Determination of Interleukins 2, 6 and some liver enzymes in gallstone patients' pre- and post- cholecystectomy

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Abstract

This study was conducted in Tikrit Teaching Hospital to elevate the serum concentration of Interleukins 2, 6 and the specific activity of liver enzymes for the gallstone patients who undergo cholecystectomy.

This study included 120 patients (92 female and 28 male) aged between 15-65.

The results showed that there is a significant difference in the Interleukins 2, 6 levels before and after the surgery; also there were significantly differences ($p \leq 0.01$) in specific activity of liver enzyme of liver in male and female before and after cholecystectomy.

Keywords: Interleukin 2, 6, liver enzymes, gallstone and cholecystectomy.

The aim of the study: Study of relationship between IL-2, IL-6 and gallstone disease before and after cholecystectomy, and study of liver enzymes in gallstone patients.

Introduction:

Gallstones (GS) are one of the most common biliary diseases. The gallstone's disease is the most common medical conditions in the United States and in developed counties in general.[1] Among biliary tract disease, it is the leading cause for inpatient admission for gastrointestinal problems. [2] [3] It is continue to be one of the major health problems in the world today, although the exact number of patients is unknown, because of the quietness of the disease when there is no complication. GS can occur anywhere within the biliary tree, including the gallbladder and the common bile duct.[4] The most accurate and noninvasive method of predicting gallstone disease was achieved with the advent of the ultrasound, which has a sensitivity/ specification of greater than 95%. However, the real prevalence of the disease remains hard to drive as the majority of patients remain asymptomatic. Recent studies indicate that only 10% - 18% of the patients with cholelithiasis develop symptoms such as nausea, vomiting and abdominal pain. [5]

Materials and Methods

The population of this study for both gender factors [male (M) & female (F)] patients age range from 16 to 65 years who are selected by depending on this study questionnaire. 10 ml of venous blood was drawn from each group subjects after (8-12) hour fasting and the serum stored at (-20 C°). We take two samples from the patients before the surgery and after 15 days of it.

Determination of interleukins 2, 6 use the quantitive determination of interleukins 2 and 6 in serum by the human interleukin (IL-2, IL-6) ELISA Kit. Serum alkaline phosphatase (ALP) was measured by Colorimetric determination using a kit (CUSABIO).

The activities of AST and ALT were calculated colorimetrically according to the method of Reitman and Frankle, utilizing a ready-made kit for this purpose. Estimation of GGT according to Persijn & Van der slik method. Standardized against recommended IFCC method.

Statistical Analysis

We use (Minitab) program for statistical analysis window, version (16.0) to calculate the mean, and standard deviation. The independent *t* test was used to compare the independent variables. Statistically, *P* value of 0.05 and 0.01 is considered significant. [6]

Result and discussion

Serum Interleukins (6)

The present study shows a significant increase ($P \leq 0.05$) in the level of Interleukins-6 between the groups of male. The significant increase is in group (A) before the cholecystectomy (75.57 ∓ 33.50 pg/ml) compared with group B (46.99 ∓ 41.69 pg/ml) and C (61.86 ∓ 48.21 pg/ml), respectively. This group (A) increases significantly ($P \leq 0.05$) (86 ∓ 33.50 pg/ml) before surgery comparing with its level after it, while there is no significant difference in groups B&C before and after surgery.

Table (1): Levels of interleukin-6 (pg/ml) according to sex and age.

Patients		Mean ±SD	
	groups	Pre	Post
Male	A	75.57± 33.50 aA	66.78 ±31.07 abB
	B	46.99 ±41.69 bcA	41.69 ± 16.05 cA
	C	61.86 ± 48.21 abA	53.26 ±4.02 bcA
Female	A	40. 56 ± 39.09 bcA	37.02 ± 35.12cA
	B	57.92 ±37.25 aA	46.06 ± 29.77 abB
	C	58.03 ±39.86 aA	45.39 ±31.68 abB

Similar letters mean the absence of significant differences. ($P \leq 0.05$)

In females group there is a significant decrease in group A (40.56 ∓ 39.09 pg/ml) ($< P \leq 0.05$) before surgery comparing to group B (57.92 ∓ 37.25 pg/ml) and C (58.03 ∓ 39.86 pg/ml), respectively. Figure (1) shows significant differences in females group before and after surgery. There was significant increase before surgery in females group B (57.92∓37.25) and group C (58.03∓39.89pg/ml) comparative with their level after surgery. In comparison between the males

and females group, results show a significant decrease (P≤0.05) in males group B (46.99 ∓ 41.69 pg/ml) and females group A (40.56 ∓ 39.09 pg/ml) pre-cholecystectomy comparing with other group in the two genders (males and females). In the patients group of two gender after the cholecystectomy results show there is a significant decrease (P≤0.05) in males group B (41.69 ∓ 16.05 pg/ml) and females group A (37.02 ∓ 35.12 pg/ml), respectively comparing with other group in the two genders.

