

## **Serum levels of several types of cytokines associated with Chronic Cholecystitis in Basrah /south of Iraq.**

Awatif H. Issa & Muthena M. Muthena

College of Science - University of Basrah

[awatifhissa@yahoo.com](mailto:awatifhissa@yahoo.com)

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### **ABSTRACT**

Acute cholecystitis is an acute inflammation of the gallbladder. A Total of 52 Cholecystectomy patients from a Al- Sadar Teaching hospital and 52 healthy volunteers were enrolled . Blood specimen collected by sterile container from both . The serum levels of pro-inflammatory cytokines (IL-1, TNF- $\alpha$  & IL-6) were significantly increased in gallstone patients sera comparing to healthy controls at ( $p<0.05$ ) .While anti-inflammatory cytokine IL-4 concentrations in gallstone patients sera showed significant reduced comparing to healthy controls at ( $p<0.05$ ). And regulator cytokine IL-17 concentrations in gallstone patients sera showed not significant reduced comparing to healthy controls at ( $p<0.05$ ), . Finally IgG concentrations in gallstone patients sera showed significant elevation comparing to healthy controls at ( $p<0.05$ ).The elevation of pro-inflammatory cytokines including (IL-1 , IL-6 & TNF- $\alpha$ ) indicated the presence of inflammatory response associated with gallstone. The reduction of anti-inflammatory cytokine (IL-4) and regulator cytokine (IL-17) indicated the presence of immune predisposition in patients for gallstone.

**Key words:** Cholecystitis, Gallstone, Cholecystectomy, Cytokines

## Introduction

Anatomically, the biliary tree is often divided into intrahepatic and extra hepatic portions that include the gallbladder epithelium. However, with respect to biliary tissue and disease, this distinction appears artificial when describing the interactions of the biliary epithelium with the immune system; it appears that biliary epithelial cells from either location have similar responses to immunogenic stimuli (Auth *et al.*, 1993). Biliary epithelial cells participate in both innate and adaptive immunity.(Harada *et al.*, 2003).

The innate immune system induces no immunologic memory to an antigen but responds instead to a variety of evolutionarily conserved patterns present in foreign antigens. In addition to

specific cellular subsets that include neutrophils , macrophages, eosinophils, basophils, mast cells, and natural killer cells, a variety of proteins also participate in innate immunity, including the complement cascade, cytokines, and proteins of the acute phase response (Akira *et al.*, 2006).

The second aspect of adaptive immunity involves the B-cell response, mediated through immunoglobulins (Igs). All Ig classes, including IgA, IgG, and IgM, are present in low concentrations in bile (Maurer *et al.*, 2009).

The type of immune response in gallbladder infection is local inflammatory response :

-Tissue macrophages recognise microbial products.

-The macrophages release cytokines and other inflammatory mediators (IL-1, TNF- $\alpha$ , IL-6) that cause vasodilation and increased vascular permeability and have chemotactic effects on monocytes and neutrophils.

-Monocytes and neutrophils are recruited to the site and there is accumulation of plasma fluid and proteins at the site, causing oedema.

-Inflammatory mediators can activate mast cells to release further mediators that amplify the response.

-If the local production of cytokines is high enough, the cytokines travel in the blood and affect other organs.

-IL-1 affects the brain, causing fever, anorexia and somnolence.

-IL-6 stimulates hepatocytes to produce acute phase proteins (Wood, 2006).

Interestingly, the source of the Igs appears to influence the ability of these proteins to nucleate solid cholesterol crystals from bile. IgM and IgA from commercial sources are incapable of nucleating supersaturated bile, whereas commercially available IgG has pronucleating activity (Harvey *et al.*, 1991 ;Upadhyia *et al.*, 1993).

The anatomic source of the Igs also appear to influence the ability of these molecules to cause cholesterol crystal phase separation. Specifically, biliary Igs seem to promote solid cholesterol nucleation more than Igs isolated from blood (Upadhyia *et al.*, 1993). Of the biliary Igs, IgM most potently nucleates supersaturated bile, whereas IgG has slightly less facility and IgA has very little activity (Harvey *et al.*, 1991 ;Upadhyia *et al.*, 1993).

Moreover, results of human studies indicated that Igs did not

contribute to formation of gallstones; in *ex vivo* bile samples from individuals with or without gallstones, there was no correlation between biliary Igs and cholesterol crystal detection times (Miquel *et al.* 1998). One limitation of these studies is that the investigators focused primarily on the effect of Ig concentrations on solid cholesterol monohydrate crystal nucleation. Although Ig concentrations could be important, it is likely that other factors are relevant to this physical-chemical interaction. For example, the antigen specificity of the Igs may be crucial. Fab fragments isolated from patients with gallstones accelerated cholesterol crystal observation times *in vitro*, whereas commercially available Fab fragments did not. These data indicate that antigen-specific Fab fragments might contribute to cholesterol nucleation *in vitro*. Therefore, merely quantifying Ig concentrations in

bile appears to provide limited functional information (Lipsett *et al.* 1994).

Recent studies have shown that mice that have only T cells (that lack B cells and Ig) develop gallstones at a greater frequency than wild-type mice. In contrast, mice possessing only B cells do not recapitulate the prevalence of gallstones observed in wild-type mice (Maurer *et al.*, 2007).

The term cytokine covers a large number of smallish proteins (usually less than 20 kDa) that serve a hormone-like function in enabling cells to communicate with each other. Most people are familiar with hormones such as insulin and growth hormone, which are produced in one organ or tissue and travel through the bloodstream to other organs where they bind to receptors on the cells of that organ and stimulate a particular response. Hormones

that are produced in one organ and act on a distant tissue are said to be acting in an endocrine manner, cytokines do not usually act in an endocrine manner; rather, they act locally. They are produced by cells in a particular tissue and act on cells in that tissue (Wood , 2006).

IL-1 and TNF- $\alpha$  have a role in secretion and absorption of the gallbladder epithelial cells (Rege, 2000). They also regulate the negative feedback loop for bile acid synthesis (Feingold et al., 1996). IL-1 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) play important roles in responses to bacterial infection (Bentley & Cerami , 1989).

