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The Role of Immunological, and Some Biochemical Variables in Patients with Obese Gallstones in Samarra City

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Abstract

In this study, obese Iraqi Samarra people with gallstones will have their levels of various immunological anti-inflammatory cytokines, antioxidant enzymes, and liver function markers examined. One hundred patient samples were gathered. in order to evaluate the levels of glutathione, MDA, and catalase (CAT) in 100 Iraqi patients with obese gallstones and 50 age-matched young adults of normal weight. This was done in order to investigate the effects of antioxidant levels and antioxidant enzymes in obese gallstone patients. The obese and normal weight groups had serum Catalase levels of 1129.7 ng/ml, 1871.5 pg/ml, and 688.6 ug/ml, respectively. The levels of the normal weight group and the obese group were significantly different. Gallstone patients had significantly higher levels of bilirubin and albumin ($P<0.05$) in the non-enzymatic liver parameters when compared to the control group; however, there was no discernible difference between their serum ALT and AST levels. Additionally, gallstone sufferers' glucose levels were significantly higher ($P<0.05$) than those of a healthy control group. Additionally, there was no discernible difference between the control group and the gallstone patients' serum total protein levels. Serum levels of the cytokines IL-10 and IL-17 (ng/l) were considerably higher in the healthy control group than in the patient group with gallstone disease. Additionally, patients with gallbladder disease had significantly higher levels of triglycerides, very low-density lipoprotein, and total cholesterol than the control group.

Keywords: Gallstones, Obese, Interleukin, Oxidative stress, Liver enzymes

Introduction

Gallstones, which come in different sizes, form in the liver and digestive system. As well as mucin gel and crystals of cholesterol monohydrate, they are made up of proteins. Some people only get a few or one big gallstone, but others get a lot of small ones (Murphy *et al*., 2020; Hao *et al*., 2025; Svensson & Lindberg, 2026). It is the most common problem with the bile system that needs to be treated in the hospital (Nimanya *et al*., 2020; Kunie *et al*., 2025). Gallstones can be put into three groups based on the main biochemicals that make them up: mixed, pigment, and cholesterol. Gallstones, which are yellow or gray stones made up of crystalline cholesterol monohydrate (Shabanzadeh, 2018; Guillen & Pereira, 2024), are mostly made of cholesterol.

A gallstone that obstructs the bile duct and results in bile congestion may harm the liver (Gu *et al*., 2020). The early and temporary increase in ALT is most likely due to transient ampulla obstruction, which results in a sudden increase in bile duct pressure and the ensuing apoptosis of liver cells (Raheem & Kakey, 2023; Morgan *et al*., 2025). For oxidative stress and human health, the antioxidant defense system consisting of catalase, malondialdehyde, glutathione peroxidase (GPx), and glutathione is essential. MDA, a biomarker for oxidative stress, is produced by lipid peroxidation. Reactive oxygen species (ROS), which are produced during either normal or aberrant cellular metabolism, must be neutralized by these enzymes (Carmo de Carvalho e Martins *et al*., 2022). Oxidative stress, which has been connected to a number of health problems, including obesity, can result from an imbalance between the body's ability to produce reactive oxygen species (ROS) and its antioxidant capacity (Jomova *et al*., 2024). Hydrogen peroxide and lipid hydroperoxides are detoxified by the selenium-containing enzyme GPx, which transforms them into alcohols and water (Salman *et al*., 2023). CAT, another antioxidant enzyme, helps convert hydrogen peroxide into molecular oxygen and water, shielding cells from oxidative damage. Recent studies have connected these antioxidant enzymes to obesity. One example is that people who are overweight or fat have higher levels of MDA and lower levels of GPx, CAT, and SOD activity (Chaudhary *et al*., 2023; Damaševičius *et al*., 2025; Nagesh *et al*., 2025).