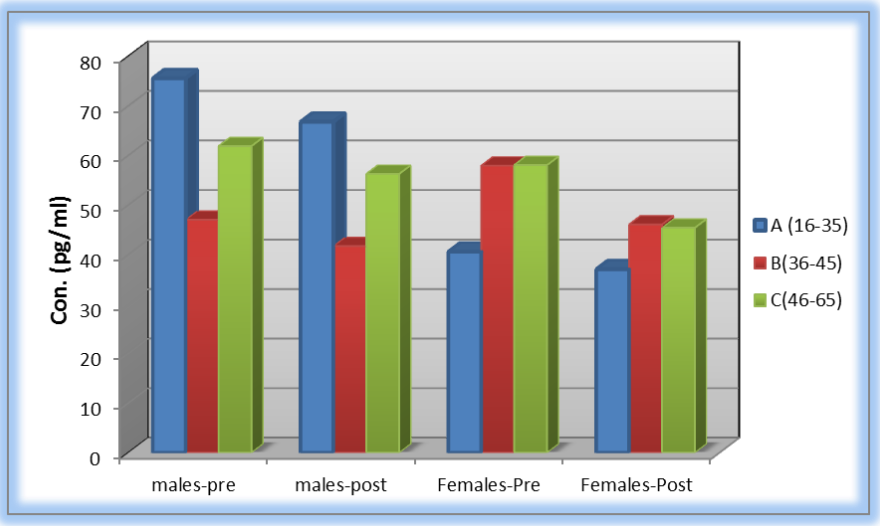


Figure (1) Serum levels of interleukin-6 (pg/ml) pre and post cholecystectomy according to age and sex.

Acute cholecytitis is an inflammation of the gallbladder that is due to biliary obstruction of the cystic duct by gall stones. [7] This obstruction causes intraluminal pressure within the gallbladder and triggers an acute inflammatory response. [2] The inflammatory response is associated with pro-inflammatory cytokine release and acute phase proteins. TNF-alpha, IL-1 and IL-6 are the largest mediators of acute phase response in humans. The first two are responsible for the activity of extrahepatic manifestations such as fever, elevation of prostaglandins, tachycardia, and accelerated catabolism. IL-6 is primarily responsible for the hepatic response, resulting in the synthesis of acute phase proteins (C-reactive protein), and activation of immunosuppressive cytokines of regulatory function.[8]

IL_6 is a pleiotropic cytokine involved in the regulation of immune responses, the acute-phase reaction, and hematopoiesis. In general, there is an increase in levels of serum IL_6 before the cholecystectomy comparing with after it. These results correspond with the study of Pei-Yuan Su *et al*., 2013 at which the levels of cytokinesis increases in Cholecystitis patients.

Cytokines such as IL_6 are small protein mediators involved in inflammatory functions. [9] The elevations of IL_6 may directly reflect the local inflammatory status of the gallbladder; some studies showed that several serum cytokine levels were increased in patients with acute cholangitis and cholecystitis. Once suspected acute cholecystitis patients are referred to hospital and the diagnosis is confirmed, and they undergo early surgery within 72 – 96 hours, they have lower complication rates, lower conversion rates, and shorter hospitals staying than patients who have delayed surgery. [10] This means that the levels of biochemical parameter start to return to its normal value after the surgery. [11]

A serum cytokine level is related to the magnitude of operative trauma and the presence of complications. Therefore, they can be used as objective biochemical markers that reflect the surgical and tissue trauma, such as IL-6, which has a serum peak between four and 48 hours after trauma, activating IL-10 and C-reactive protein, which have their serum levels increased until up to 30 days post trauma. [12]