The biliary fluid of patients with cholangitis has increased concentrations of the inflammatory cytokines TNF- $\alpha$  and IL-6 suggesting that bacterial infection leads to an increase in cytokines released into the bile (Rosen *et al.*,

1995). For example, the proinflammatory cytokines, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), and interleukin-6 (IL-6) are frequently detected in the sera of patients with septic shock. These cytokines appear to play a critical role in the development of septic shock ( Detrick *et al.*, 2008 ).

IL-6 regulates the inflammatory system by upregulating the hepatic synthesis of acute-phase reactants such as CRP (Ridker *et al.*, 2000). Several specific cytokines have been implicated in cholesterol and lipoprotein metabolism. One of these cytokines is interleukin-4 (IL-4), which appears to have an expression that is restricted to CD4<sup>+</sup> T lymphocytes, mast cells, and subtypes of natural killer cells (Paul & Ohara , 1987). A study on mice showed the effect of IL-4 in gallstone formation and cholesterol

and lipoprotein metabolism (Kinget *et al.*, 2002).

IL-17 is a cytokine that induces mobilization and activation of neutrophils and triggers the production of proinflammatory cytokines and chemokines by a broad range of cellular targets (Korn *et al.*, 2009). Recent studies indicate a novel IL-17-dependent protective mechanism of  $\gamma\delta$  T cells that acts against intracellular bacterial infections in the mouse (Bonneville *et al.*, 2010). Human Th17 cells are characterized by the production of IL-17 and differentiate from naive T cells (Th0). Th17 cells are part of the mucosal host defense system and have a major role in protection against bacterial infections. IL-17-positive cells identified as Th17 cells are mainly present at the interface of inflamed portal tracts and accumulated around damaged interlobular bile ducts in cases of

the chronic inflammation of bile ducts (Harada *et al.*, 2009).

In cultured cholangiocytes, many mucin genes are regulated by inflammatory mediators. Study in cell culture system have shown that mucin gel accumulation and mucin gene expression increased following exogenous addition of LPS and TNF- $\alpha$  (Zen *et al.*, 2002). In another study, LPS and other pathogen-associated molecular patterns were found to interact with TLR2 or TLR4 on the surface of biliary epithelium. This interaction induced expression of *Cdx2*, a gene whose product is associated with intestinal metaplasia of epithelial cells; CDX2 in turn induced expression of *Muc2*. Furthermore, MUC2 protein expression is increased in the gallbladders of humans with gallstones. (Ikeda *et al.*, 2007 ; Sakamoto *et al.*, 2007 )

## **Materials and Methods**

The specimens were collected from (104) persons including (52) patients whose ages ranged from (11-66) years submitted to the cholecystectomy operation in Al-sader Teaching Hospital in Basrah province through the period from Nov. 2011 to Feb. 2012 and 52 matching healthy volunteers whose ages ranged from (16-52) years, who served as controls for blood specimens. After giving informed consent all patients and controls answered as fill-up questionnaire . The data then were statistically analyzed.

Five ml of blood were withdrawn from each patients and control placed in plain tube , let in room temperature to be coagulated , then it was centrifuged at 3000 rpm . the serum was collected in sterile eppendorff tubes and stored at -20 °C until it use (Collee *et al.*, 1996).

The concentrations of cytokines (IL-1 $\alpha$  , IL-4 , IL-6 , IL-17 &

TNF- $\alpha$ ) were evaluated in serum of patients and control groups by ELISA technique according to the instruction provided by manufacturer.

The concentrations of Immunoglobulin IgG were evaluated in serum of patients and control groups by radial immunodiffusion plate technique according to the instruction provided by manufacturer.

The results were evaluated by the analysis of the variance (ANOVA) ,p-values at levels ( $p \leq 0.05$ ) was considered to be statistically significant. This calculation was carried out according to Statistical Package for Social Science (SPSS version 19) and the least significant difference (LSD) at level less than 0.05 by using Gene State 2009.

## Results and Discussion

### Evaluation of Cytokines :

The results of cytokines (IL-1 $\alpha$ , TNF- $\alpha$ , IL-6, IL-4&IL-17) were measured by using Enzyme-Linked ImmunoSorbent Assay (ELISA) specific kits for evaluation of their concentrations in 52 serum specimens from gallstone patients under study.

### **Evaluation of IL-1 $\alpha$ Cytokine :**

IL-1 $\alpha$  concentrations calculate-ed according to the manufacturer's standard curve Figure (1). The results of IL-1 $\alpha$  concentrations in gallstone patients sera showed significant elevation comparing to healthy controls. The range of IL-1 $\alpha$  concentrations in gallstone patients sera was (1.518-26.024) pg/ml while in healthy controls was(3.564-12.732) pg/ml, the higher concentration mean according to the age was (7.8326  $\pm$  4.25293) pg/ml at the patients of (21-40) age group comparing to the healthy controls was (5.8328  $\pm$  1.33944) pg/ml at the same age

group. And the higher concentration mean according to the gender was (8.7149 $\pm$  3.33710) pg/ml at the males patients comparing to the male healthy controls was (5.7057 $\pm$  2.69712) pg/ml. Table (1).

