

# Effect of cholecystectomy on serum oxidative stress and nitric oxide pre and post operation

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## Abstract

**Background:** The objective of this study was to determine the changes in oxidative and antioxidant in three stages: preoperative, postoperative and long periods for cholecystectomy.

**Methods:** Blood samples were collected from two groups of females (Women who will undergo their cholecystectomy as well as women who have been undergoing from cholecystectomy for a long time) with a total number of 128 women and 64 control females at AL-Hussein Teaching Hospital in Thi-Qar Governorate. from November 2017 to May 2018. Serum was separated by centrifugation and MDA, NO, Cp, Alb were measured. The data were analyzed using SPSS.

**Results:** The levels of serum NO, MDA and Cp were significantly increased in all patient groups in comparison with control groups ( $p \leq 0.05$ ). It was found no significant differences in the concentration of serum NO, MDA and Cp between each of post (short and long terms) groups ( $p \leq 0.05$ ). Also there were significant differences in the concentration of serum NO, MDA and Cp between each of post (short and long terms) groups and control groups ( $p \leq 0.05$ ). While levels of serum Alb was significantly decreased in all patient groups in comparison with control groups ( $p \leq 0.05$ ). It was found no significant differences in the concentration of serum Alb between each of post (short and long terms) groups ( $p \leq 0.05$ ). Also it was found significant differences in the concentration of serum Alb between each of post (short and long terms) and control groups ( $p \leq 0.05$ ).

**Conclusions:** Free radical-induced lipid peroxidation associated with a decrease in serum antioxidant capacity pre patient's cholecystectomy compared with post operation where less oxidative stress

**Keywords:** Cholecystectomy, oxidative stress, malondialdehyde, nitric oxide, ceruloplasmin, albumin.

## INTRODUCTION

Bile is an important physiological fluid of the human body that is synthesised by hepatocytes in the liver<sup>(1)</sup>. The Cholelithiasis is defined as a presence or formation of gallstones in the common bile duct (CBD)<sup>(2)</sup>. The basis for which is the impaired metabolism of cholesterol, bilirubin and bile acids, which is characterized by the formation of gallstones in the hepatic bile duct, common bile duct<sup>(3)</sup>. The prevalence of cholecystitis is approximately of 13% to 19% in Thi -Qar during the period between 2012-2017. Most cases are asymptomatic, as gallstones are usually discovered incidentally during routine imaging for other abdominal conditions. Although small stones can be spontaneously eliminated to the duodenum through the ampulla of Vater, the narrow termination of the bile duct frequently leads to the impaction of stones<sup>(4)</sup>. Gallstones and diseases of the biliary tract affect more than 10% of the adult population<sup>(5)</sup>. which can be caused by either primary stones that originated from the bile duct or by secondary stones that migrated from the gallbladder, and can lead to obstructive jaundice, cholangitis, or pancreatitis<sup>(2)</sup>, can be lethal. Moreover, surgery to remove the gallbladder in these patients, in an attempt to relieve the symptoms, gives variable results<sup>(5)</sup>.

Laparoscopic surgery is a surgical technique in which operations in the abdomen are performed through small incisions in the abdominal<sup>(6)</sup>. Today, more than 90% of cholecystectomies are performed laparoscopically. The procedure has been found to be superior to open cholecystectomy with less morbidity and mortality<sup>(7)</sup>. Currently, CO<sub>2</sub> is usually used for insufflation due to its low cost, non-flammability, chemical stability<sup>(8)</sup>. Cholecystectomy increased during two years past to approximately of 13% to 17% in Thi -Qar. The CO<sub>2</sub> pneumoperitoneum causes an increase in intra-abdominal pressure with a consecutive elevation of the diaphragm which can result in hyperventilation<sup>(9)</sup>. when the laparoscopic procedure is completed, abdominal deflation is performed this reduces the intra-abdominal pressure and increases splanchnic perfusion<sup>(10)</sup>.