Calculous cholecystitis, as the name suggests, is an inflammatory disease associated with immune lymphocyte migration and cytokine production (Liu *et al*., 2018; Kowalski *et al*., 2024). On the other hand, little is known about interleukin-based cellular signaling. Previous studies have shown that adult gallbladder tissues express IL-2, IL-7, IL-8, IL-10, and IL-17 for a number of reasons (Shengelia *et al*., 2012). However, little is known about the morphological development of cholecystitis and its

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proinflammatory profile in children. Both proinflammatory and anti-inflammatory qualities are present in the cytokine L-10 (Özdemir *et al*., 2022). Monocytes and B cells create it, which aids in the survival and production of antibodies by B cells (Singh *et al*., 2019; Novak & Dvorak, 2025). Other cytokines, such as IL-8, are inhibited by IL-10. By inhibiting the expression of the macrophage MHC protein, it may also aid in the reduction of excessive inflammation. It has been demonstrated that elevated IL-10 increases the risk of gallstones (Al-Qahtani *et al*., 2024; Huber *et al*., 2025). The condition known as gallstones and its risk factors have not received much attention in the Iraqi city of Samarra. Thus, the current study sought to determine the relationship between risk factors and obese individuals as well as to examine immunological anti-inflammatory cytokines, antioxidant enzymes, and liver function indicators in Iraqi Samarra patients with gallstones. A better understanding of the risk factors may help detect people with gallstone disease (GSD) and reduce the risk of GSD in particular situations. To identify risk factors for GSD, we carried out case-control studies in the Samarra patient community.

Materials and Methods

Subjects

In Samarra City, case-control research was carried out. This study included one hundred patients who were classified as having fat gallstones. Additionally, a sample of fifty seemingly healthy people was drawn from the general community for each participant between January 2025 and February 2026.

After that, the samples were centrifuged for five minutes at 3,000 rpm to extract the plasma from the blood. The samples were then examined. Fifty control subjects provided the 100 samples. Ten milliliters of blood were extracted from young adults aged fifteen to thirty-five and placed in a gel tube. Ten milliliters of blood were drawn early in the morning after each subject had fasted for ten to twelve hours. After that, this amount of blood was kept in a gel tube at 23 to 27 degrees Celsius for an hour. After that, the blood was spun at 3,000 x g for five minutes to get the serum out of it. There were two halves of the serum. The first part was five milliliters long and was used to look at molecular outcome factors in the blood. To enable further immunological data processing, the remaining three milliliters of the second half were moved to Eppendorf tubes and frozen at -20 degrees Celsius. Techniques for store-bought enzyme-linked immunosorbent tests (ELISAs).

The pharmaceutical lab in Samarra City used an automatic biochemical analyzer (Cobas C 111) to measure ALT, AST, ALP, FBG, bilirubin, and albumin levels. measurement of alkaline phosphatase (ALP). A number of hepatobiliary and bone disorders can be identified with the use of elevated serum alkaline phosphatase. Alkaline phosphatase levels 10 to 12 times the upper reference limit (URL) may indicate a biliary blockage; these levels often return to baseline after the obstruction is removed.

Principle

Colorless para-nitrophenyl phosphate (p-NPP) is hydrolyzed by the test sample's alkaline phosphatase to produce inorganic phosphate and para-nitrophenol, a yellow phenoxide form at alkaline pH. The rate at which absorbance at 404 nm increases is clearly correlated with the alkaline phosphatase activity of the test sample. The test sample is given the ideal amounts of zinc and magnesium ions to activate the alkaline phosphatase.

Calculating the Concentrations of HDL Cholesterol, Triglycerides, and Serum Total Cholesterol

According to Spinreact kits, the enzymatic approach was used to measure total cholesterol, triglycerides, and HDL cholesterol.

Determination of Immunological Markers (Interleukin)

A variety of serum interleukins, such as proinflammatory cytokines (IL-17) and anti-inflammatory cytokines (IL-10), can be found using the sandwich enzyme-linked immunosorbent assay (ELISA) method, which is made in China by BT LAB (Bioassay method Laboratory). The ability of the ELISA method to identify antigens is well known.

Statistical Analysis

The statistics were stored in a computerized database system. The statistical analysis was carried out with the assistance of the computer application known as SPSS version 20, which stands for Statistical Package for Social Sciences. Every parameter under investigation was expressed using the mean ± standard deviation (SD). The significance of the differences between the sets was assessed using the t-test. A P value of less than 0.05 was considered significant.

Results and Discussion

Table 1 displayed the antioxidant enzyme results. The glutathione level was substantially greater ($p<0.03$) in obese gallstones (559.6±1129.7 ng/ml) compared to normal weight (1714.2±6427.2 ng/mL). The obese gallstones group had significantly ($p<0.001$) higher serum MDA levels (9852.2 ±1871.5 pg/ml and 375.0 ±220.3 pg/ml, respectively) than the normal weight group. The mean serum Catalase concentration was significantly lower ($p<0.001$) in the obese gallstones group (1188.4 ±688.6 ug/ml) than in the normal weight group (17613.6 ±1 65.1 ug/ml).