In a study on the cytokine response in patients undergoing cholecystectomy, it was observed an increase in IL-6 and IL-10 both in laparoscopic and in open procedures, but with significantly higher levels in the open group. In our analysis there is also a significant increase in levels of IL-6 in the both procedure. [13]

Another studies found the increase of IL_6 with a distinct delay after the observed changes of endotoxin plasma level with a significantly higher plasma level in the major surgical group. [14]

The cytokines are produced during inflammatory processes, and the stimulators of the production of acute phase proteins participate with them. [15] IL_6 is the chief stimulator of the production of must acute phase proteins, the patterns of IL_6 production and of acute phase response differ in different inflammatory conditions. The systematic stress response is also mediated by inflammatory mediator originating from the surgical wound of these mediators; IL_6 has a crucial role in the induction and control of acute phase protein synthesis, particularly C-reactive protein.

Levels of Serum Interleukin_2

Results of figure (2) showed that there is a significant decrease in male group C (103.63 ± 13.89 pg/ml) before surgery (P≤0.05) in IL_2 levels comparing with group A&B. While there are no significant differences between male group pre and post cholecystectomy except group C (103.63±13.89pg/ml) (P≤0.05) which decrease to (93.51±12.2pg/ml) after cholecystectomy . In female group, before the surgery there is a significant decrease (P≤0.05) in group A (168.30 ± 35.13 pg/ml) comparing with group B&C.

Table (2): Level of interleukin-2 (pg/ml) according to sex and age.

Patients		Mean ±SD	
	groups	Pre	Post
Male	A	134.94± 34.75 aA	124.95 ±37.09 abA
	B	120.74 ±37.47 abA	111.21 ± 35.91 bcA
	C	103.63 ± 13.89 bcA	93.51 ±12.2 cB
Female	A	168.30 ± 35.13 bcA	137.13 ± 33.42cB
	B	175.52 ±28.52abA	156.37 ± 28.08 bcB
	C	180.19 ± 23.90 aA	176.69 ± 25.32 bcA

Similar letters mean the absence of significant differences. (P≤ 0.05)

Also, we noted from figure (2) that there is a significant increase in female group C post cholecystectomy (P≤0.05) (176.69∓25.32 pg/ml) comparing with other group. Results show a significant increase in Il-2 level before cholecystectomy in female groups A (168.30 ± 35.13 pg/ml) and B (175.52 ±28.52 pg/ml) when compared with its level after it. The present results show a significant increase (P≤0.05) in Il-2 levels in female groups before surgery which was A (168.30 ± 35.13), B (175.52 ±28.52), and C (180.19 ± 23.90) respectively, in comparison with its level in male group.

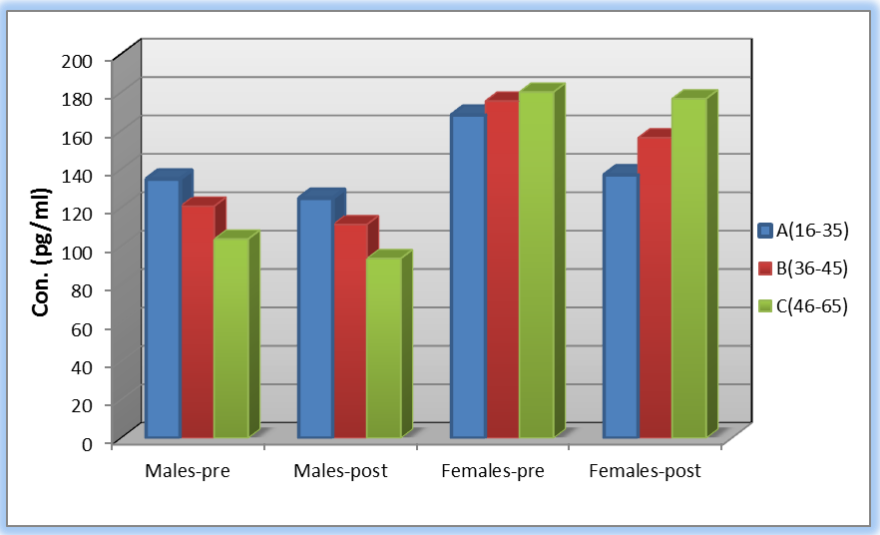


Figure (2): Serum levels of interleukin-2 (pg/ml) pre and post cholecystectomy according to age and sex.