Cholecystectomy has long been considered as a safe procedure, while secondary effects have been overlooked<sup>(11)</sup>. where the gallbladder is a 'controller' operating in concert with key

pathways governing metabolic homeostasis<sup>(12)</sup>. Thus, several pathophysiological and clinical changes are anticipated after cholecystectomy procedure associated with loss of reservoir-concentrating function of the gallbladder<sup>(13,14)</sup>. The intestine will act as the major bile acid reservoir and bile acid synthesis will show a twofold increase<sup>(15)</sup>. Different studies in mice and humans showed that cholecystectomy increased the enterohepatic recirculation rates of bile acids, in particular during fasting<sup>(16)</sup>. Accelerated intestinal recycling is associated with increased secretion rates of bile acids and cholesterol in bile, after cholecystectomy. Therefore, although the bile acid pool is not enlarged, fat is digested and absorbed normally in cholecystectomized patients<sup>(17)</sup>. In keeping with an increased exposure of the bile acid pool to intestinal bacteria as a result of a more rapid enterohepatic circulation, cholecystectomy also increases bacterial deconjugation and dehydroxylation of bile acids, and thereby the proportion of secondary bile acids<sup>(18,19)</sup>. Despite conflicting results, there is agreement about the increased incidence of colon cancer after cholecystectomy Secondary bile acids, known to have a role in colon cancer promotion, have been considered responsible since cholecystectomized patients have high proportions of Secondary bile acids in their bile acids pool<sup>(20)</sup>.

The gallbladder mucosal inflammation consists of infiltration with phagocytes that generate reactive oxygen species, increased concentrations of oxygen-derived products is called Oxidative stress. The hydroxyl radical is an important oxidant that can abstract hydrogen atoms from polyunsaturated fatty acids, a reaction that can start lipid peroxidation<sup>(21)</sup>. Hydroxyl radicals stimulate the release of glycoproteins, such as mucin from gallbladder epithelium, resulting in the formation of cholesterol crystal nucleation<sup>(22, 23)</sup>. Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (MDA)<sup>(24)</sup>. MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations<sup>(25)</sup>. MDA is an end-product of the radical-initiated oxidative decomposition of poly-unsaturated fatty acids<sup>(25)</sup>.

Chemically reactive oxidants and radicals such as hydrogen peroxide, oxyradicals and nitric oxide are produced during

inflammation Recently, attention has been focused on nitric oxide (NO) as an endogenous mutagen<sup>(26)</sup>. Nitric oxide, a reactive radical gas, controls various vital physiological functions in the body. This small inorganic molecule plays a variety of roles such as maintenance of vascular tone, neurotransmission, and host defense by destroying microbes<sup>(27)</sup>. Organic nitrate reduces the pressure of the sphincter of Oddi and to relax the gallbladder, but evidence was obtained that endogenous NO is produced by the gallbladder tissues and that NO may serve as a mediator of non-adrenergic, non-cholinergic (NANC) nerves in the control of the contractions of the gallbladder and the sphincter of Oddi<sup>(28)</sup>.

Studies others have defined the role of trace elements such as iron, calcium, zinc and copper in the formation of gallstones<sup>(29)</sup>. Out of these trace elements, iron deficiency has been shown to alter the activity of several hepatic enzymes leading to increased bile cholesterol saturation and promotion of crystal formation<sup>(30)</sup>. Iron acts as a cofactor for Nitric Oxide Synthase (NOS), which synthesizes Nitric Oxide (NO) and is important for the maintenance of basal gallbladder tone and normal relaxation<sup>(31)</sup>. The NOS acts as a calcium-calmodulin dependent enzyme. Hence, a deficiency in serum calcium causes deranged function of NOS resulting in altered gallbladder motility, leading to biliary stasis and subsequently increased crystal formation in bile<sup>(29)</sup>. Thus, deficiencies of serum iron and serum calcium can lead to increased risk of gallstone disease<sup>(32)</sup>.