Table 1. Comparing oxidative stress and antioxidant enzymes in groups with obese gallstones and healthy controls.

Antioxidants	Groups	N	Mean	Std. Deviation	P value
Glutathione, ng/ml	Patients	**100**	559.6	1129.7	0.03
	control	**50**	1714.2	6427.2	
MDA, pg/ml	Patients	**100**	9852.2	1871.5	0.001
	control	**50**	375.0	220.3	
Catalase, ug/ml	Patients	**100**	1188.4	688.6	0.001
	control	**50**	17613.6	1 65.1	

Gallstone patients and controls had significantly different serum ALT and AST levels, although there was no significant difference in ALP levels, according to the present results in **Table 2**. In

contrast to the control group, gallstone patients had significantly higher levels of bilirubin and albumin (P<0.05) in the liver non-enzymatic parameters. Additionally, gallstone sufferers' glucose levels were significantly higher (P<0.05) than those of a healthy control group. Additionally, there was no appreciable change between the control group and gallstone patients' serum total protein levels.

Table 2. Liver function comparison between gallstone patients and controls

Liver functions tests	Group	N	Mean	Std. Deviation	P value
ALT, U/l	Patients	**100**	28.61	2.87	0.002*
	Control	**50**	19.8	1.19	
AST, U/l	Patients	**100**	29.84	3.12	0.04*
	Control	**50**	20.31	1.13	
ALP, U/l	Patients	**100**	78.75	3.98	0.4
	Control	**50**	98.19	0.21	
Bilirubin, mg/dl	Patients	**100**	0.71	0.02	0.001*
	Control	**50**	0.37	0.01	
FBG, mg/dl	Patients	**100**	126.43	3.17	
	Control	**50**	89.63	1.87	
Total protein, g/dL	Patients	**100**	6.02	0.18	0.29
	Control	**50**	8.98	0.14	

Fasting blood glucose (FBG), aspartate aminotransferase (AST), aspartate transaminase (ALT), and aspartate aminotransferase (ALP) were used to evaluate the P value. * Critical

According to this study, patients with gallbladder illness had higher levels of cholesterol, and their levels of triglycerides, total cholesterol, and very low-density lipoprotein were significantly (p<0.05) higher than those of the control group. When compared to the control, the concentrations of high- and low-density lipoprotein did not differ significantly **(Table 3)**.

Table 3. The concentration of lipid profile comparison between gallstone patients and controls

Immunological markers	Groups	N	Mean	Std. Deviation	P value
Cholesterol, mg/l	Patients	100	281.32	22.91	**0.01****
	Control	50	243.65	19.82	
TG, mg/l	Patients	100	193.76	54.12	**0.01****
	Control	50	148.54	43.63	
HDL, mg/l	Patients	100	42.65	6.86	**0.2**
	Control	50	45.87	5.98	
VLLD, mg/l	Patients	100	38.12	12.65	**0.4**
	Control	50	34.28	11.54	
LDL, mg/l	Patients	100	198.54	38.26	**0.01****
	Control	50	178.65	29.21	

*** Significant difference at P< 0.05**
****Highly significant difference at P< 0.01**

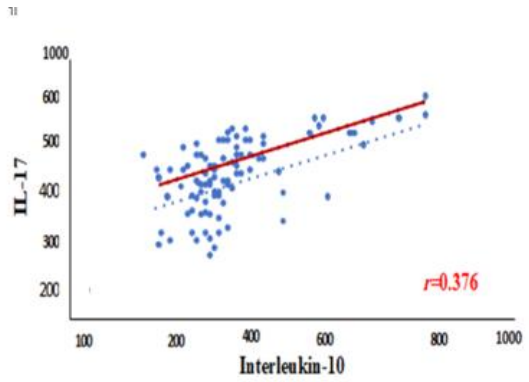
Table 4 indicates that the healthy control group had considerably greater serum levels of the cytokines IL-10 and IL-17 (ng/l) than the patient group with gallstone disease.