IL_2 also called –T- cells growth factor; it is a kind of lymphoid factor with immune regulatory effect which can promote T-cell- dependent immune response. [16] In general, the results in the current study, the results showed increase in the serum level of IL_2 even after the cholecystectomy over the normal value. It is known, the cholesystitis is an inflammatory disease and one of the reasons for the affliction of this disease is bacterial agents.

Elective surgical wounds and trauma elicit have similar physiological responses. These include stress, hormone release, interaction of intermediary metabolism, and fluid balance, negative nitrogen balance and increased hepatic production of acute phase proteins. [17]

Activity of ALT, AST, ALP and GGT

Results of male groups of ALT activity before the surgery, as shown in figure (3) indicate that there are no significant differences between all groups, while there is a significant increase (P≤0.05) in all males group before the surgery comparing with the activity of enzyme after it, which was A (33.62 ∓ 6.17 U/L); B (32.59 ∓ 4.88 U/L), and C (33.115 ∓ 4.76 U/L), respectively. In female group before cholecystectomy there is a significant increase (p≤ 0.05) in the activity of ALT in group C (32.64 ∓ 2.34 U/L) comparing with groups A and B. Results also show a significant increase in female group before the surgery (p≤ 0.05) which are A (30.35 ∓ 3.9 U/L), B (31.76 ∓ 2.58 U/L) and C (32.64 ∓ 2.34 U/L) respectively comparing with the activity of enzyme after the surgery.

Table (3) Activity of ALT & AST (U/I) according to age and sex.

GR.		ALT. U/I Mean ± SD.		AST U/I Mean ± SD	
		pre	post	pre	post
Male	A	33.62±6.17aA	23.73±3.04bB	40.65±5.22aA	29.84±2.33bB
	B	32.59±4.88aA	24.63±2.22bB	38.84±4.57aA	29.43±4.21bB
	C	33.115±4.76aA	24.59±2.88bB	41.80±4.96aA	31.06±4.00bB
Female	A	30.35±3.96bA	23.03±3.08dB	35.30±6.09aA	25.21±3.74bB
	B	31.76±2.58abA	24.78±2.27cdB	37.308±7.20aA	26.12±5.05bB
	C	32.64±2.34aA	25.15±2.12cB	36.42±4.27aA	26.002±3.27bB

Similar letters mean the absence of significant differences. (P ≤ 0.05)

For AST activity, the results of figure (4) showed a significant increase (P≤ 0.05) in patients group (M&F) before surgery comparing to the activity of enzyme after it, which was in male groups A (40.65 ∓5.22 U/L), B (38.48 ∓4.57 U/L), and C (41.80 ∓4.96 U/L), respectively and in females group, was A (35.30 ∓6.09 U/L), B (37.308 ∓7.20 U/L), and C (36.42 ∓4.27 U/L), respectively.

The results of the present study showed a significant increase (p≤ 0.05) in ALP in patients group (M&F) before surgery comparing to the activity of enzyme after it, which was in male groups A (103.80 ∓38.39 U/L), B (83.50 ∓8.47 U/L), and C (82.20 ∓4.96 U/L), respectively and in female groups was A (106.07 ∓28.26 U/L), B (99.90 ∓13.39 U/L), and C (99.68 ∓11.89 U/L) respectively. Also result showed a significant increase (p≤ 0.05) in males group A (103.80 ∓38.39 U/L) comparing with groups B and C, in comparing between males and females group before surgery the result showed a significant decrease in male groups B (83.50 ∓8.47 U/L), and C (82.20 ∓4.96 U/L) comparing with the other groups of (M&F). There was no significant difference between M&F group after surgery.

Table (4) Activity of ALP & GGT (U/I) according to age and sex.

GR.		ALP. U/I Mean ± SD		GGT U/I Mean ± SD	
		Pre	post	pre	post
Male	A	103.80±38.39aA	67.40±17.41cB	39.96±4.22bA	24.80±3.03eB
	B	83.50±8.47bA	56.37±9.98cB	37.95±5.85cA	26.98±4.16deB
	C	82.20±7.35bA	51.70±8.79cB	43.16±3.77aA	28.11±2.90dB
Female	A	106.07±28.26aA	69.96±19.97bB	26.02±4.75cA	14.60±2.45eB
	B	99.90±13.39aA	65.37±9.37bB	32.17±5.59aA	17.73±3.19dB
	C	99.68±11.98aA	62.45±10.63bB	28.18±5.47bA	16.70±2.68dB