For protect the cells and organ systems of the body against free radical damage therefore, antioxidants deactivating free radicals before they attack cells. The antioxidants can be endogenous or obtained exogenously eg, as a part of a diet or as dietary supplements<sup>(33)</sup>. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive<sup>(34)</sup>. Albumin is considered an antioxidant. It is involved in the scavenging of oxygen free radicals, which have been implicated in the pathogenesis of inflammatory diseases this may be related to the abundance of sulfhydryl (-SH) groups on the albumin molecule<sup>(35)</sup>. Alb is a single polypeptide consisting of 585 amino acids with M.W. of approximately 66,248 Dalton, synthesized by the liver. It is most abundant in human plasma. Usually, it constitutes about 55– 60% of all plasma proteins and has a serum half-life of about 20 days<sup>(36)</sup>. Hypoalbuminemia is a major culprit in gallbladder thickening alone or as a secondary mechanism in patients with cirrhosis, heart failure or renal disease. Other speculated mechanisms of gallbladder wall thickening in the disease states above are increased portal venous pressure. Gallbladder wall thickening is often evident in adenomyomatosis, cholecystitis and gallbladder cancer as well<sup>(37)</sup>. Ceruloplasmin (Cp) is a major antioxidant glycoprotein that are synthesized in many tissues, Cp inhibits the peroxidation of membrane lipids catalyzed by metal ions, such as iron and copper, and it protects polyunsaturated fatty acids in red blood cell membranes from active oxygen radicals<sup>(38)</sup>. The molecular weight of human Cp is described to be 151 KDa<sup>(39)</sup>. Cp is synthesized mainly in the liver as a single chain polypeptide, and after the incorporation of six atoms of copper in the biosynthetic pathway, it is secreted into the plasma as an  $\alpha_2$ -glycoprotein. Cp is an acute phase protein and is synthesized in response to the tissue damage and inflammation.<sup>(40)</sup>, playing important anti-inflammatory roles, as a copper transporter from hepatocytes to other tissues, a regulator for hepatic iron mobilization as well as a scavenger of free radicals and superoxide<sup>(41)</sup>.

The aim of this study is to investigate the oxidative stress by measuring the lipid peroxidation marker (MDA) and NO, to

evaluate serum antioxidant status in the mentioned disease by measurement (ceruloplasmin (Cp), and albumin (Alb)) preoperative and postoperative cholecystectomy and compared with control group, Comparison between short and long term after cholecystectomy.

## SUBJECTS AND METHODS

This study has been conducted at AL-Hussein Teaching Hospital in Thi-Qar, Biochemistry Laboratory in College of Science, at the period between 10/11/2017 to 5/5/2018. It included (192) women, (64) control and (64) patients before operation and 24 hr after operation and (64) long term for a period ranging from 3 months to 3 years, their ages were between 20 -69 years. Those patients were diagnosed by specialist doctors as uncomplicated, symptomatic gallstones (cholelithiasis), who underwent elective laparoscopic cholecystectomy. Patients with respiratory, hepatic, cardiovascular (ischemic heart disease and hypertension), diabetes were excluded from this study.

### Blood Sampling

Five ml of blood vein puncture were drawn from each fasting patient and control. the sample left at room temperature for 10 minutes to clotted then centrifuged for at 3000 rpm for 10min, the serum samples were separated and stored at (-20°C) for later measurement of biochemical parameters, unless used immediately.

### Determination of Serum Malondialdehyde (MDA):

Determinations of serum MDA level that consider as a lipid peroxidation marker were performed according to the method of Fong<sup>(42)</sup>. MDA concentrations were calculated, using the molar extinction coefficient of MDA ( $\epsilon_{MDA}$ ) equal to  $1.56 \times 10^5 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ . MDA formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.

### Determination of Serum Nitric Oxide (NO):

Determination of serum NO level that consider as a lipid peroxidation marker were performed according to the method of Dervisevic<sup>(43)</sup> concentrations were calculated, using the standard curve of NO. NO is a unique chemical messenger produced from L- arginine by the enzyme nitric oxide synthase (NOS)<sup>(44)</sup>.

### Determination of Serum Ceruloplasmin (CP)

Serum Cp concentration was measured by the method of Menden et al<sup>(45)</sup> which using the extinction coefficient of Cp ( $\epsilon_{Cp}$ ) equal to (0.68) to calculate its concentration.

### Determination of Serum Albumin (Alb)

The bromocresol green (BCG) method, colorimetric method<sup>(46)</sup>, is the simplest technique which have been developed to determine Alb concentration.

### Statistical analysis

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean  $\pm$  standard deviations (mean  $\pm$  SD). One-way ANOVA was used to compare parameters in different studied groups. P-values ( $P \leq 0.05$ ) were considered statistically significant.

