

# Study of Biological Indicators and Biochemical Changes Related to Gallstones Formation in Patients in Kirkuk City

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## Abstract

The study included (29) patients with gallstones who were visiting the New Kirkuk Hospital and whose condition was confirmed by conducting pathological examinations and ultrasound examination (sonar) in comparison to (29) healthy subjects.

This study aimed on estimating of some chemical variables in blood serum, including measurement of total Bilirubin, direct and indirect Bilirubin. In addition, measuring the activities of Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT), blood sugar, in relation to the percentage of patients consuming soft drinks, and the state of intestines and stool samples in both male and female for different age groups.

A comparison was made between patients with gallstones and was confirmed to have jaundice and patients with gallstones who did not develop jaundice. A significant increase in blood sugar, the levels of total Bilirubin, direct and indirect Bilirubin, Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) was observed regardless to the patient's gender, rate of soft drink consumption, and the bowel condition didn't have effect in forming gallstone in comparison to healthy subjects.

Comparison between patients with gallstones and healthy people has revealed the presence of significant differences in the bowel condition between two groups. Very high levels of total Bilirubin, direct and indirect Bilirubin, Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) in patients with lethargy of the intestine and indigestion. However, there were no significant differences in the percentage of blood sugar or the amount of soft drinks consumed between two groups. Gender has no effect on the results obtained.

**Keywords:** *biological indicators, biochemical changes, gallstones formation*

## Introduction

Gallstones are one of the most common diseases affecting the bile duct and it has been estimated that about 10-15% of adults have this disease in the USA. 80% of cases are without noticeable symptoms<sup>1</sup>. Gallstones are formed as a result of saturation of the bile fluid with some dissolved salts. For example, when the concentration of cholesterol is high in the liquid, the substance will precipitate and small crystals will form<sup>2</sup>. Sometimes the stone is formed as small as a grain of sand. A gallbladder may contain one or several dozen stones at a time, at other times, the sedimentation is in the form of a clay material called "biliary mud"<sup>3</sup>. The

formation of stones in the gallbladder and the bile duct is due to several reasons, the most important of which is the inactivity of the gallbladder, increased concentration of salt materials in the bile fluid, such as an increase in the concentration of cholesterol, increased bilirubin concentration. The increasing concentration of any additive in the liquid ultimately causes the liquid to become saturated with those additives. These substances settle at the bottom of the gallbladder and form small crystals that grow until they become a large stone<sup>4</sup>.

Gallstones that are discovered by chance and do not cause pain can be left unintended, because most patients who do not have symptoms of gallstone may

not have any problems. However, when the patient suffers from pain due to the stone, then these stones must be treated<sup>5</sup>. Treatment methods for gallstones are, removal of the gallbladder by conventional surgery<sup>6</sup>. As for laparoscopic surgery, it is preferred over the conventional surgery for lack of pain, faster recovery, and patient able to leave hospital in a very short time. Aesthetically, the wounds in the abdominal wall are small without any abnormalities. Laparoscopic removal of the gallbladder is the currently approved method and the surgeon resorts to conventional surgery in limited cases only<sup>7</sup>.

There are some oral medications that can help dissolve stones in some limited cases. However, it may take months or years for the stone to completely dissolve, such as Ursodeoxycholic acid<sup>8</sup>.

Sound wave therapy can be used to divide a large stone into small, soluble stones if the stone is less than 2 cm. This method is used in cases where laparoscopic and conventional surgery is very dangerous for the patient. The risks of this fragmentation are that some of the broken pieces may still be large and block the bile duct or the general collecting duct, causing inflammation of the gallbladder<sup>9</sup>.

As for herbs, there is no conclusive scientific evidence for their effectiveness, and there is a drug on the market named (Gallbladder cleanse) suppose it dissolves the pebbles, and it contains olive oil, some herbs, and fruit juice, however, there is no conclusive scientific evidence of the effectiveness of this formula<sup>9,10</sup>.

## Materials and Methods

This study was conducted in New Kirkuk Hospital in Kirkuk Governorate for the period from September – to December 2019. The study included (29) blood samples for patients with gallstones and (29) blood samples for healthy subjects, for different age groups, ranging between (25-46) years.

### Sample collection and laboratory tests

Blood samples were taken when patients with gallstones were visited the hospital and underwent surgery to remove their gallbladder. Blood samples were obtained from a vein of 10 cm<sup>2</sup> from each patient

and blood was placed in EDTA (ethylene diamine tetra acetic acid) substance-free test tubes. The blood was left at room temperature for 20 minutes, after which the blood serum was separated using a quick centrifuge at 3000 rpm for 10 minutes. Serum was drawn with a fine pipette and placed in test tubes for biochemical tests.

