OSI), which is the ratio of (TOS) to (TAS) might be a more precise index of oxidative stress in the body because it is a comprehensive measurement of TAS and TOS (Wu *et al*., 2017). Besides, these results are in accordance with findings of Ridha and Taher, (2013) who reported that patients with gallstone are exposed to a potent oxidative stress with elevation in some liver function enzymes and increase oxidative stress markers may play a role in the progression of inflammatory changes in the gallbladder. While, results of present study are disagreement with Arslan Onuk*et al*., (2019) who suggested that no statistically significant differences were observed in (TOS) levels among (AC) patients.

Table (2): The mean values of serum total oxidant status (TOS)(U/ml)levelsof acute cholecystitis patients and control subjects according to age range for both genders.

Total oxidant status (TOS) (U/ml)				
Age range (Years)	**Control**		**(AC) Patients**	
	Male	**Female**	**Male**	**Female**
≤ 30	4.708+0.62 Aa	5.407+0.72 Aa	12.160+1.34 Ba	14.492+0.56 Ca
31-45	5.356+0.70 Aa	5.424+0.60 Aa	13.741+1.12 Ba	14.869+0.47 Ba
46-60	5.442+0.48 Aa	5.247+0.52 Aa	15.123+0.76 Ba	15.624+0.43 Ba
≥ 60	4.172+0.31 Aa	5.666+0.55 Aa	15.717+0.73 Ba	14.908+0.79 Ba
LSD=1.719				

Data= Mean ± S. E. M.
Different capital letters denote to significant differences between groups (P≤0.05).
Different small letters denote to significant differences within groups (P≤0.05).
Similar capital and small letters denote to non-significant differences (P>0.05).
LSD = Least significant difference.

Table (3) illustrate statistical data of serum catalase levels as enzymatic endogenous antioxidant in (AC) patients and control volunteers. Results of present study refer to the statistically significant decrease in serum catalase (U/ml) levels for (AC) patientsin all age range (≤30, 31-45, 46-60, and ≥ 60 years) for both gender in comparison to healthy subjects. In addition, serum catalase (U/ml) levels exhibit no statically significant differences (p>0.05) within all age range and also between male and female in patients groups. In contrast, significant differences were observed in healthy participants (p≤0.05) according to the age ranges and gender too. The clearly reduction in serum catalase levels may be due to impairment of bile flow which leads to progressive liver cirrhosis. If not treated properly, liver transplantation is required in most cases (Hsiao *et al*., 2008).

Meanwhile, oxidative stress is associated with the cholestasis pathogenesis and lipid peroxidation which responsible for the tissue injury. So, patients with jaundice have higher levels of plasma lipid peroxidation than patients without jaundice because of impairment of bile flow which resulting in the accumulation of toxic bile salts within the hepatocytes (Chen *et al*.,2015).The findings of current study were consistent with the study conducted by Chen *et al*., (2015) who refers to the low levels of (CAT) enzyme in patients with biliary atresia cholestasis and significant increase in the enzymatic antioxidant activity after liver transplantation may refer to their role as a key enzymes in the protection of the liver from harmful free radical reactions. Moreover, Xu *et al*., (2012) refers to relationship between extrahepatic cholestatic and highly susceptible oxidative stress that leads to decrease enzymatic antioxidant concentration in patients with gallstone diseases.

Table (3): The mean values of serum catalase levels (U/ml) of acute cholecystitis patients and control subjects according to age range for both genders.

Catalase (CAT) (U/ml)				
Age ranges (Years)	**Control**		**(AC) Patients**	
	Male	**Female**	**Male**	**Female**
≤ 30	49.414±4.20 Aa	47.904±4.68 Aac	14.030±1.96 Ba	14.647±1.13 Ba
31-45	48.106±2.47 Aa	40.352±2.84 Bb	16.158±1.88 Ca	17.229± 0.89 Ca
46-60	45.476±4.13 Aa	43.811±3.91 Aab	18.749±1.82 Ba	14.777±1.06 Ba
≥ 60	49.036±5.41 Aa	51.862±3.26 Ac	16.280±1.48 Ba	17.443±1.57 Ba
LSD=4.920				

Data= Mean ± S. E. M.
Different capital letters denote to significant differences between groups (P≤0.05).
Different small letters denote to significant differences within groups (P≤0.05).
Similar capital and small letters denote to non-significant differences (P>0.05).
LSD = Least significant difference.