## RESULT

In this study, we estimated the levels of NO, MDA, CP and Alb, Women patients and comparison between short and long term after cholecystectomy and also with compared with control group. The levels of serum NO, MDA and Cp showed a significant increase in all patient groups in comparison with control groups ( $p \leq 0.05$ ). Table 1, 2 and 3 shows a significant increase in the concentration of serum NO, MDA and Cp in the groups pre and each of post (short and long terms) in comparison with control group ( $p \leq 0.05$ ). It was found no significant differences in the concentration of serum NO, MDA and Cp between each of post (short and long terms) groups ( $p \leq 0.05$ ). Also it was found

significant differences in the concentration of serum NO, MDA and Cp between each of post (short and long terms) groups and control groups ( $p \leq 0.05$ ). While levels of serum Alb showed a significant decrease in all patient groups in comparison with control groups ( $p \leq 0.05$ ). The results in table 4 shows a significant decrease in the concentration of serum Alb in the groups pre and each of post (short and long terms) groups in comparison with

control group ( $p \leq 0.05$ ). It was found no significant differences in the concentration of serum Alb between each of post (short and long terms) groups ( $p \leq 0.05$ ). Also it was found significant differences in the concentration of serum Alb between each of post (short and long terms) groups and control group ( $p \leq 0.05$ ).

Table 1: Concentration of serum Malondialdehyde in all studies groups.

Group of Age	NO.	MDA conc.( $\mu\text{mol/L}$ )				Lsd
		Con	Pre	Post		
				Short term	Long term	
A: (20-45)	37	2.41 $\pm$ 0.60 <sup>c,A</sup>	3.10 $\pm$ 0.82 <sup>a,A</sup>	2.72 $\pm$ 0.29 <sup>b,A</sup>	2.78 $\pm$ 0.29 <sup>b,A</sup>	0.17
B: (46-69)	27	2.45 $\pm$ 0.28 <sup>c,A</sup>	3.20 $\pm$ 0.58 <sup>a,A</sup>	2.93 $\pm$ 0.80 <sup>b,A</sup>	2.80 $\pm$ 0.52 <sup>b,A</sup>	0.25
LSD		0.21	0.21	0.19	0.12	

NO: The number of each class, Con: control groups, pre: Patients group pre operation at least 20 minutes, post (short term): 24 hr after operation, post (short term): postoperative long term for a period ranging from 3 months to 3 years, non-identical superscript (a,b or c ...etc).were considered significantly differences ( $P \leq 0.05$ ) to compare horizontally, and with non-identical superscript (A, B or C...etc.)were considered significantly differences ( $P \leq 0.05$ ) to compare vertically., LSD: Low significantly differences.

This table shows no significant differences in the concentration of serum MDA between A and B groups ( $p \leq 0.05$ ).

Table 2: Concentration of serum Nitric oxide in all studies groups.

Group of Age	NO.	NO conc.( $\mu\text{mol/mL}$ )				Lsd
		Con	Pre	Post		
				Short term	Long term	
A: (20-45)	37	7.60 $\pm$ 0.85 <sup>c,A</sup>	13.80 $\pm$ 1.06 <sup>a,A</sup>	9.49 $\pm$ 1.51 <sup>b,A</sup>	9.60 $\pm$ 1.22 <sup>b,A</sup>	0.35
B: (46-69)	27	7.50 $\pm$ 0.88 <sup>c,A</sup>	13.73 $\pm$ 1.03 <sup>a,A</sup>	9.45 $\pm$ 1.11 <sup>b,A</sup>	9.70 $\pm$ 1.24 <sup>b,A</sup>	0.47
LSD		0.25	0.31	0.39	0.36	

Legend as in table 1.

This table shows no significant differences in the concentration of serum NO between A and B groups ( $p \leq 0.05$ ).

Table 3: Concentration of serum Ceruloplasmin in all studies groups.

Group of Age	NO.	Cp conc. (mg/L)				Lsd
		Con	Pre	Post		
				Short term	Long term	
A: (20-45)	37	2.71 $\pm$ 0.38 <sup>c,A</sup>	3.52 $\pm$ 0.98 <sup>a,A</sup>	3.05 $\pm$ 0.57 <sup>b,A</sup>	3.22 $\pm$ 0.75 <sup>b,A</sup>	0.18
B: (46-69)	27	2.87 $\pm$ 0.66 <sup>c,A</sup>	3.57 $\pm$ 0.71 <sup>a,A</sup>	3.09 $\pm$ 0.30 <sup>b,A</sup>	3.25 $\pm$ 0.37 <sup>b,A</sup>	0.20
LSD		0.18	0.25	0.13	0.17	

Legend as in table 1.

This table shows no significant differences in the concentration of serum Cp between A and B groups ( $p \leq 0.05$ ).

Table 4: Concentration of serum Albumin in all studies groups.

