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By Maysaa Saleh Mahdi

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Original Article

The differences in pro and anti-inflammatory cytokine levels between patients with cholecystitis and apparently healthy individuals

Maysaa Saleh Mahdi[1], Ali Abdul kadhim Jasim[2]

[1] Clinical Laboratories Department, Applied Medical Sciences College, Kerbala University, Kerbala, Iraq

[2] Biology Department, College of Sciences, Kerbala University, Kerbala, Iraq.

Maysaa Saleh Mahdi **ORCID ID**: 0000-0002-6075-1530

Ali Abdul kadhim Jasim **ORCID ID**: 0000-0003-0456-4340

CORRESPONDING AUTHOR:

Maysaa Saleh Mahdi

Email ID: maysa.alhamdany@gmail.com

Clinical Laboratories Department, Applied Medical Sciences College, Kerbala University, Kerbala, Iraq

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ABSTRACT

Background and objectives. Interleukins has an important role in the inflammatory process of cholecystitis and orchestrate both acute and chronic phases of the disease. This study aimed to evaluate the serum level of cytokine (IL-10, IL-37, TGF-β1) as anti-inflammatory cytokines and (Il-17, IL-23) cytokines as pro int- inflammatory cytokines in patients with acute and chronic cholecystitis comparing with control healthy individuals.

Materials and methods. A Case-Control study was conducted. Out of 179 patients and apparently healthy individuals were enrolled. Blood samples were collected to be used in determination of cytokines level by ELIZA technique.

Results. There were statistically significant differences in the mean level of the studied cytokines. The results revealed that the level of all tested serum interleukin in control healthy individuals were higher than Acute and chronic cholecystitis patients (except for IL-17). There was significant positive correlation among the studied interleukins. ROC analysis revealed that TGF-β1 has the best discrimination between patients and control groups.

Conclusions. The presence of significant increase in cytokine level in healthy subject in comparison to acute and chronic cholecystitis may reflect the inappropriate immune response in diseased patients. Non-significant difference in the level of the tested cytokines according to stone number suggest that gall stone do not cause variation in circulating inflammatory profile. Anti/pro-inflammatory cytokines ratio calculation in this study reflect that the amount of anti-inflammatory cytokines were higher than the amount of pro-inflammatory cytokines which may reflect stronger systemic anti-inflammatory response.

Keywords: acute cholecystitis, chronic cholecystitis, pro-inflammatory cytokines, anti-inflammatory cytokines

Abbreviations:

IL	Interleukin
Jan	January
Feb	February
TGF- β1	Transforming growth factor-Beta 1
ELIZA	enzyme-linked immunosorbent assay

Treg	regulatory T cells
MHC	major histocompatibility complex
SPSS	Statistical Package for Social Science
SD Error	Standard Error
ROC	Receiver Operating Characteristics
AUC	Area under curve

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INTRODUCTION

Cytokines play crucial role in the regulation of different biological responses, such as inflammation [1]. Some of them had proinflammatory effect that results in the initiation of inflammation and recruitment of inflammatory cells for controlling the inflammation. However, this process may result in tissue damage if there is an excessive cytokine production. Persistent interleukin production results in persistent inflammation (Chronic inflammation) and leads to certain complications. Inflammation of the gallbladder is an early sign of gallstone development. An abrupt inflammation of the gallbladder is known as cholecystitis. Gallbladder inflammatory diseases affect up to 15% of the population in developed nations. Surgical intervention is usually necessary due to the difficult course of cholecystitis [2]. There are several variations and etiologies of gallbladder disease. Chronic and acute cholecystitis [3]. The most widespread form of gallbladder disease is chronic cholecystitis, which is gallbladder inflammation that continue, because of multiple recurring incidents of gallstone and unrepaired tissue damage. While Acute cholecystitis is defined as an acute inflammation of the gall bladder wall. It constitutes 3-10% of acute abdominal patients [4]. The role of immune system on development of calculus and cholecystitis is mediated by cell-mediated immunity (Th1 cell) exerting its effect on formation of cholesterol gallstone and local inflammation [5]. The first proinflammatory immune response is interceded by the cells of the innate immune system and then followed by the compensatory anti-inflammatory response which is interceded by the cells of adaptive immune system that may display the host to various postoperative septic complications if uncontrolled [6].