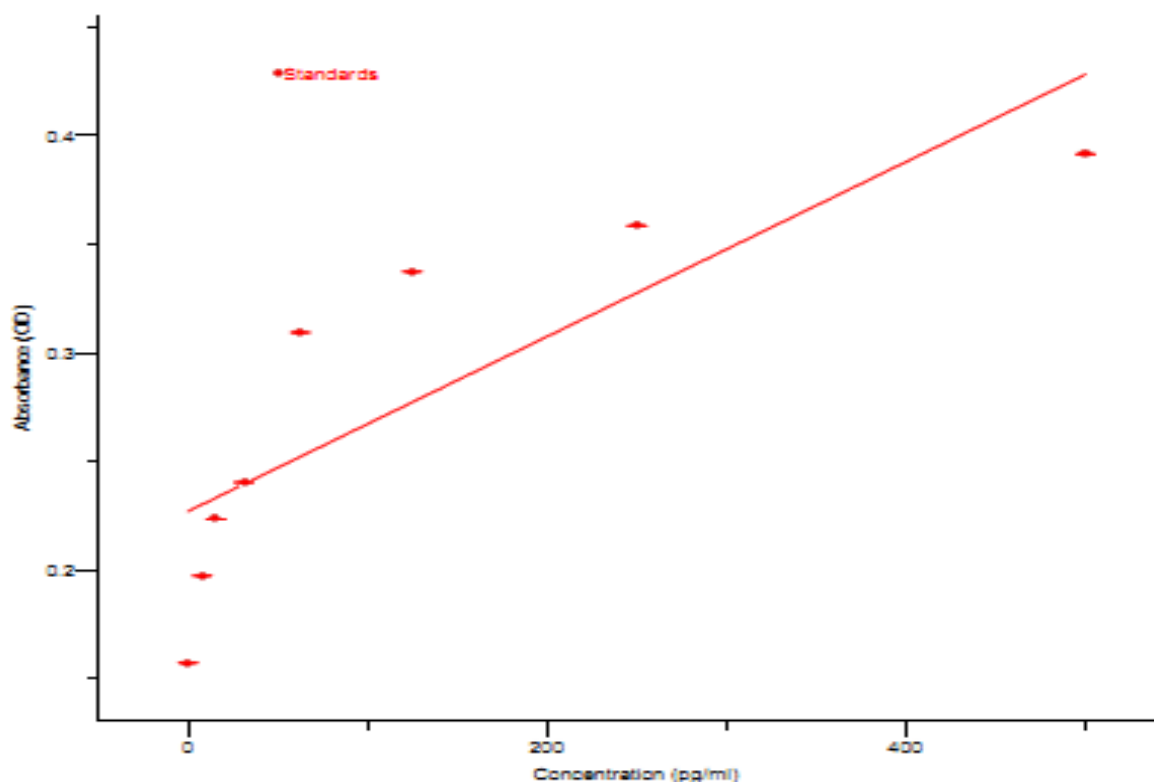
The pro-inflammatory role of IL-1 in inflammation was that it can trigger fever by enhancing prostaglandin E2 (PGE2) synthesis by the vascular endothelium of the hypothalamus and can stimulate T cell proliferation.

In addition, IL-1elicits the release of histamine from mast cells at the site of inflammation. Histamine then triggers early vasodilation and increase of vascular permeability. The pro-inflammat-ory effects of IL-1can be inhibited by IL-1 receptor antagonist (IL-1Ra), originally referred to as IL-1 inhibitor. IL-1Ra is produced by immune complex or IL-4-stimulated macrophages (Feghali



&Wright , 1997).Rege (2000) reported that increasing of IL-1 $\alpha$  in gallbladder inflammation in cultured human gallbladder epithelial cells and effect in absorptive function of the gallbladder wall and that may play a role in

gallstone pathogenesis.Prystvosky&Rege (1997) indicated that adding LPS to the bile of guinea pig gallbladders in vivo led to increased IL-1concentration in the bile.



Abs. = 0.2276 + 0.0004025 Conc.

Figure (1): The standard curve shown the IL-1 $\alpha$  standards concentrations (0-500) pg/ml and their O.D that used in determination of IL-1 $\alpha$  levels in patients with gallstone disease and healthy controls.

Table (1): Statistical analysis of IL-1 $\alpha$  concentrations (pg/ml) at gallstone patients and healthy controls sera.

Gender and age groups	Patients and control groups	Mean	Std. Deviation	Std. Error Mean
(1-20) age group	Control n=6	7.2550	3.49313	1.42606
	Patients n=8	8.5454	3.95711	1.39905
(21-40) age group	Control n=10	5.8328	1.33944	0.42357
	Patients n=21	7.8326	4.25293	0.92807
(41-60) age group	Control n=10	7.7445	2.88570	0.91254
	Patients n=22	8.1704	5.61633	1.19751
(61above) age group	Control n=1	3.5640	-	-
	Patients n=1	4.1170	-	-
The males	Control n=8	5.7057	2.69712	0.95358
	Patients n=11	8.7149	3.33710	1.00617
The females	Control n=19	7.2222	2.56261	0.58790
	Patients n=41	7.8256	5.08483	0.79412
Total	Control n=27	6.7729	2.64627	0.50928
	Patients n=52	8.0137	4.75362	0.65921