### Measurement of total serum Bilirubin

A ready kit from bioMerieux (French company) was used the procedure described by Doumas and his colleagues<sup>12</sup> to measure the total bilirubin concentration in the blood serum. Using blank tube contained of 1 ml of working solution, standard tube that contained 1 ml of work solution and 10 $\mu$ L of standard solution, then the test tube that contained 1 ml work solution and 10 $\mu$ L of the test sample. The tubes were shaken well, and then incubated at 37°C. The optical absorbance intensity was measured by spectrophotometer at wavelength 450 nm.

### Direct measurement of bilirubin in serum

Direct bilirubin was measured using a ready-made assay kit from Randox. Two tubes were used, one called blank which contained 0.20 ml of reagent 1, 2 ml of Sodium chloride(9g/l), and 0.20 ml of the sample. While the sample tube contained 0.20 ml of reagent 1, 2 ml of Sodium chloride(9g/l), 0.20 ml of the sample and 1 drop (0.05) ml of reagent 2. The tubes were mixed and left for 5 minutes at a temperature 20- 25°C. The absorbance of the sample was measured at 530 nm wavelength against a blank solution. The amount of direct Bilirubin is calculated based on the following equation:

$$CB(\text{m}\backslash\text{dl}) = 14.4 * A_{cb} \text{ where } A \text{ is the absorbance.}$$

### Measurement of indirect Bilirubin in serum

The amount of indirect Bilirubin is calculated from calculating the difference between the amount of total Bilirubin and direct Bilirubin from the following equation:

$$\text{Amount of indirect Bilirubin} = \text{total Bilirubin} - \text{direct Bilirubin}$$

### Measuring blood sugar level

The level of glucose in the blood serum was determined by using the enzymatic method by using the ready-made kit from the German company biocon,

and following the methods described by Passing *et al*<sup>13</sup>. The blank tube contained of 1 ml work solution only, the standard tube contained 1 ml of work solution and 10 $\mu$ L of standard solution, finally the test tube which contained 1 ml work solution and 10 $\mu$ L of the sample. The tubes were shaken well, then incubated at 37 $^{\circ}$  C and the optical absorbance intensity was measured by a spectrophotometer at wavelength 540 nm.

#### **Measuring Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT)**

A ready-made analysis kit from a Randox-UK-company was used according to the method described<sup>14</sup>. (0.5 ml) of buffer base solution added to (1.0 ml) of blood serum, the solution mixed well and incubate in 37 $^{\circ}$ C, then (0.5 ml) of the staining solution DNPH was added, mixed well, and left for 20 minutes at a temperature (20-25)  $^{\circ}$ C. Then 0.5 ml of NaOH solution was added at a concentration of 0.4 N. The solution was mixed and left for 5 minutes. After that, the absorbance intensity was read against the blank solution at the wavelength of 546 nm. The activity of the enzyme was calculated in international units / liter (I.U / L).

#### **Statistical analysis**

Data on the study samples were collected and statistically analyzed using the (SAS). It calculated mean and standard error (mean  $\pm$ S.D). The significant differences were tested using cut off parameter *p*-Value of (0.05).

### **Results and Discussion**

Table (1) demonstrates the results obtain from comparing healthy subjects and gallstone patients and significant differences has reported between gallstones patients and healthy people in the levels of total Bilirubin, direct Bilirubin, Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT). While no significant differences were recorded in indirect Bilirubin ratio, blood sugar or the effect of drinking soft drinks between two groups. The reason for the significant increase in the levels of Bilirubin and liver enzymes over the normal levels is due to the presence of blockage in the bile ducts and gallbladder due to the presence of gallstones and deficiency in hepatocytes<sup>15</sup>. Table (2) indicates the study of chemical variables in

blood serum among healthy males and females (without gallstones). No significant differences between two groups were observed. The absence of a significant increase in the chemical variables for healthy people is due to no existence of gallstones that cause blockage in the bile ducts and that the chemical parameters and enzymes remained within the normal range<sup>16</sup>.

Table (3) demonstrates the chemical variables in the serum of gallstones patients in both males and females. No significant differences were found in the levels of total Bilirubin, direct Bilirubin, Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) and blood sugar level. The percentage of drinking soft drinks or the bowel condition among males and females who were diagnosed with gallstones was not affected. An increase in the percentages of chemical changes in close proportions without significant differences between the sexes was noticed, this is in line with what Sherlock has described<sup>17</sup>.

Table (4) indicates the chemical changes in serum of male patients than in healthy male. There were high significant differences in direct Bilirubin and Aspartate Aminotransferase (AST/GOT). There were no significant differences in total Bilirubin, blood sugar percentage, Alanine Aminotransferase (ALT/GPT), consumption of soft drinks and bowel condition among male patients and healthy male. The increase in some liver enzymes and other variables is due to a disorder or obstruction in the metabolism of Bilirubin and damages to some liver cells<sup>20,21</sup>.