Many cytokines were participated in the immune response of cholecystitis such as IL-10, IL-37 and TGF-β1 that were derived from T helper cells, monocytes, macrophages and dendritic cells and regulatory T cells (Treg) are the major source [7-10]. which act as anti-inflammatory cytokines.

While IL-23 and IL-17 act as pro- inflammatory cytokines. TGF-β1 is known to influence gallstone development, as well as transition and activation of gallbladder cells. In human cholesterol gallstones is associated with production of TGF-β and it is related to gallbladder fibrosis and inflammation [7]. Human Interleukin-37 (IL-37), a novel cytokine which is one-member of IL-1 family, has the ability to inhibit inflammation and immune response by inhibiting the production of pro-inflammatory cytokines [11].

IL-10 is a cytokine that have both pro- and anti-inflammatory functions. The activities of IL-8 which has a chemotactic activity as well as other cytokines are inhibited by IL-10. It is capable of downregulating the expression of the MHC protein in monocytes, which attributes to the reduction of inappropriate inflammation [12]. It has been determined that the risk for gallstones formation is enhanced with high levels of IL-10 [13].

Little is known about the association of gallstone existence with circulatory inflammatory cytokines. Additionally, Evaluation of cytokines level in both acute and chronic cholecystitis and compare this level with control group might possibly aid in understanding the role of these cytokines in cholecystitis and aid potential therapeutic interventions. Thus, this study aimed to evaluate and compare the level of cytokines, (IL-10, IL-37, TGF-β1) as anti-inflammatory cytokines and (Il-17, IL-23) cytokines as pro-inflammatory cytokines in patients with acute and chronic cholecystitis in comparison to healthy individuals.

MATERIALS AND METHODS

A case-control study was conducted. This study was approved by Ethical committee at the faculty of science, Department of Biology in Kerbala University. One hundred seventy-nine patients and apparently healthy individuals were enrolled. Both age and sex were matched between patients and healthy individuals. Serum samples were collected between the period from January 2023 to February 2024 from healthy subjects and patients with acute and chronic cholecystitis who were undergo cholecystectomy after submitting to al-Safeer and Al Kafeel Hospital in Kerbala Province. Verbal acceptance was taken from each participant before blood collection. The concentrations of cytokines (IL-10, IL-17, IL-23, IL-37 & TGF-β1) were evaluated in serum of patients and control by Enzyme-Linked Immunosorbent Assay (ELISA) technique using BT LAB Kit according to the instruction provided by manufacturer. Statistical Package for Social Science (SPSS version 24) was used to analyze data. Normality test was done first to explore the normally distributed variables. Kruskal Wallis Test was used to find the significance among the tested cytokines. Bivariate correlation was used to find the presence of positive and negative association among tested cytokines. Receiver Operating Characteristics (ROC) curve analysis was used to determine the

discriminatory power of the tested cytokines. The statistical significance level was considered at *P-value* <0.05.

RESULTS

Out of 179 participants were enrolled in this study which were classified to 92 (51.3%) patients and 87 (48.6%) were apparently healthy individuals. Patients were classified as chronic cholecystitis 83 (90.2%) and Acute cholecystitis 9 (9.8%). The mean age of the diseased patients and control group were 47.14 ± 15.69 and 44.68 ± 14.42, respectively. Table 1 shows the sex distribution of the participant with odds ratio for female 1.062.

Table 1. Distribution of the participants according to sex

Disease-Control		Sex N (%)		Total
		Male	Female	
Control		30 (34.5)	57 (65.5)	87 (100)
Disease	Acute	4 (44.4)	5 (55.6)	9 (100)
	Chronic	24 (28.9)	59 (71.1)	83 (100)
	Disease Total	28 (30.4)	64 (69.6)	92 (100)
Odds Ratio for female		1.062		
Female/ male Ratio		2.28:1		

There were significant differences in the serum level of the tested cytokines between patients and control except for IL17. All the tested cytokines were significantly higher in healthy control group than diseased patient group, as in Table 2.