Table (4) describe the data of serum glutathione peroxidase1 (GPX1) concentrations (pg/ml) as enzymatic endogenous antioxidant in both (AC) patients and control volunteers. The results refer to considerably significant decrease in serum (GPX1) concentrations for both gender of (AC) patients in the third and fourth age ranges (46-60 and ≥ 60 years) as compared with control subjects. Where as, in early age group the data were exhibit non-significant differences (p≥0.05) in serum (GPX1) for both gender of (AC) patients in comparison to control subjects. On the other hand, depending on the gender the serum (GPX1) (U/ml) of patients group were decreased significantly in female as compared with male in the second age range (31-45years). Additionally, although the serum (GPXl) level shows no significant differences within all age groups in healthy participants. While, in (AC) patients subjects there was clearly significant decrease (p≤0.05) in the third and fourth age ranges as compared with first age group of patients.

Table (4): The mean values of serum glutathione peroxidase1 (GPX1) of acute cholecystitis patients and control subjects according to age range for both genders.

Glutathione peroxidase1 (GPX1) (pg/ml)				
Age range (Years)	**Control**		**(AC) Patients**	
	Male	**Female**	**Male**	**Female**
≤ 30	93.580±2.29 Aa	96.237±2.66 Aa	88.650±2.21 Aa	92.621±1.87 Aa
31-45	97.789±2.50	95.185±2.16	93.635±2.17	62.996±1.27

	Aa	Aa	Aa	Bb
46-60	97.653±1.99 Aa	96.463±2.19 Aa	59.245±2.96 Bb	63.853±1.58 Bb
≥ 60	98.180±2.07 Aa	93.448±2.92 Aa	62.499±4.36 Bb	65.766±1.84 Bb
LSD= 3.977				

Data= Mean ± S. E. M.
Different capital letters denote to significant differences between groups (P≤0.05).
Different small letters denote to significant differences within groups (P≤0.05).
Similar capital and small letters denote to non-significant differences (P>0.05).
LSD = Least significant difference.

On the other hand, serum superoxide dismutase1(SOD1)(pg/ml) levels for the (AC) patients and the healthy participants are listed in table (5). The data of present study indicated to significant decreased in serum (SOD1) levels within all age groups (≤30, 31-45, 46-60, and ≥ 60 years) for both gender of (AC) patients in comparison with healthy volunteers. Where as, the results exhibit no significant differences in serum (SOD1)concentration within all age ranges for both study groups (control and AC patients). In addition, the serum (SOD1)(pg/ml) levels showed no significantly variation between male and female for both study participants.

Table (5): The mean values of serum superoxide dismutase1 (pg/ml) levelsof acute cholecystitis patients and control subjects according to age ranges for both genders.

Superoxide dismutase1 (SOD1) (pg/ml)				
Age ranges (Years)	**Control**		**(AC) Patients**	
	Male	**Female**	**Male**	**Female**
≤ 30	104.806± 2.54 Aa	103.717± 2.06 Aa	69.180± 3.19 Ba	68.601±1.30 Ba
31-45	100.435± 1.74 Aa	100.064+ 1.15 Aa	69.617±1.94 Ba	68.897±1.08 Ba
46-60	99.735± 0.82 Aa	99.981±1.02 Aa	66.027±1.27 Ba	67.688±1.21 Ba
≥ 60	100.429± 1.89 Aa	101.477± 2.38 Aa	70.893±3.71 Ba	70.120±1.61 Ba
LSD= 3.722				

Data= Mean ± S. E. M.
Different capital letters denote to significant differences between groups (P≤0.05).
Different small letters denote to significant differences within groups (P≤0.05).
Similar capital and small letters denote to non-significant differences (P>0.05).
LSD = Least significant difference.

However, results of current study are in line with a recent research has conductedin Tikrit university, Iraq , by Sadiem*et al*., (2014) who refer to significant decreased of superoxide dismutase (SOD) concentration in patients with gallstone diseases. As well as, similar finding

were shown by Kaur and Kaur (2010) they found highly significant decrease in antioxidant enzymes activities (SOD) in patients with gallstone comparing with controls. Also, Terz, (2010) and Dixit, (2012) and have the same findings. Thus, oxidative stress markers are useful in the pathogenesis and diagnosis of cholelithiasis. So, in cholelithiasis patients antioxidant enzyme such as (SOD) decreased significantly due to highly increase of myeloperoxidase (MPO) as oxidative stress markers (Sadiem*et al*., 2014). Under normal physiological conditions (ROS) are produced during metabolic process in the body. (ROS) formation and elimination are balanced by the action of antioxidant enzymes. This balance is important for maintaining proper cellular states. A moderate increase in (ROS) can stimulate cell growth. However, excessive (ROS) generation will contribute to cellular injury, such as damage to DNA, protein. Elevated serum (SOD) levels might be regulated in order to response to oxidative stress, and then may predict excessive (ROS) state in the carcinogenesis (Pham *et al*., 2009).

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