Table 4. Gallstone patients' and controls' levels of IL-10 and IL-17 were compared

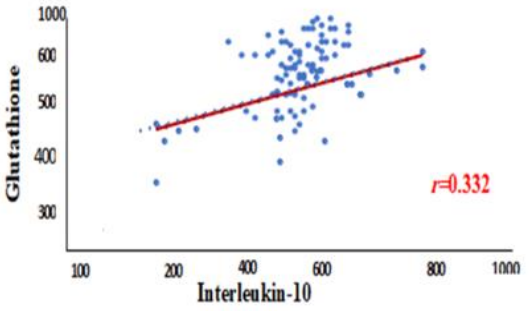
Immunological markers	Group	N	Mean	Std. Deviation	P value
IL-10, ng/l	Patients	100	366.35	12.19	**0.01***
	Control	50	312.87	11.18	
IL-17, ng/l	Patients	100	139.243	5.98	**0.01***
	Control	**50**	**112.853**	**6.65**	

Correlations between Parameters in Patients with Obese Gallstones

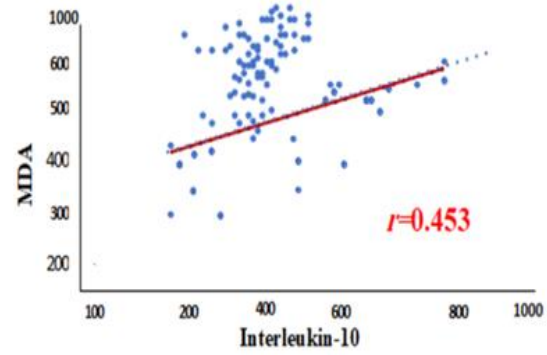
The result presented in **Figure 1a** shows that there is a positive correlation between interleukin-10 and interleukin-17 in obese gallstone patients (r=0.3761). The result presented in **Figure 1b** shows that there is a positive correlation between interleukin-10 and Glutathione in obese gallstone patients (r=0.332). The result presented in **Figure 1c** shows that there is a positive correlation between interleukin-10 and MDA in obese gallstone patients (r=0.453). The result presented in **Figure 1d** shows that there is a positive correlation between interleukin-10 and Catalase in obese gallstone patients (r=0.432).



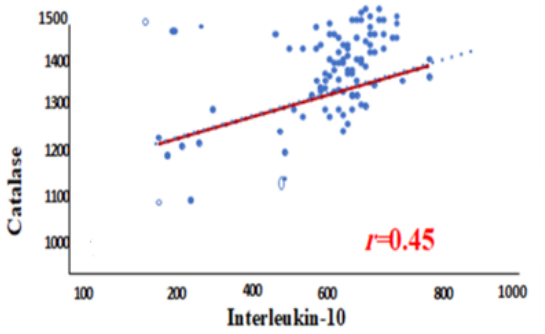
a)



b)



c)



d)

Figure 1. The correlation results between interleukin-10 and other measured parameters in obese gallstone patients: (a) interleukin-17, (b) Glutathione, (c) MDA, and (d) Catalase

Glutathione is made up of three different amino acids: cysteine, glutamic acid, and glycine. This antioxidant has a number of vital roles in the body, including immunological response and cleaning, as well as protecting cells from oxidative damage (Salman *et al*., 2023; Kajanova & Badrov, 2024; Salem *et al*., 2025). Conversely, obesity with gallstones is a condition in which an individual has excess body fat, which may be detrimental to their health. There may be a connection between glutathione levels and being overweight, according to recent research. Compared to the healthy groups, the levels of glutathione were much lower in our investigation. According to 2015 research published in the Journal of Clinical Endocrinology & Metabolism, glutathione levels were lower in fat individuals than in non-obese individuals. Insulin resistance and other metabolic issues that are often present in obese individuals have been connected to these decreased levels (Swinburn *et al*., 2011; Kwatra *et al*., 2024; Novak & Jelic, 2025).

Research that looked at the connection between human adipose tissue catalase activity and obesity was published in 2008 in the Journal of Clinical Endocrinology and Metabolism. Comparing the adipose tissue of lean and obese people revealed a striking difference in catalase activity. In addition, when induced to become obese by a high-fat diet, individuals with obese gallstones had reduced catalase activity in their adipose tissue. Because reduced catalase activity causes oxidative stress and inflammation in adipose tissue, the authors hypothesized that this would hasten the development of obesity-related health problems (Furukawa *et al*., 2008; Altowijri, 2024; Zairkhanova *et al*., 2024; Abdoul-Latif *et al*., 2025; Parveen & Ghosh, 2025).

New studies show that there is a strong link between MDA levels and being overweight. The Journal of Clinical Biochemistry and Nutrition released a study that looked at MDA levels and the link between fat and gallstones in Korean women. Although MDA levels were significantly greater in obese women than in non-obese women, the results of the study showed that oxidative stress may play a role in the growth of both fat and gallstones (Lee *et al*., 2010; Aksoy & Akaydin, 2024; Nguyen *et al*., 2025).