Table 2. Serum level differences among the studied groups according to disease and control

		IL10	IL17	IL23	IL37	TGF-β1
Disease (N=92)	Mean	324.2868	110.1798	185.7132	54.3457	661.8011
	SD. error	13.28476	6.82206	6.28183	2.51074	71.17993
	Mean rank	76.86	84.11	65.25	53.20	49.15
Control (N=87)	Mean	356.7317	111.7842	241.0295	98.1136	3358.6046
	SD. error	11.17945	5.86156	12.33984	5.88524	194.65001
	Mean rank	103.90	96.23	116.17	128.91	133.20
Kruskal Wallis Test		0.000*	0.118*	0.000*	0.000*	0.000*

SD Error =standard Error, * significant ≤0.05

Classification of patients according to acute and chronic cholecystitis, IL17 and TGFβ1 were higher in acute cholecystitis patients. Whereas, serum level of IL10, IL23 and IL37 were higher in patients with chronic cholecystitis in comparison to patients with acute disease. Significant difference in

serum level of inflammatory cytokines were found between acute/ chronic versus control group, as shown in Table 3.

Table 3. Serum cytokine level differences according to acute and chronic cholecystitis

		IL10	IL17	IL23	IL37	TGF-β1
Control (N=87)	Mean	360.4704	113.9342	243.1084	99.0305	3434.6305
	SD. Error	11.06096	5.82515	12.26420	5.87693	197.64452
	Mean Rank	106.37	98.17	117.39	129.12	133.29
Acute (N=9)	Mean	308.0254	149.0304	164.6924	52.4739	1304.2078
	SD. Error	12.59114	33.02787	13.51652	5.30845	440.64866
	Mean Rank	76.11	105.67	48.33	55.72	75.72
Chronic (N=83)	Mean	326.0501	105.9671	187.9926	54.5487	592.1426
	SD. Error	14.66382	6.59521	6.77944	2.72966	59.77746
	Mean Rank	74.35	79.73	65.81	52.71	46.17
Kruskal Wallis Test		0.000*	0.044*	0.000*	0.000*	0.000*

SD Error =standard Error, * significant ≤0.05

In the current study, there were no significant differences in the serum level of the tested cytokines according to the gallstone existence and their number. All the tested cytokines were higher in a calculus group than calculus group. Comparable level was seen among patients with multiple stone group and single stone group. However, higher level was seen in patients with multiple stones, as shown in Table 4.

Table 4. The impact of gallstone on inflammatory cytokines

Stone Number		IL10	IL17	IL23	IL37	TGF-β1
Single (N=14)	Mean	318.95	96.08	183.31	52.90	581.98
	SD. Error of Mean	18.79	8.54	17.54	3.69	73.84
	Mean Rank	49.5	44.50	40.93	49.71	49.29
Multiple (N=75)	Mean	321.78	111.56	184.21	54.10	669.52
	SD. Error of Mean	15.59	8.06	6.74	2.96	85.98
	Mean Rank	44.75	46.15	46.65	45.03	44.99
A calculus (N=3)	Mean	411.60	141.43	234.30	67.17	841.24
	SD. Error of Mean	80.51	40.40	48.72	11.75	181.47257
	Mean Rank	76.33	64.67	68.67	68.33	71.33
Kruskal Wallis Test		0.12	0.47	0.26	0.29	0.22

This study found positive significant correlation between anti-inflammatory cytokines and pro-inflammatory cytokines as shown in Table 5.

Table 5. Correlation between cytokines among patients with cholecystitis

		IL10	IL17	IL23	IL37	TGF-β1
IL10	Pearson Correlation	1	0.457**	0.465**	0.407**	0.340**

	Sig. (2-tailed)		0.000	0.000	0.000	0.000
IL17	Pearson Correlation		1	0.548**	0.490**	0.451**
	Sig. (2-tailed)			0.000	0.000	0.000
IL23	Pearson Correlation			1	0.879**	0.688**
	Sig. (2-tailed)				0.000	0.000
IL37	Pearson Correlation				1	0.752**
	Sig. (2-tailed)					0.000
**. Correlation is significant at the 0.01 level (2-tailed).						