### Evaluation of IL-6 Cytokine :

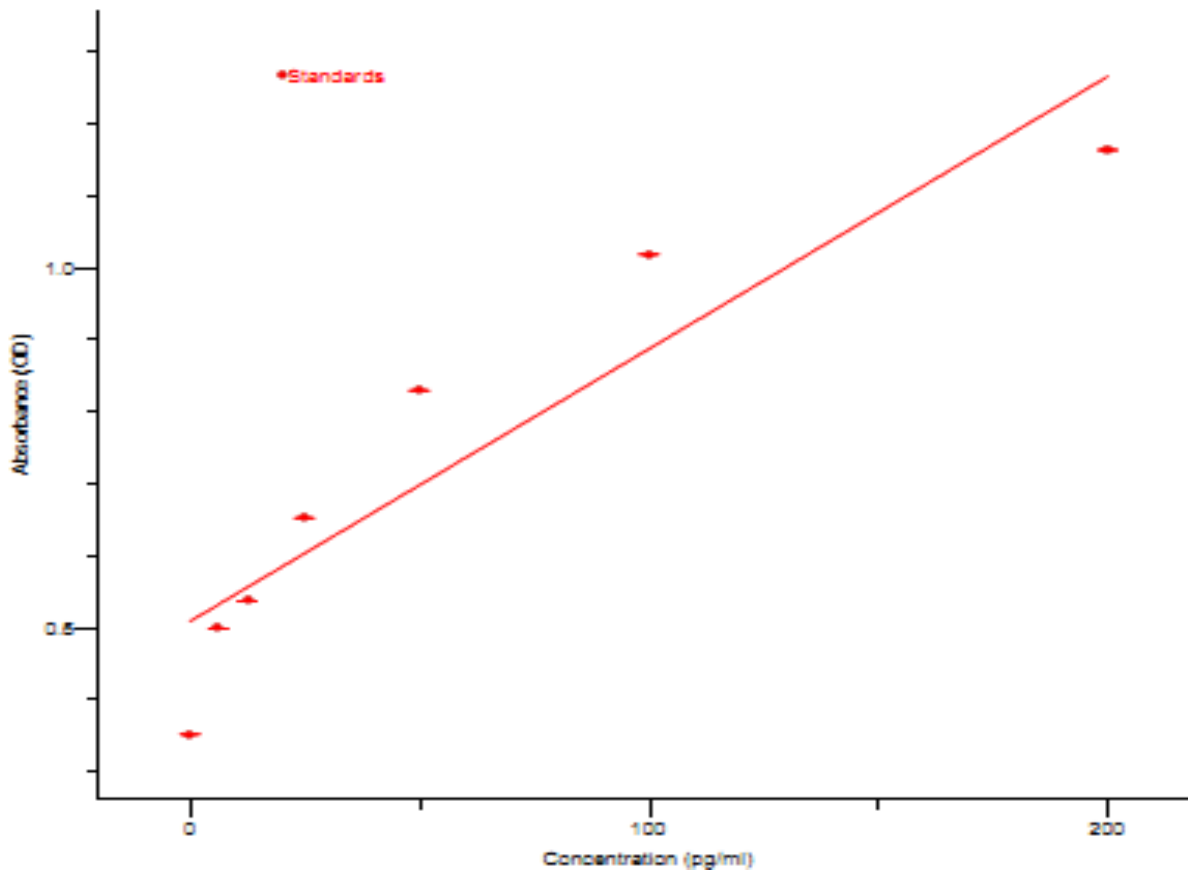
IL-6 concentrations calculated according to the manufacturer's standard curve Figure (2). The results of IL-6 concentrations in gallstone patients sera showed significant elevation comparing to healthy controls. The range of IL-6 concentrations in gallstone patients sera was (3.933-18.081) pg/ml while in healthy controls was (3.392-8.892) pg/ml, the higher concentration mean according to the age was (10.3258  $\pm$  5.42999) pg/ml at the patients of (1-20) age group comparing to the healthy controls was (6.4938  $\pm$  1.36320) pg/ml at the same age group. The higher concentration mean according to the gender was (9.5522  $\pm$  3.63795) pg/ml at the males patients comparing to the male healthy controls was (5.5852  $\pm$  1.79364) pg/ml Table (2).

IL-6 is pro-inflammatory in chronic inflammatory diseases (Yamamoto *et al.*, 2000). It is

important to the transition between acute and chronic inflammation (Kaplanski *et al.*, 2003). IL-6 operates to control the extent of tissue inflammatory responses, in chronic diseases IL-6 not only serves as an inducer of acute phase reactions but also is an important player in eliciting cellular immune responses to affected cells and mucosal humoral responses directed against reinfection (Ramsay *et al.*, 1997). IL-6 levels are elevated in inflammatory diseases in humans, expression of IL-6 is enhanced at the site of inflammation, IL-6 is produced and plays a key role in the acute phase response as defined by a variety of clinical and biological features such as the production of acute phase proteins (Gabay , 2006) . In addition to the IL-6 stimulation of acute phase protein synthesis by the liver, it acts as a growth factor for mature B cells and induces their final maturation

into antibody-producing plasma cells and it is involved in T cell activation and differentiation (Feghali&Wright , 1997).Rosen *et al.* (1997) reported that the biliary fluid of patients with cholangitis

has increased concentrations of the inflammatory cytokines like IL-6 suggesting that bacterial infection leads to an increase in cytokines released into the bile.



$$\text{Abs.} = 0.5102 + 0.003786 \text{ Conc.}$$

Figure (2): The standard curve shown the IL-6 standards concentrations (0-200) pg/ml and their O.D that used in determination of IL-6 levels in patients with gallstone disease and healthy controls.

Table (2): Statistical analysis of IL-6 concentrations (pg/ml) at gallstone patients and healthy controls sera.

Gender and age groups	Patients and control groups	Mean	Std. Deviation	Std. Error Mean
(1-20) age group	Control n=6	6.4938	1.36320	0.55652
	Patients n=8	10.3258	5.42999	1.91979
(21-40) age group	Control n=10	5.2446	1.43126	0.43154
	Patients n=21	8.1694	2.85441	0.62288
(41-60) age group	Control n=10	5.5353	1.55715	0.46950
	Patients n=22	7.9931	2.75991	0.58841
(61above) age group	Control n=1	3.4930	-	-
	Patients n=1	4.9190	-	-
The males	Control n=8	5.5852	1.79364	0.63415
	Patients n=11	9.5522	3.63795	1.09688
The females	Control n=19	5.5406	1.45080	0.31659
	Patients n=41	8.0453	3.25477	0.50831
Total	Control n=27	5.5529	1.51925	0.28212
	Patients n=52	8.3641	3.36004	0.46595

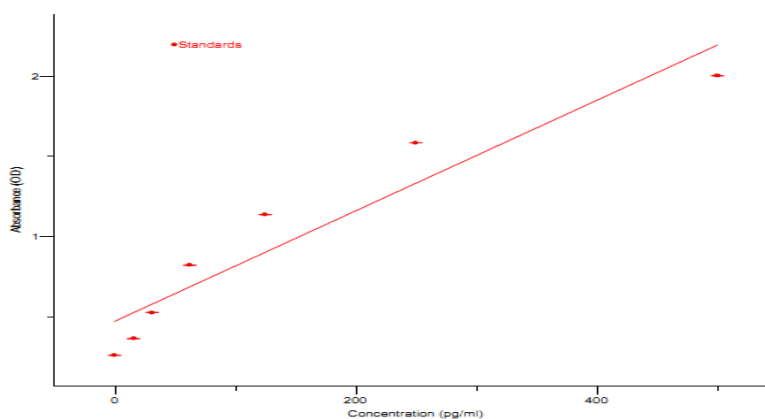
### Evaluation of TNF- $\alpha$ Cytokine :