The current study revealed a positive and statistically significant link between GSD patients and higher levels of liver function enzymes, such as ALT, AST, and serum total bilirubin, confirming earlier results (Khurshid & Mohammed, n.d.). Gallstone (GS) development and serum ALP levels showed a non-significant association, although there was no statistically significant correlation between GS creation and elevated ALP levels. Nonetheless, the agreement with research findings showed that those with GSD typically had higher serum ALP levels (Song *et al*., n.d.; Jannath *et al*., 2024; Elango & Govindaraju, 2025). Gallstone patients had non-significantly decreased serum total protein levels, which correlated with the findings of this investigation (Hamad & Kakey, 2014). The current study's findings showed that blood glucose levels were statistically significantly higher in gallstone patients. The findings supported the findings of the majority of earlier research, which demonstrated that GSD was highly prevalent in diabetics (Jiang *et al*., 2023; Liedekerke *et al*., 2025). Gallstones are more common in diabetics because of decreased gallbladder motility, changed cholesterol nucleation, and bile supersaturation (Gu *et al*., 2023).

Interleukins control both the acute and chronic stages of gallstone disease and have a significant impact on the inflammatory process in these patients. It triggers the innate or acquired immune response, which either neutralizes the harmful stimuli and starts tissue healing, or it continues as a long-term, chronic process that both destroys and restores tissue (Spasovski *et al*., 2022; Dobrzynski *et al*., 2024).

Both proinflammatory and anti-inflammatory interleukins, IL-10 and IL-17, interact with each other in a complex and changing way, affecting how the immune system works and how people with gallstones keep their balance (Al-Qahtani *et al*., 2024). Negative feedback loops are one way that proinflammatory and anti-inflammatory interleukins work together to stop bad things from happening. Anti-inflammatory interleukins may be made with the help of proinflammatory interleukins to lower inflammation that is too high. To lower tissue damage and inflammation, for example, making less IL-10 and more IL-17 and IL-6 has been linked to less tissue damage (Chen *et al*., 2019). In order to determine whether gallstone patients' immune responses were suitable, this study examined proinflammatory and anti-inflammatory cytokines. The results of the study showed that IL17 and IL-10 were more prevalent than proinflammatory cytokines, which may suggest a more systemic anti-inflammatory response. The best capacity to distinguish between patients and controls was shown by the IL-17 and IL-10 ratios.

These findings supported the previous report (Atman, 2006). The relationship between gallstone development and lipid profile was studied in 2006. It is thought that aberrant bile synthesis from the liver is the cause of supersaturation of cholesterol (Dennis *et al*., 2005). For cholesterol stones, which are mostly made up of cholesterol (Portincasa *et al*., 2006), the idea of cholesterol supersaturation as a cause for gallstone formation has been stressed. High cholesterol makes it harder for the gallbladder to empty. High overall cholesterol is not always linked to gallstones, but gallstones happen when there is too much cholesterol in the blood. Triglyceride amounts that are too high may make it harder for the liver to empty. Although the exact process by which cholesterol stones develop is unknown, it most likely results from a complicated change in hepatobiliary function. In comparison to bile salts and phospholipids, bile containing cholesterol stones contains more cholesterol (Williams *et al*., 2008). resulting in the formation of cholesterol crystals. Such bile is referred to as "lithogenic" or "supersaturated."Oestrogens and conditions that disrupt the intrahepatic circulation of bile salts, ileal disease, resection or bypass, and cholestyramine therapy decrease the concentration of bile salts in bile. Although all of these variables are linked to an increased risk of stones, some individuals with cholesterol supersaturation do not develop stones, indicating the significance of other factors (Stamatelou & Goldfarb, 2023).

Conclusion

Patients with fat gallstones had higher levels of MDA than control groups, although their levels of glutathione and catalase were found to be lower. Patients with obese gallstones may experience serious, potentially fatal consequences that can affect people of all ages. Unquestionably, obesity raises oxidative stress. As evidenced by rising MDA, glutathione, and catalase activity, rising BMI increased lipid peroxidation. Low-grade systemic inflammation may have boosted oxidative status by causing the production of free radicals, which in turn increased lipid peroxidation. When compared to individuals with cholecystitis, healthy subjects had much higher levels of cytokines, which may indicate an improper immune response. As well, patients with gallbladder disease had significantly higher levels of triglycerides, very low density lipoprotein, and total cholesterol than the control group.

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