ROC curve analysis revealed that TGF-β1 had the highest discriminatory ability between disease and control cases among the tested cytokines, Area under Curve (AUC was 97.1), Followed by IL37 (AUC was 92.5), Whereas, IL17 has the lowest one (AUC was 58.9) (Figure 1).

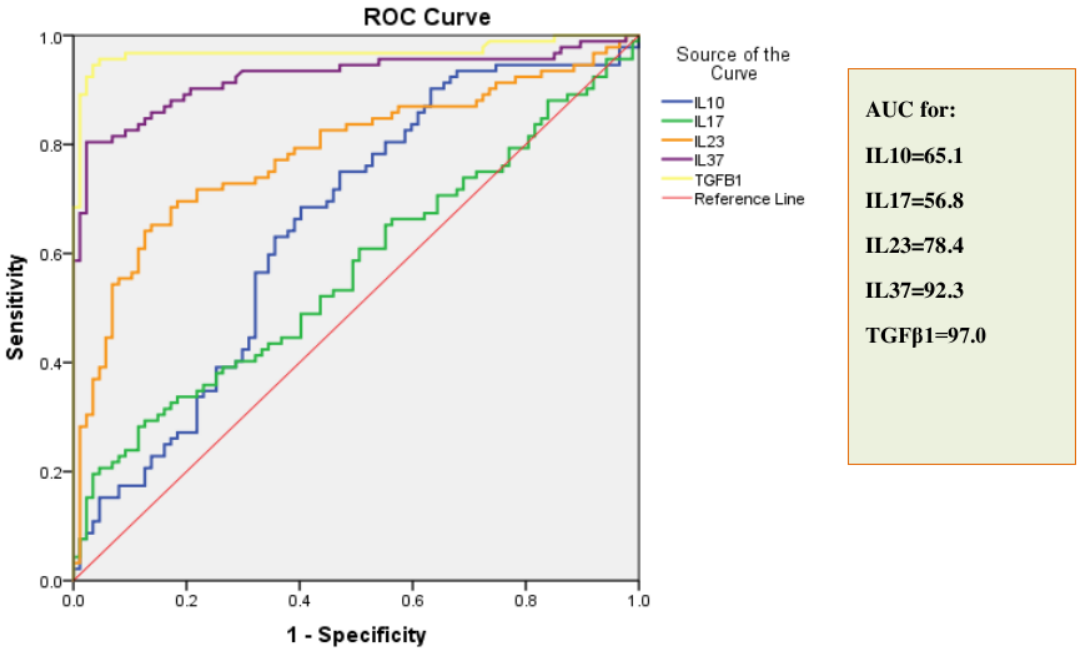

Figure 1. ROC curve analysis of inflammatory cytokine according to disease and control group. AUC=Area under curve; IL=Interleukin; TGF-β1= Transforming growth factor β1

According to acute/chronic cases, the tested cytokines showed Lower discrimination was found between acute and chronic patients using inflammatory cytokines. However, TGF-β1 had the highest discriminatory ability (AUC was 68), Followed by IL17 (AUC was 62.7), Whereas, IL23 has the lower one (AUC was 36.1) (Figure 2).