TNF- $\alpha$  concentrations were calculated according to the manufacturer's standard curve Figure (3). The results of TNF- $\alpha$  concentrations in gallstone patients sera showed high significant elevation comparing to healthy controls. The range of TNF- $\alpha$  concentrations in gallstone patients sera was (8.67-45.454) pg/ml while in healthy controls was (8.84-15.43) pg/ml. The higher concentration mean according to the age was (16.0898  $\pm$  7.35902) pg/ml at the patients of (41-60) age group comparing to the healthy controls was (10.9106  $\pm$  2.34818) pg/ml at the same age group. And the higher concentration mean according to the gender was (17.5498  $\pm$  9.98655) pg/ml at the males patients comparing to the male healthy controls was (10.1986  $\pm$  2.05893) pg/ml Table (3).

Many of the pro-inflammatory effects of TNF- $\alpha$  can be explained on the basis of TNF- $\alpha$ 's effects on vascular endothelium and endothelial leukocyte interactions, in response to TNF- $\alpha$  endothelial cells promote inflammation (Bradley , 2008). Chronic inflammation mediated by cytokines such as IL-1 and TNF- $\alpha$ , may not just be a consequence of gallstones but a precursor (Rege , 2000). One of the major biological roles of TNF- $\alpha$  is in the host defense to bacterial, viral and parasitic infections, physiologically TNF- $\alpha$  is important for the normal response to infection, but inappropriate or excessive production can be harmful (Bradley , 2008). The influence of lipopolysaccharide (LPS) a bacterial component on apomucin expression in cultured murine biliary epithelial cells was

examined with emphasis on the participation of tumor necrosis factor TNF-  $\alpha$ , it was found that LPS up-regulated the expression of MUC2 and MUC5AC in cultured murine biliary epithelial cells, LPS also induced the expression of TNF- $\alpha$  in biliary epithelial cells and its secretion into the culture medium (Zen *et al.*, 2002). The up-regulation of these apomucins was inhibited by pretreatment with TNF- $\alpha$  antibody, TNF-  $\alpha$  alone also induced the overexpression of MUC2 and MUC5AC in cultured biliary epithelial cells. The accumulation of TNF-  $\alpha$  in the gallbladder bile of patients with

cholangitis may be due in part to the apical release of TNF-  $\alpha$  by biliary epithelial cells (Savard *et al.*, 2002).Rege (2000) reported that increasing of the inflammatory cytokines IL-1 $\alpha$  and TNF-  $\alpha$  directly affected gallbladder epithelial cell absorptive function, and that may play a role in gallstone pathogenesis.Rosen *et al.* (1997) reported that the biliary fluid of patients with cholangitis has increased concentrations of the inflammatory cytokine TNF- $\alpha$  suggesting that bacterial infection leads to an increase in cytokines released into the bile.



$$\text{Abs.} = 0.4774 + 0.003438 \text{ Conc.}$$

Figure (3): The standard curve shown the TNF- $\alpha$  standards concentrations (0-500) pg/ml and their O.D that used in determination of TNF- $\alpha$  levels in patients with gallstone disease and healthy controls.

Table (3): Statistical analysis of TNF- $\alpha$  concentrations (pg/ml) at gallstone patients and healthy controls sera.

Gender and age groups	Patients and control groups	Mean	Std. Deviation	Std. Error Mean
(1-20) age group	Control n=6	9.3695	0.40962	0.16722
	Patients n=8	14.2404	9.34787	3.30497
(21-40) age group	Control n=10	10.1994	1.68443	0.50787
	Patients n=21	15.9348	10.43835	2.27783
(41-60) age group	Control n=10	10.9106	2.34818	0.70800
	Patients n=22	16.0898	7.35902	1.56895
(61above) age group	Control n=1	9.6140	-	-
	Patients n=1	11.0850	-	-
The males	Control n=8	10.1986	2.05893	0.72794
	Patients n=11	17.5498	9.98655	3.01106
The females	Control n=19	10.3072	1.79545	0.39180
	Patients n=41	15.1358	8.54833	1.33502
Total	Control n=27	10.2773	1.83435	0.34063
	Patients n=52	15.6464	8.82378	1.22364

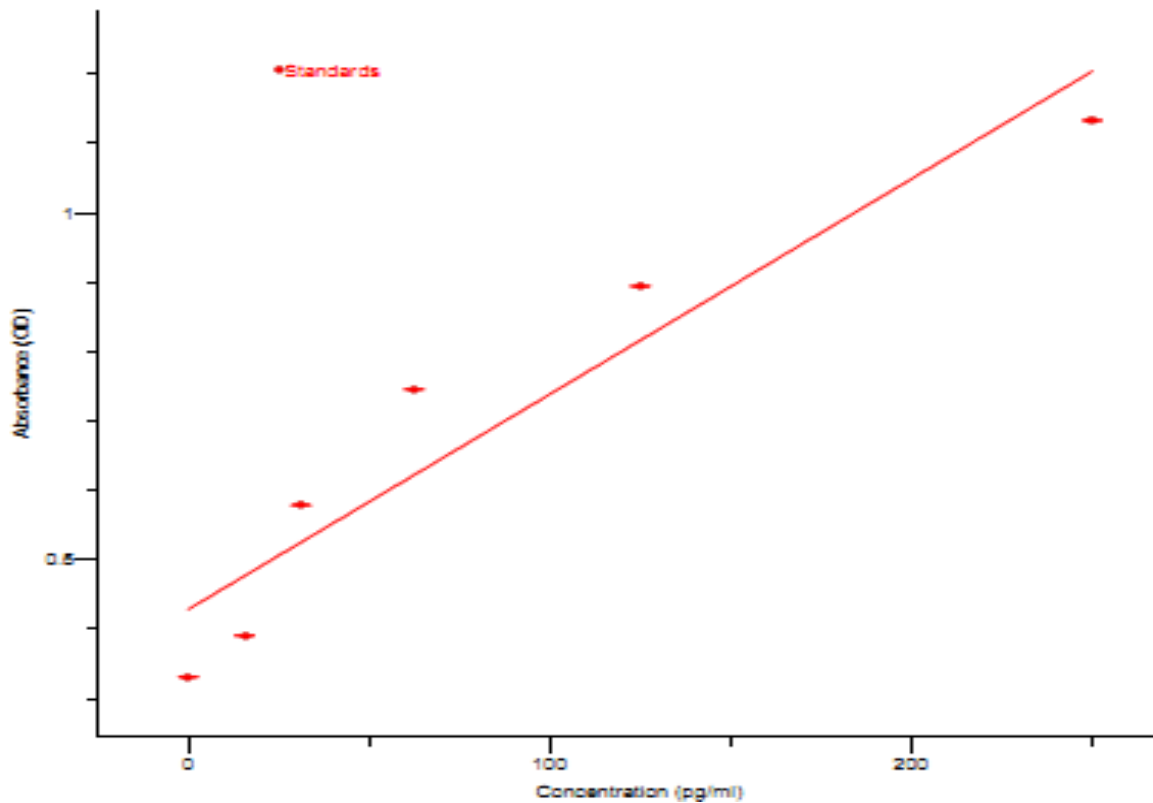
### Evaluation of IL-4 Cytokine:



IL-4 concentrations were calculated according to the manufacturer's standard curve Figure (4). The results of IL-4 concentrations in gallstone patients sera showed significant reduction comparing to healthy controls. The range of IL-4 concentrations in gallstone patients sera was (7.082-14.958) pg/ml while in healthy controls was (7.222-15.08) pg/ml, the lower concentration mean according to the age was (7.1660) pg/ml at the patients of (61 above) age group comparing to the healthy controls was (10.75) pg/ml at the same age group. The lower concentration mean according to the gender was  $(7.7992 \pm 0.88688)$  pg/ml at the males patients comparing to the male healthy controls was  $(9.4191 \pm 2.32385)$  pg/ml Table (4).

A potential mechanism underlying the effect of IL-4

deficiency may be the decreased ability to generate endogenous repressors of enzymes involved in bile acid synthesis, this suppressed ability to generate bile acid would lead to cholesterol super saturation and gallstone formation (King *et al.*, 2002). IL-4 appears to exhibit some anti-inflammatory properties by inhibiting the production of several pro-inflammatory cytokines such as IL-1, IL-6 and TNF- $\alpha$  (Miossec , 1993). IL-4 has an inhibitory effect on human PMNs-derived TNF- $\alpha$  release after lipopolysaccharides (LPS) stimulation, a coordination of pro-inflammatory mediators and anti-inflammatory mediators is essential to regulate and to recover from inflammation (Lee *et al.*,2002). King *et al.* (2002) reported by using murine experiment that IL-4 deficiency predisposes gallstone formation.



Abs. = 0.4299 + 0.003104 Conc.

Figure (4): The standard curve shown the IL-4 standards concentrations (0-300) pg/ml and their O.D that used in determination of IL-4 levels in patients with gallstone disease and healthy controls.

Table (4): Statistical analysis of IL-4 concentrations (pg/ml) at gallstone patients and healthy controls sera.

Gender and age groups	Patients and control groups	Mean	Std. Deviation	Std. Error Mean
(1-20) age group	Control n=6	9.1210	2.74832	1.03877
	Patients n=8	8.3464	1.11594	0.39455
(21-40) age group	Control n=10	9.2120	2.26445	0.68276
	Patients n=21	8.0843	1.72032	0.37540
(41-60) age group	Control n=10	9.3774	2.40153	0.69326
	Patients n=22	7.8787	1.67156	0.35638
(61above) age group	Control n=1	10.7500	-	-
	Patients n=1	7.1660	-	-
The males	Control n=8	9.4191	2.32385	0.77462
	Patients n=11	7.7992	0.88688	0.25602
The females	Control n=19	9.2585	2.38202	0.50785
	Patients n=41	8.0681	1.73075	0.27030
Total	Control n=27	9.3051	2.32752	0.41804
	Patients n=52	8.0200	1.58851	0.22029

### Evaluation of IL-17 Cytokine :

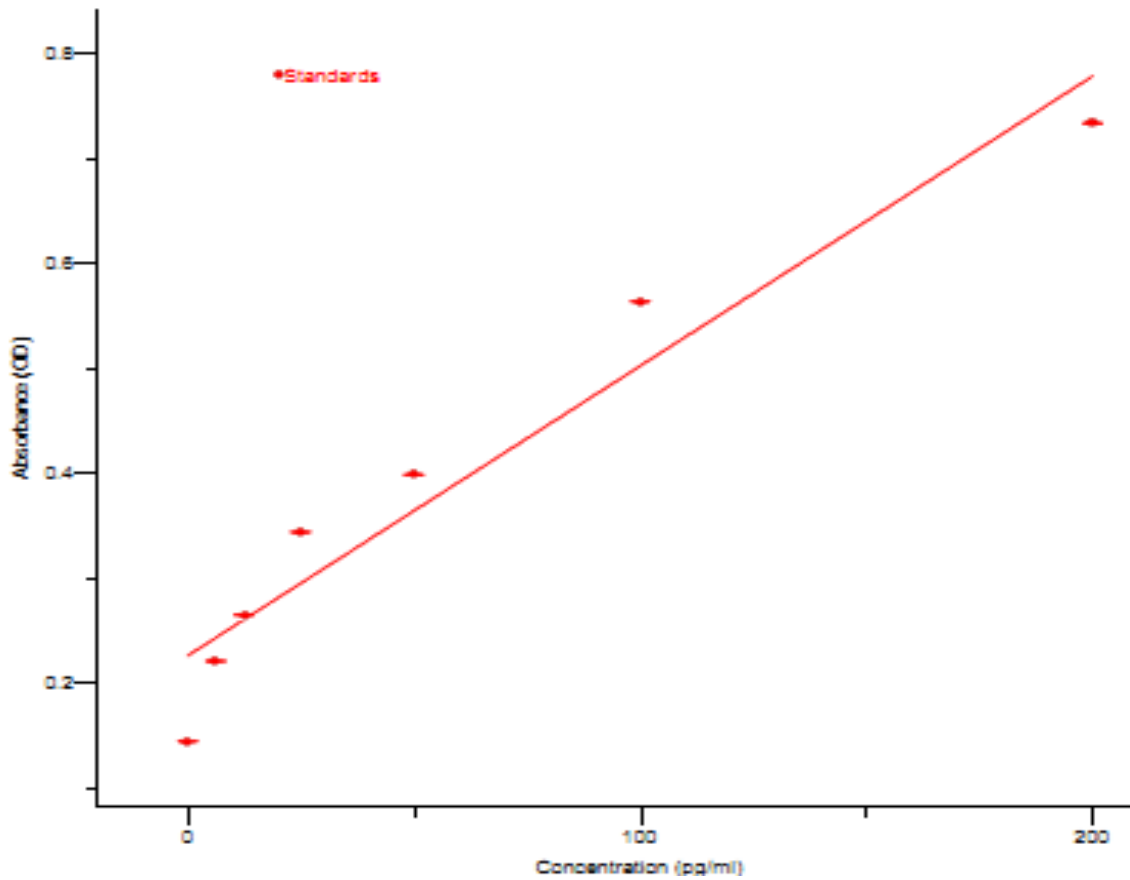
IL-17 concentrations were calculated according to the manufacturer's standard curve Figure (5).The results of IL-17