Similar letters mean the absence of significant differences. (P ≤ 0.05)

For GGT activity the result of the present study showed a significant increase (p≤ 0.05) in patients groups (M&F) before surgery comparing to the activity of enzyme after it, which was in male groups A (39.96∓4.22 U/L), B (37.95 ∓5.85 U/L), and C (43.16 ∓3.77 U/L), respectively and in female groups was A (26.02 ∓4.75 U/L), B (32.17 ∓5.59 U/L), and C (28.8 ∓5.47 U/L), respectively. There was a significant increase in male groups C (43.16 ∓3.77 U/L) before operation comparing with groups A&B, and there was a significant increase in female group B (32.17 ∓5.59 U/L) pre cholecystectomy comparing with groups A&C.

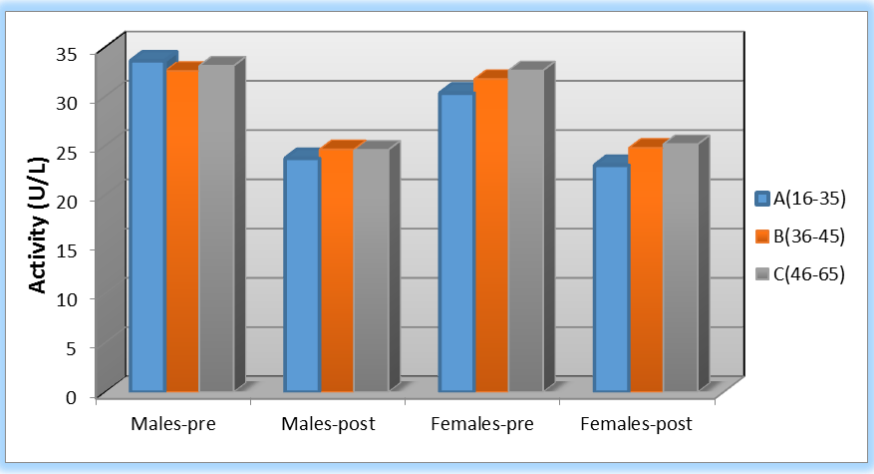


Figure (3): Activitys of S.ALT (U/L) pre and post cholecystectomy according to age and sex.

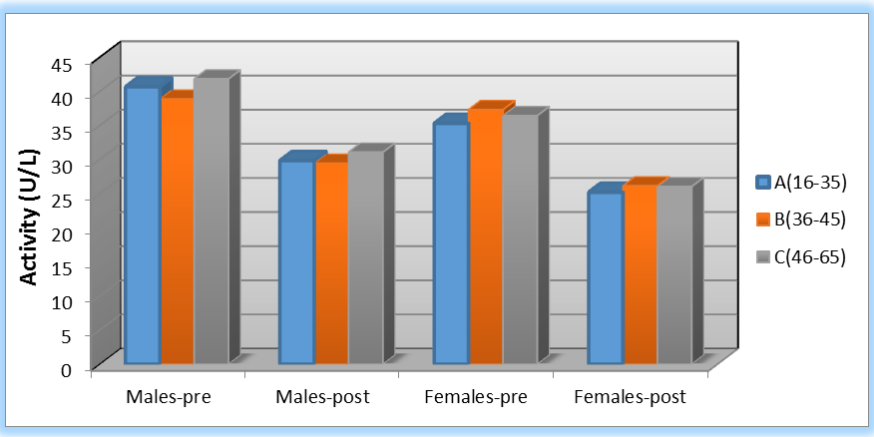


Figure (4): Activity of S.AST (U/I) pre and post cholecystectomy according to age and sex.

Before operative treatment a significant increase ($p \leq 0.05$) in ALT & AST activity was seen, this may be due to that occurrence of biliary lithiasis which may lead to the sub-clinical cholestatic liver impairment and this may help physicians to follow up those patients. [18] Occurrence of gallstones was positively correlated with the rise of serum transaminases activity, and these results are in agreement with a finding by Hafiz *et al*. [19]

Our findings correlate with the finding of the previous studies but frequently was found to be much higher as compared to Zare *et al*., AL-Kataan, Erhan and Suleyman, [20] [18] [21] found a significant increase in the activity of AST, ALT, and ALP after 24 hour of laparoscopic cholecystectomy, but the activity of these enzymes are return to its normal value after several days and this result agrees with the present study. Our findings highlight the fact that due to GS disease the liver is inflamed and damaged which simultaneously causes rise in hepatic enzymes in blood.