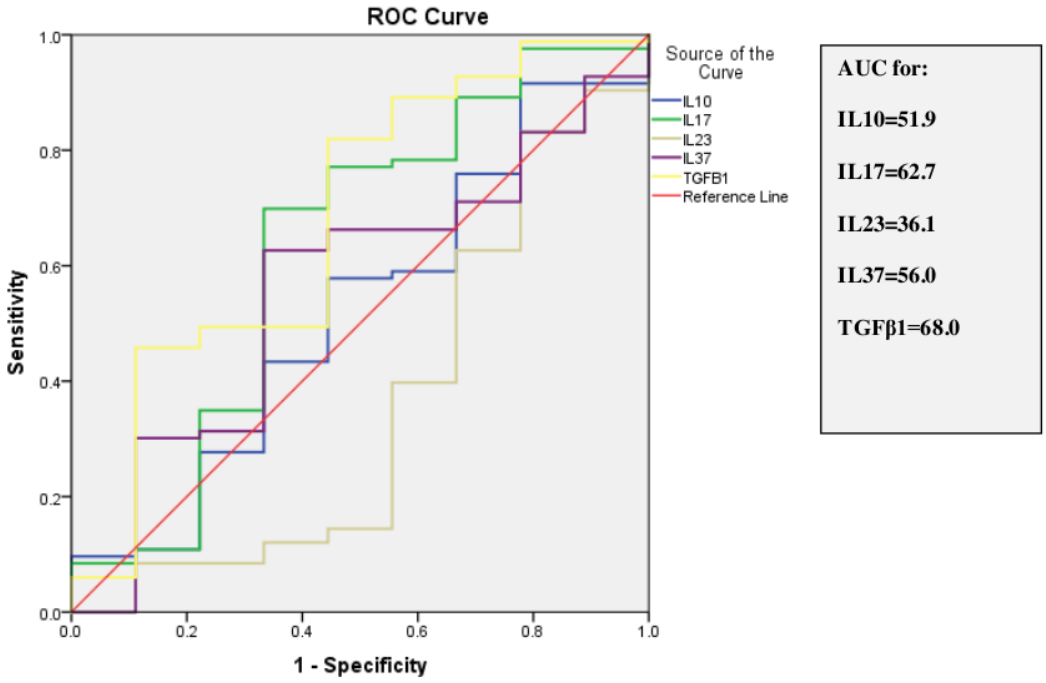

Figure 2. ROC curve analysis of inflammatory cytokine according to Acute and chronic group. AUC=Area under curve**;** IL=Interleukin; TGFβ1= Transforming growth factor β1

Similarly, ROC analysis revealed lower discrimination according to the number of Stones using inflammatory cytokine, as shown in Figure 3.