concentrations in gallstone patients sera showed nosignificantreduction compared to healthy controls. The range of IL-17 concentrations

in gallstone patients sera was (4.765 -10.832) pg/ml while in healthy controls was (5.368 - 10.225) pg/ml, the lower concentration mean according to the age was (5.372) pg/ml at the patients of (61 above) age group comparing to the healthy controls was (7.017) pg/ml at the same age group. And the lower concentration mean according to the gender was (5.9922 ± 1.24763) pg/ml at the females patients comparing to the female healthy controls was (6.8873 ± 1.21402) pg/ml Table (5).

Evidence has been accumulating to suggest that one of the molecules that may serve as a mediator of the T cell response to pathogens is IL-17, it may serve as a modulator of early immune responses to pathogens, and as such may be an important element of host defense (Dumont , 2003). Th17 cells characterized by the

secretion of IL-17 are implicated in the chronic inflammation of bile ducts and the presence of Th17 cells around bile ducts is causally associated with the biliary innate immune responses to (PAMPs) pathogen-associated molecular patterns (Harada &Nakanuma, 2010). lipopolysaccharide has been found to induce the release of IL-17 from T cells, but it required the presence of macrophages (Miyamoto *et al.*, 2003). Increased expression of IL-17 has been detected in *Helicobacter pylori*-colonized gastric mucosa (Luzzaet *al.*,2000). IL-17 appears to stimulate predominantly the production of cytokines that either specifically attract neutrophils to the site of inflammation or stimulate granulopoiesis in bone marrow (IL-6, G-CSF, GM-CSF), moreover IL-17 was found to induce IL-1 and TNF- $\alpha$  in macrophages (Jovanovicet *al.*,1998).



Abs. = 0.2273 + 0.002764 Conc.

Figure (5): The standard curve shown the IL-17 standards concentrations (0-200) pg/ml and their O.D that used in determination of IL-17 levels in patients with gallstone disease and healthy controls.

Table (5): Statistical analysis of IL-17 concentrations (pg/ml) at gallstone patients and healthy controls sera.

Gender and age groups	Patients and control groups	Mean	Std. Deviation	Std. Error Mean
(1-20) age group	Control n=6	8.0190	1.29350	0.52807
	Patients n=8	6.9010	2.36894	0.83755
(21-40) age group	Control n=10	6.7095	1.02852	0.31011
	Patients n=21	5.8849	1.12275	0.24500
(41-60) age group	Control n=10	6.5340	1.23277	0.37169
	Patients n=22	6.0287	0.92905	0.19807
(61above) age group	Control n=1	7.0170	-	-
	Patients n=1	5.3720	-	-
The males	Control n=8	7.0221	1.41123	0.49895
	Patients n=11	6.4648	1.58629	0.47828
The females	Control n=19	6.8873	1.21402	0.26492
	Patients n=41	5.9922	1.24763	0.19485
Total	Control n=27	6.9245	1.24676	0.23152
	Patients n=52	6.2011	1.43264	0.25326

### Evaluation of Immunoglobulins (IgG)

The technique of radial immune diffusion (RID) by using specific plate for IgG was applied to evaluate its concentrations. The

results of IgG concentrations in gallstone patients sera showed significant elevation comparing to healthy controls. The range of IgG

concentrations in gallstone patients sera was (277.8 - 3176.5) mg/dl while in healthy controls was (934.1 -1694.2) mg/dl. The higher concentration mean according to age was ( $1237.3 \pm 252.39493$ ) mg/dl at the patients of (1-20) age group comparing to the healthy controls was ( $971.80 \pm 53.31585$ ) mg/dl at the same age group. The higher concentration mean according to the gender was ( $1537 \pm 768.70223$ ) mg/dl at the females patients comparing to the female healthy controls was ( $1376.3 \pm 299.44309$ ) mg/dl Table (6).

All Ig classes, including IgA, IgG, and IgM, are present in low

concentrations in bile, however biliary epithelial cells have only minimal participation in Ig secretion (Maurer *et al.*, 2009). Harvey *et al.* (1991) study have shown that biliary Igs, particularly IgM and IgG, promote the nucleation of solid cholesterol crystals, IgM and IgA from commercial sources are incapable of nucleating supersaturated bile, whereas commercially available IgG has pronucleating activity. And Jiao *et al.* (2000) study showed remarkable decrease of serum IgG after cholecystectomy operation.

Table (6): Statistical analysis of IgG concentrations (mg/dl) at gallstone patients and healthy controls sera.