Group of Age	NO.	ALb conc. (g/dL)				Lsd
		Con	Pre	Post		
				Short term	Long term	
A: (20-45)	37	3.89 $\pm$ 0.49 <sup>a,A</sup>	2.83 $\pm$ 0.23 <sup>c,A</sup>	3.18 $\pm$ 0.53 <sup>b,A</sup>	3.12 $\pm$ 0.51 <sup>b,A</sup>	0.13
B: (46-69)	27	3.86 $\pm$ 0.35 <sup>a,A</sup>	2.88 $\pm$ 0.26 <sup>c,A</sup>	3.22 $\pm$ 0.45 <sup>b,A</sup>	3.13 $\pm$ 0.41 <sup>b,A</sup>	0.12
LSD		0.12	0.07	0.14	0.13	

Legend as in table 1.

This table shows no significant differences in the concentration of serum Alb between A and B groups ( $p \leq 0.05$ ).

## DISCUSSION

Laparoscopic cholecystectomy is one of the most common elective surgical procedures in the world. Laparoscopic surgery has a number of advantages compared with open surgery. However, insufflations of the peritoneal cavity with carbon dioxide (CO<sub>2</sub>) can be associated with negative consequences. Insufflation during surgery and subsequent desufflation at the end of surgery creates an ischemia–reperfusion situation due to the effects on splanchnic circulation. Thus, it increases in lipid peroxidation these result agree with previous study like (47, 48) examined the effects of cholecystectomy on oxidative stress.

Some of the hypotheses concerning increase or decrease in oxidative stress response suggest that these may be the effects of operative treatment as a stressor and impact of intraabdominal pressure in patients undergoing laparoscopic operation (49), examined the effects of intraabdominal pressure increase on lipid peroxidation and protein oxidation in patients undergoing laparoscopic cholecystectomy. One of the factors which also might be correlated with higher oxidative stress is the length of operation (50), examined the impact of length of operation in patients undergoing laparoscopic and open cholecystectomy on oxidative stress response, and they found higher increase of

TBARS (thiobarbituric acid reacting substances) and decreasing levels of TAS (total antioxidant status) in patients who had longer operative time. Decreasing levels of TAS might correlate with progressive antioxidant system depletion caused by surgical trauma.

Ceruloplasmin is an enzyme which has a role as an oxidant or antioxidant depending on the existence of Fe ions. It also stops lipid peroxidation by direct oxidation of cations. As a result, membrane lipid oxidation is controlled. This defense system consists of Cofactors which are element enzymes and low weighted molecular compounds. Ceruloplasmin makes up 95% of the total Cu contained in these isotopic elements. Cu makes up the main compound of the metalloproteins responsible for oxidation-reduction reactions and essential cofactor for many enzymes, including cytochromes, but it is toxic in its unbound form. The vast majority of serum copper is transported bound to ceruloplasmin<sup>(51)</sup>. Ceruloplasmin is an inflammation-sensitive protein and an acute phase reactant which rises in inflammatory diseases<sup>(52)</sup>. In our study, we found that in patients pre cholecystectomy increase in ceruloplasmin levels in comparison with control group and a slow return to the normal. The possible cause responsible for this elevation was related to increase hepatic synthesis of the protein in consequence to cytokine and leukocyte endogenous mediator stimulants that are released during an acute phase response to tissue damage.

Albumin behaves as antioxidant, this is supported by Sengupta et al<sup>(53)</sup> who suggested that decrease in the levels of this antioxidant accelerate the lipid peroxidation. Proteins may be damaged by specific interactions of oxidants or free radicals with particularly susceptible amino acids. The organic response after surgical trauma is mainly dependent on the extent of surgery and is mediated through a variety of mechanisms that include insulin resistance<sup>(54)</sup>. Moreover, the acute-phase response was also influenced by the extent of preoperative fasting used. Patients undergoing a standard fast showed a significant decline in negative acute-phase proteins such as ALB response to tissue damage.<sup>(55)</sup>

### CONCLUSION

From the data presented in this study, we could obtain the following conclusions: - This study revealed Lipid peroxidation and oxidative stress is more prominent among patients pre patient's cholecystectomy compared with post operation and healthy individuals, and this is mostly the leading cause of Gallbladder inflammation. this lipid peroxidation reflected by MDA and NO were obvious in this study. While Cp was high and Alb was low because of response to tissue damage

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