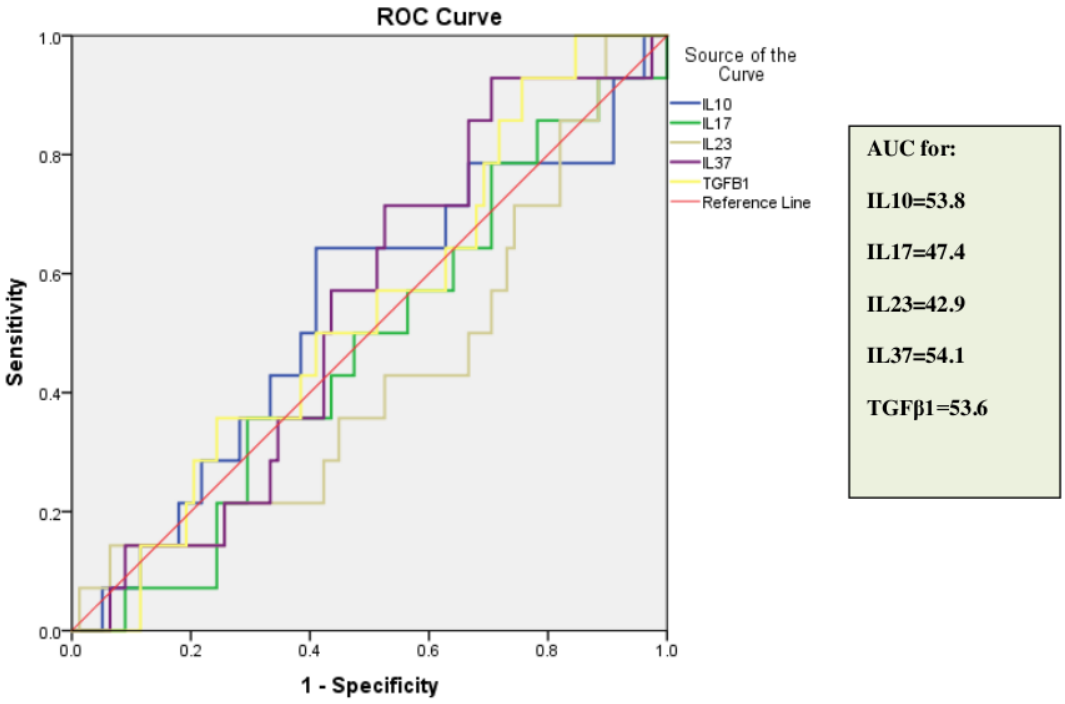

Figure 3. ROC curve analysis of inflammatory cytokine according to stone number. AUC=Area under curve; IL=Interleukin; TGFB1= Transforming growth factor β1

Anti-inflammatory/ pro-inflammatory cytokine balance among patients with cholecystitis

Cytokines are classified into anti-inflammatory and Pro-inflammatory cytokines. The balance between these two classes shapes the generation of the appropriate immune response and it is crucial for effective managements of diseases. Some cytokines had been shown to affect the development of inflammation in gallbladder. However, the balance between pro-inflammatory and anti-inflammatory cytokines with certain types of diseases like cholecystitis has not been studied previously. Calculation of balance between Anti-inflammatory cytokines and pro-inflammatory cytokines and comparing these levels between acute and chronic cases revealed the presence of significant difference in the balance between TGFβ1over IL17 and IL23 ratio and between IL37 over IL17 ratio, as shown in Table 6.

Table 6. Anti-inflammatory/ pro-inflammatory cytokine balance among patients with cholecystitis

		IL10overIL17	IL10overIL23	TGF-β1 overIL17	TGF-β1overIL23	IL37overIL17	IL37overIL23
Acute (N=9)	Mean	2.76	1.96	7.42	6.95	0.42	0.32
	SD. Error	0.424	0.154	0.950	1.783	0.054	0.020
	Mean Rank	35.11	58.11	44.54	69.67	29.89	62.22
Chronic	Mean	3.43	1.79	5.45	3.12	0.56	0.30
(N=83)	SD. Error	0.153	0.064	0.269	0.264	0.011	0.269

	Mean Rank	47.73	45.24	64.56	43.99	48.30	44.80
Kruskal Wallis Test		0.178	0.170	0.033*	0.006*	0.049*	0.063

SD Error =standard Error, * significant ≤0.05

ROC curve analysis for the studied ratios revealed that the TGF-β1 over IL-17 and IL-23 had the highest ability in discrimination between patients and control group AUC for TGF-β1 over IL-17=99.4 and TGF-β1 over IL-23=97.2 followed by AUC IL-37 over IL-17=88.2 and IL-37 over IL-23=87.2 followed by AUC for IL-10 over IL-17=45.3 and IL-10 over IL-23= 67.0 as shown in Figure 4.