Gender and age groups	Patients and control groups	Mean	Std. Deviation	Std. Error Mean
(0-20) age group	Control n=6	971.80	53.31585	37.70000
	Patients n=8	1237.3	252.39493	89.23508
(21-40) age group	Control n=10	1371.2	456.86169	323.05000
	Patients n=21	1501.4	859.39830	187.53608
(41-60) age group	Control n=10	1310	213.03972	122.99854
	Patients n=22	1533.7	696.41300	148.47575
(61above) age group	Control n=1	1087.2	-	-
	Patients n=1	1167.2	-	-
The males	Control n=8	1049.5	100.31906	50.15953
	Patients n=11	1210.9	384.50181	115.93166
The females	Control n=19	1376.3	299.44309	149.72155
	Patients n=41	1537	768.70223	120.05112
Total	Control n=27	1212.9	270.65706	95.69172
	Patients n=52	1468	714.51012	99.08473

**The relationship between immune response and chronic diseases associated with gallstone:**

The higher concentration means of cytokines according to the chronic diseases associated with gallstone wherein IL-1 $\alpha$  concentration was (8.251 $\pm$ 5.892) pg/ml at the patients

had chronic diseases associated with gallstone, IL-6 concentration was(8.804 $\pm$ 3.940) pg/ml at the patients had chronic diseases associated with gallstone, TNF- $\alpha$



concentration was  $(17.604 \pm 8.934)$  pg/ml at the patients had chronic diseases associated with gallstone, IL-4 concentration was  $(9.305 \pm 2.327)$  pg/ml at the healthy controls, IL-17 concentration was  $(6.924 \pm 1.246)$  pg/ml at the healthy controls. The higher concentration mean of IgG according to the chronic diseases was  $(1493.7 \pm 842)$  mg/dl at the patients had chronic diseases associated with gallstone Table (7).

Diseases such as diabetes mellitus seem to facilitate the development of gallstone formation secondary to increased triglyceride levels associated obesity as well as promoting gallbladder hypomotility and stasis (Shizuka *et al.*, 1999). Insulin resistance predisposes to gallstone formation, suggesting altered

cholesterol and bile salt metabolism (Nervi *et al.*, 2006). Hepatic insulin resistance may act by enhancing hepatic cholesterol secretion, depressing bile salt synthesis and impairing gallbladder motility (Biddinger *et al.*, 2008). Hypertension associated with high cholesterol diet (Ibrahem, 2002). The decreasing in HDL cholesterol and increasing in triglycerides carry an increased risk of developing stones (Petitt *et al.*, 1981). The increased production of bilirubin caused by hereditary spherocytosis, thalassaemia, sickle cell disease, liver cirrhosis, malaria, and ineffective erythropoiesis and be risk factors of gallstone disease (Grunhage & Lammert, 2006).

Table (7): The cytokines and IgG concentrations according to chronic diseases associated with gallstone (IL-1 $\alpha$  L.S.D = 0.302 , IL-6 L.S.D = 0.621, TNF- $\alpha$  L.S.D = 1.323, IL-4 L.S.D = 0.229, IgG L.S.D = 102.54).

Chronic diseases Cytokines and IgG	No chronic diseases associated with gallstone (M $\pm$ SD)	Chronic diseases associated with gallstone (M $\pm$ SD)	Healthy controls (M $\pm$ SD)
<b>IL-1<math>\alpha</math> (pg/ml)</b>	7.903 $\pm$ 4.313	8.251 $\pm$ 5.892	6.772 $\pm$ 2.646
<b>IL-6 (pg/ml)</b>	8.186 $\pm$ 3.137	8.804 $\pm$ 3.940	5.553 $\pm$ 1.519
<b>TNF-<math>\alpha</math> (pg/ml)</b>	14.853 $\pm$ 8.776	17.604 $\pm$ 8.934	10.277 $\pm$ 1.834
<b>IL-4 (pg/ml)</b>	8.116 $\pm$ 1.811	7.784 $\pm$ 0.822	9.305 $\pm$ 2.327
<b>IL-17 (pg/ml)</b>	6.029 $\pm$ 1.292	6.247 $\pm$ 1.434	6.924 $\pm$ 1.246
<b>IgG (mg/dl)</b>	1457.6 $\pm$ 668	1493.7 $\pm$ 842	1212.9 $\pm$ 271

### Conclusion:

- The elevation of pro-inflammatory cytokines including (IL-1 , IL-6 & TNF- $\alpha$ ) indicated the presence of inflammatory response associated with gallstone. The reduction of anti-inflammatory cytokine (IL-4) and regulator cytokine (IL-17) indicated the presence of immune

predisposition in gallstone patients.

### Recommendation:

- Immunohistochemistry study must be done to the gallbladder tissue samples in gallstone patients to view the local immune response.

- Attempt to study immune-modulation to prevent gallstone formation.

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تقدير مستوى المدورات اللمفاوية المصاحبة لالتهاب المرارة في البصرة /جنوب العراق

عواطف حميد عيس و مثنى محمد مثنى

جامعة البصرة/كلية العلوم

#### الخلاصة

يعد التهاب المرارة من الالتهابات الحادة ..

جمعت عينات دم من 52 مريض يخضع للعملية (استئصال المرارة) في مستشفى الصدر التعليمي و52 اصحاء متطوعين لغرض السيطرة. جمعت عينات الدم في قناني معقمة.

كان مستوى المدورات اللمفاوية البادئة للالتهاب والمتمثله (IL-1, TNF- $\alpha$  & IL-6) مرتفعة وبفارق معنوي عن السيطرة عند مستوى الاهمية ( $p < 0.05$ ) بينما المضادة للالتهاب والمتمثله بال IL-4 منخفضة وبفارق معنوي عن امصال السيطرة أما ال IL-7 و الذي يعد من المدورات اللمفاوية المنظمة منخفضا ولكن ليس بفارق معنوي ( $P < 0.05$ ).

كما ارتفع مستوى IgG في امصال المرضى مقارنة بالسيطره.