Table (5) indicates the chemical variables in the blood serum of the female patients in comparison to the healthy female. Very high significant differences were found in the levels of total Bilirubin, direct Bilirubin, Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) in the female patients, while no significant differences in blood sugar percentage, consumption of soft drinks and bowel condition among two groups. This increase in the chemical parameters in the female patients might be due to the influence of the gender factor, genetics, digestive disorders, rapid weight loss, in addition to chronic diseases that help in the development of gallstones in female<sup>22-24</sup>.

**Table 1: Biological indicators in healthy subjects and gallstone patients**

	Blood sugar		GPT		GOT		Indirect Bilirubin		Direct Bilirubin		Total Bilirubin	
	Mean+_std. deviation	sig	Mean+_std. deviation	sig	Mean+_std. deviation	sig	Mean+_std. deviation	sig	Mean+_std. deviation	sig	Mean+_std. deviation	sig
Healthy subjects	106.0833+_19.1309	0.939	12.2083+_4.6995	0.000	10.2083+_4.6155	0.005	0.6208+_0.1215	0.063	0.1958+_7.506	0.000	0.8208+_0.156	0.006
Patients	106.6207+_29.4684		5.7586+_3.4605		17.3793+_11.0725		0.8021+_0.4540		0.4345+_0.2859		1.2400+_0.7012	

**Table 2: Chemical variables in blood serum among healthy males and females (without gallstones).**

	Blood sugar		GPT		GOT		Indirect Bilirubin		Direct Bilirubin		Total Bilirubin	
	Mean+_Stander deviation	sig	Mean+_Stander deviation	sig	Mean+_Stander deviation	sig	Mean+_Stander deviation	sig	Mean+_Stander deviation	sig	Mean+_Stander deviation	sig
Healthy male	101.1250+_9.8043	0.381	13.3750+_4.6579	0.402	9.3750+_2.7742	0.544	0.6250+_0.1035	0.909	0.2000+_9.258	0.852	0.8375+_0.1506	0.720
Healthy female	108.562+_22.2859		11.6250+_4.759		10.6250+_5.340		0.6188+_0.1328		0.1937+_6.80		0.8125+_0.1628	

**Table 3: The difference of chemical variables between males and females gallstones patients**

	Blood sugar		GPT		GOT		Indirect Bilirubin		Direct Bilirubin		Total Bilirubin	
	Mean+_stander deviation	sig	Mean+_stander deviation	sig	Mean+_stander deviation	sig	Mean+_stander deviation	sig	Mean+_stander deviation	sig	Mean+_stander deviation	sig
Male patients	109.222+_26.4186	0.756	6.666+_4.582	0.352	18.777+_14.437	0.657	0.8467+_0.5221	0.730	0.4867+_0.3051	0.519	1.344+_0.8267	0.600
Female patients	105.4500+_31.324		5.3500+_2.8704		16.7500+_9.569		0.7820+_0.4332		0.4110+_0.2817		1.193+_0.6553	

**Table 4: serum of male patients than in healthy male**

	Blood sugar		GPT		GOT		Indirect Bilirubin		Direct Bilirubin		Total Bilirubin	
	Mean+_stand devaition	sig	Mean+_stand devaition	sig	Mean+_stand devaition	sig	Mean+_stand devaition	sig	Mean+_stand devaition	sig	Mean+_stand devaition	sig
Healthy male	101.125+_9.8043	0.427	13.375+_4.657	0.009	9.3750+_2.774	0.091	0.6250+_0.1035	0.258	0.2000+_9.258	0.022	0.8375+_0.1506	0.109
Male patients	109+_22.26.418		6.666+_4.5826		18.777+_14.437		0.8467+_0.5221		0.486+_0.3051		1.344+_0.826	

**Table 5: Chemical variables in the blood serum of the female patients in comparison to the healthy female**

	Blood sugar		GPT		GOT		Indirect Bilirubin		Direct Bilirubin		Total Bilirubin	
	Mean+_sta. devaition	sig	Mean+_sta. devaition	sig	Mean+_sta. devaition	sig	Mean+_sta. devaition	sig	Mean+_sta. devaition	sig	Mean+_sta. devaition	sig
Healthy Female	108.56+_22.285	0.740	11.625+_4.759	0.000	10.6250+_5.340	0.029	0.6188+_0.1328	0.156	0.1937+_0.8005	0.000	0.8125+_0.1628	0.030
Female patients	105.45+_31.324		5.350+_2.870		16.750+_9.569		0.7820+_0.433		0.4110+_0.2817		1.193+_0.553	

## Conclusion

Gallstones are one of the most common diseases affecting the bile duct. This study aimed on estimating of some chemical variables in blood serum among gallstone male and female patients in comparison to healthy subjects. The significant increase in the levels of Bilirubin and liver enzymes over the normal levels is due to the presence of blockage in the bile ducts and gallbladder due to the presence of gallstones and deficiency in hepatocytes. While no significant differences in blood sugar percentage, consumption of soft drinks and bowel condition among two groups. Gender has no effect on the results obtained.