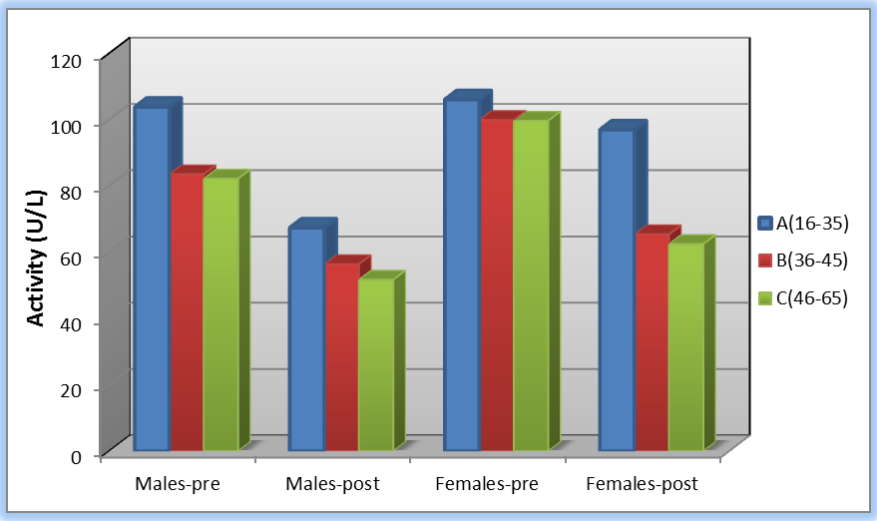


Figure (5): Activity of S.ALP (U/L) pre and post cholecystectomy according age and sex.

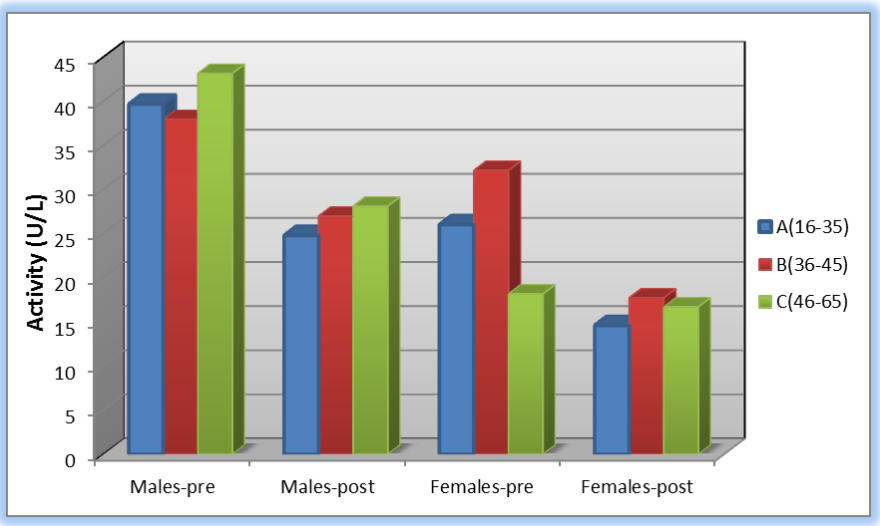


Figure (6): Activity of S.GGT. U/L. pre and post cholecystectomy according age and sex.

Pre cholecystectomy and post it activity of AST, ALT, GGT, ALP, have been investigated in various studies to determine the physiological basis of hepatic malfunction. [22] However significant elevations after LC compared with OC have been defined for only AST and ALT activity. Time controlled studies have shown that these enzyme elevations last for about 3 days postoperatively and the significant between LC and OC values fade away after 2 days. [23]

This discrepancy of liver transaminases from the past to the present time may be related to environmental, cultural, or social factors. [24] They found in a study on GS patients after laparoscopic chol. A significant increase in AST and ALT activity, but they returns to its normal activity within 72 hours, and this result agree with that of our present study. Despite the increase of AST and ALT activity, we didn't note any clinical problems obviously related to this change. Specifically, there was no increase in plus rate of temperature or any clinical signs to indicate a common duct injury. Furthermore, enzymes returned to its normal values within several days therefore, we can conclude that these biochemical data are not clinically significant.