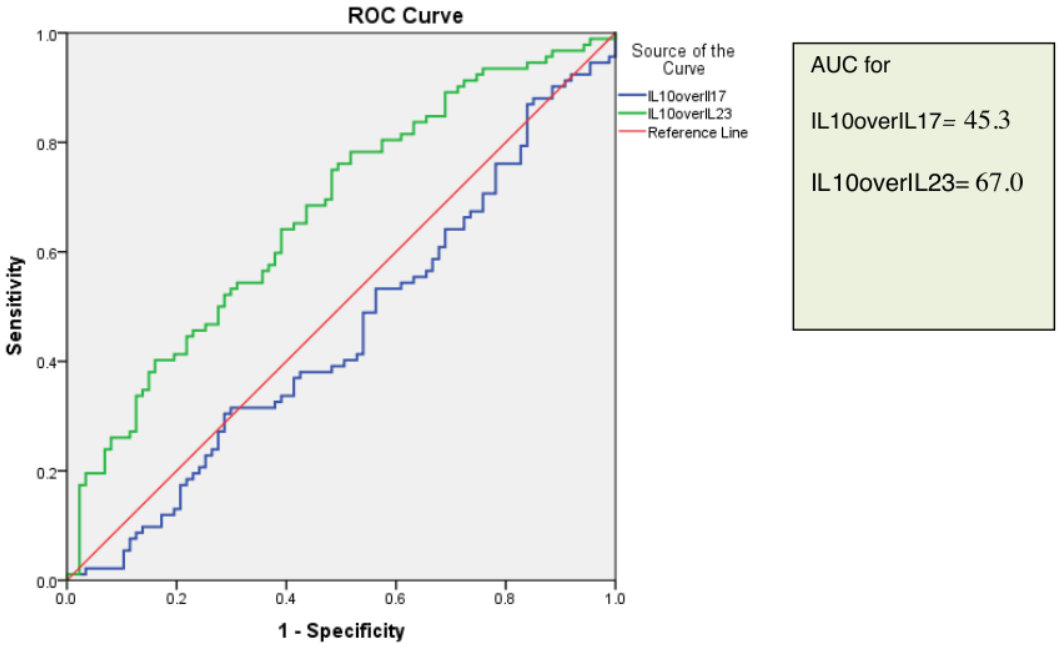

Figure 4A: ROC curve analysis of the tested cytokine balance. AUC=Area under curve; IL=Interleukin

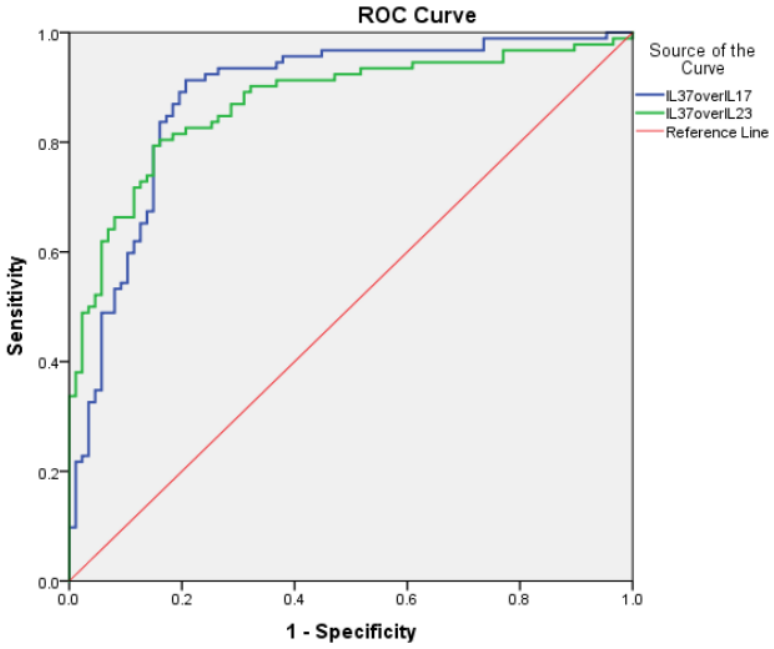

Figure 4B: ROC curve analysis of the tested cytokine balance. AUC=Area under curve; IL=Interleukin.