**Acknowledgements :** This study was supported by both authors financially. Special thanks for New Kirkuk Hospital in Kirkuk city for their help providing patient samples and collecting Anthropometric data.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Agriculture and all experiments were carried out in accordance with approved guidelines.

## References

- 1- Saqib A, Shaikh S, Sodhar J. GALL STONES. The Professional Medical Journal. 2014; 21(02): 386-390.
- 2- Vázquez, M, Rigotti, A, Zanlungo, S. Molecular mechanisms underlying the link between nuclear receptor function and cholesterol gallstone formation. Journal of lipids, 2012.
- 3- Magnuson, T, Lillemo, K. Altered bile composition during cholesterol gallstone formation: cause or effect?. Journal of Surgical Research. 1990; 48(6): 584-589.
- 4- Krawczyk M, Lütjohann, D. Phytosterol and cholesterol precursor levels indicate increased cholesterol excretion and biosynthesis in gallstone disease. Hepatology. 2012; 55(5): 1507-1517.
- 5- Wu X, Liang X, Du, Y, Zhang, Y. Prevention of gallstones by Lidan Granule: Insight into underlying mechanisms using a guinea pig model. Biomedical reports. 2016; 5(1): 50-56.
- 6- Grady D. Doctors try new surgery for gallbladder removal. The New York Times. 2007; 20.
- 7- Chan K, Yeh, TS. Laparoscopic cholecystectomy for early gallbladder carcinoma: long-term outcome in comparison with conventional open cholecystectomy. Surgical Endoscopy And Other Interventional Techniques, 2006; 20(12): 1867-

- 1871.
- 8- Leuschner U, Lindenthal B. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. *Hepatology*, 52(2): 472-479.
  - 9- Tazuma S, Unno, M, Igarashi Y. Evidence-based clinical practice guidelines for cholelithiasis 2016. *Journal of gastroenterology*, 2017; 52(3): 276-300.
  - 10- Zhang, J. Z., Zhang, X. L., Liang, X. Q., Gu, H. G., & Zhu, P. T. (2008). Effects of different Chinese herbal medicines on biochemical parameters in guinea-pig with pigment gallstones. *Zhong xi yi jie he xue bao= Journal of Chinese integrative medicine*, 6(8), 856-859.
  - 11- Li Z, Lin, Z, Lu, Z, Feng, Z. Coix seed improves growth performance and productivity in post-weaning pigs by reducing gut pH and modulating gut microbiota. *AMB Express*. 2019; 9(1): 115.
  - 12- Dourmas B, Perry, BW, Sasse, E, Straumfjord J. Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. *Clinical chemistry*. 1973; 19(9): 984-993.
  - 13- Passing H, Bablok, W. A general regression procedure for method transformation. *J Clin Chem Clin Biochem*, 1988; 26(11): 783-790.
  - 14- Orhan D, Orhan, N, Ergun, E. Hepatoprotective effect of *Vitis vinifera* L. leaves on carbon tetrachloride-induced acute liver damage in rats. *Journal of ethnopharmacology*. 2007; 112(1): 145-151.
  - 15- Newsome, P, Cramb R. Guidelines on the management of abnormal liver blood tests. *Gut*. 2018; 67(1): 6-19.
  - 16- Lammert F, Acalovschi, M. EASL Clinical Practice Guidelines on the prevention, diagnosis and treatment of gallstones. 2016.
  - 17- Sherlock S. *Virus hepatitis. Diseases of the liver and biliary system*. 1997.
  - 18- Sherlock, S, Dooley, J. *Diseases of the liver and biliary system*. John Wiley & Sons. 2008.
  - 19- Schuppan, D, Afdhal, N. Liver cirrhosis. *The Lancet*. 2008; 371(9615): 838-851.
  - 20- Kwo P, Cohen, S. ACG clinical guideline: evaluation of abnormal liver chemistries. *American Journal of Gastroenterology*. 2017; 112(1): 18-35.
  - 21- Giannini E, Testa, R, Savarino, V. Liver enzyme alteration: a guide for clinicians. *Cmaj*. 2005; 172(3): 367-379.
  - 22- Bonfrate L, Wang, D, Garruti, G. Obesity and the risk and prognosis of gallstone disease and pancreatitis. *Best practice & research Clinical gastroenterology*. 2014; 28(4): 623-635.
  - 23- Nakeeb A, Comuzzie, A, Martin L. Gallstones: genetics versus environment. *Annals of surgery*. 2002; 235(6): 842.
  - 24- Stinton L, Myers R. Epidemiology of gallstones. *Gastroenterology Clinics*, 2010; 39(2): 157-169.