The mechanism for these changes in liver enzymes is unclear and we postulate several possible explanations:

1. Increased intra-abdominal pressure should be proved by further studies in patients in whom pneumoperitoneum is created for some purposes other than LC.
2. The "squeeze" pressure effect on the liver by the traction of the gallbladder may free these enzymes into the blood stream.
3. Prolonged use of diathermy to the liver surface and spread of heat to the liver parenchyma. Some surgeons use diathermy to excise the gallbladder while performing open cholecystectomy. We, however ,could find no reference in the literature to postoperative enzyme level compared with those after cholecystectomy performed without the use of diathermy.
4. Pulling on the gallbladder creates a transient kink in the extra hepatic ducts, which could induce an increase in the endoluminal pressure and a subsequent increase in enzyme level.
5. Another issue is the passage of a small stone. This, indeed, would be hard to prove under any circumstances but from other studies we know that the rate of undiscovered stones ranges from 1.2% to12.4%.[5]
6. Inadvertent clipping of the right branch of the hepatic artery, or any other aberrant arterial branch is supplying blood to the liver. This, however, should be followed by a massive increase in liver enzymes and usually has clinical implications.

After 15 -30 days of cholecystectomy, all these effects will diminish due to the half of the present enzymes finish. [18] So, this can give account why ALT activity declined to near normal activity.

Gamma glytamayl transpeptidase (GGT) is a liver enzyme that has traditionally been measured to detective liver health and function the normal biologic role of GGT is to reconstitute glutathione, the body master antioxidant. [25]There is a significant increase in GGT activity in GS patients before surgery comparing with the activity of it after surgery. It knows that liver disorder can be accured with GS disease; GS disease is known to cause liver disease and derangement of its enzymes.

The results of the present study agree Tawfic which found similar results. [26] Certainly elevation of serum GGT belong on the list of biomarker linked to the metabolic syndrome. The results of our study also, agree with the findings, which have showed that chronic liver disease is risk factor for gallstone disease.

Figure (5) showed a significant increase in ALP activity in GS patients' before surgery, and it was the highest in female groups comparing with male

groups. This increase of the ALP activity in females is In agreement with the finding by. [27] This increase of serum ALP in female groups might be owing to the increase bone turn over or simultaneous formation of steroid in these females than males.

The finding of this study is in agreement with the findings by AL-Ktaan, Tawfic, which they found similar results. [18] [26] They found that among the components of the LFTs, alkaline phosphatase was a better indicator for choledocholithiasis than bilirubin. [26] While they found in their study that the differences of ALP activity before and after surgery in GS patients cannot be causally related to the process of GS formation, instead they could reflect the presence of some biliary obstruction known to occur with gallbladder disease. [28] This increase of ALP activity begins to reduction by the time after surgery and return to its normal values after disappearance of obstruction.

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دراسة مستوى المركبات الخلوية 2, 6 وبعض انزيمات الكبد لدى مرضى حصى المرارة قبل وبعد عملية الاستئصال

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الملخص

صممت هذه الدراسة لإيجاد التغيرات في تركيز نوعين من المركبات الخلوية وهما 2, 6 وبعض انزيمات الكبد لمرضى حصى المرارة والذين خضعوا لعملية استئصالها (بنوعيها المنظارية باستخدام المنظار والمفتوحة عن طريق احداث شق في البطن). تضم هذه الانزيمات كل من انزيمات وظائف الكبد (الانزيمات الناقلة للأمين GGT,AST,ALT, انزيم الفوسفات القاعدي ALP). شملت الدراسة 120 مريضاً (92 اناث, و28 ذكور) تتراوح اعمارهم ما بين 16–65 سنة.

اظهرت النتائج ان هناك اختلافات معنوية في تركيز الانترلوكينات (2,6) قبل وبعد اجراء العملية. كذلك اظهرت النتائج ان هناك اختلافات معنوية ($P \leq 0.05$) في فعالية انزيمات الكبد قبل وبعد اجراء عملية استئصال المرارة لكل المجاميع المرضية من كلا الجنسين.

الكلمات الدالة: المركبات الخلوية 2, 6, انزيمات الكبد, حصى المرارة, عملية استئصال المرارة.