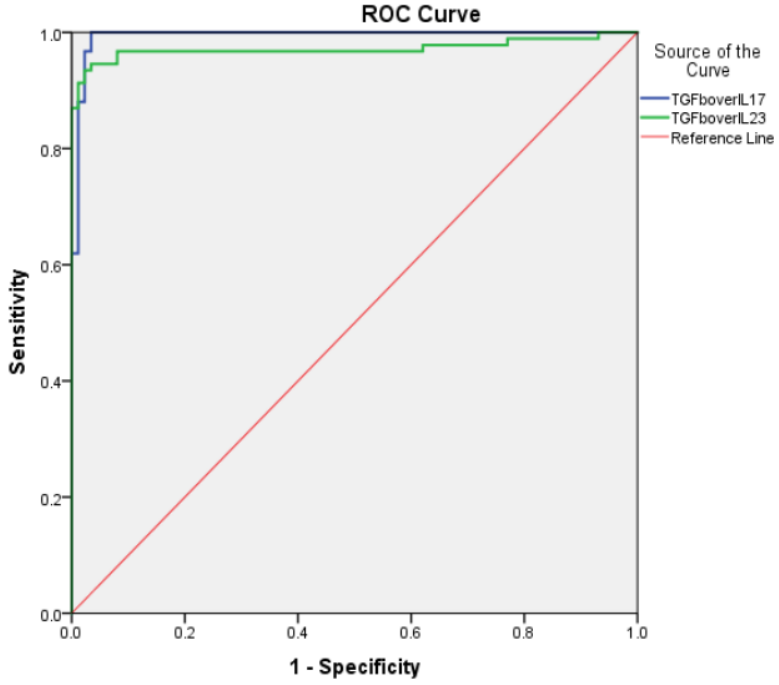

Figure 4C: ROC curve analysis of the tested cytokine balance. **AUC**=Area under curve**;** IL=Interleukin;

TGFβ1=Transforming Growth factorβ1.

DISCUSSION

Cholecystitis is a prevalent disease in hepatobiliary surgery departments. Different managements for different severity degrees of cholecystitis have different influences on prognosis. Therefore, correctly assessing the severity, classification, and staging of cholecystitis in clinical practice is particularly important for the timely treatment and prognosis of cholecystitis.

This study revealed high incidence of chronic verses acute laparoscopic cholecystectomy (LC) as shown in Table 1. A similar finding was reported by Ali et. al., (2021) [14]. Chronic cholecystitis tends to be more prevalent due to the gradual nature of its development and the chronic presence of gallstones or other underlying factors that lead to long term irritation of the gall blader. The mean age of the patients and control group were 47.14 which agree with several previous studies [15,16]. The female to male ratio was 2.28/1 (female 69.6% and male 30.4%) and the Odds ratio for female sex was 1.06. (Table 1). Comparable results was documented by Wattoo 2010 [16]. This might possibly reflect the impact of female sex on cholecystitis.

Cytokines are critical mediators that oversee and regulate immune and inflammatory responses via complex networks and serve as biomarkers for many diseases. Quantification of cytokines has significant value in both clinical medicine and biology as the levels provide insights into physiological and pathological processes and can be used to aid diagnosis and treatment.

To the best of our knowledge, this study was the first case-control study that examined the association between Anti-inflammatory cytokines (IL-10, IL-37 and TGFβ1) and the Pro inflammatory cytokines (IL-17 and IL-23) among acute and chronic cholecystitis patients with healthy control.

This study revealed that all of the tested cytokines (Except for IL-17) were significantly higher in healthy control group than diseased patient group, as shown in Table 3. The level of IL-17 was higher in patients with acute cholecystitis in comparison to chronic cholecystitis and to healthy individuals. This might possibly reflect the inappropriate immune response in patients enrolled in this study in comparison to healthy individuals. It has been noticed that some cytokines (like IL-37) could be transferred in to inside the cells which may cause low serum level of these cytokines especially in inflammatory diseases [17]. Interestingly, in a study done by Fehrenbacher J.C.et.al, patients with acute cholecystitis had higher levels of IL-10 than patients with chronic cholecystitis in Interstitial fluid from gallbladder tissue [18].

It has been documented that the concentration of IL-37 is extremely low [17] while its concentration in inflammatory diseases is elevated [19].

It has been documented that the increase in TGF-β1 levels in Acute gallbladder inflammation patients as TGF-β1 induces the morphogenesis of gallbladder epithelial cells and lead to inflammation [7].

This study showed that higher non-significant levels of all tested cytokines were found in acalculous patients, as shown in Table 4. Zhiwei Liu *et al*. suggest that gallstones do not cause variation in the circulating inflammatory profile. It is generally known that inflammation plays a role in the development of gallstones, even if the function of cytokines in the gallbladder and their impact on gallstone formation are unclear. For instance, inflammation may change the metabolism of the number of proteins and lipids; these modifications may change how cholesterol and bile acids are metabolized and raise the levels of bile salts, which can lead to the development of gallstones [13]. It has been documented that Il-17 has decreased in patients with cholelithiasis. Increased IL-10 has been proven to heighten the risk of developing gallstones [12].

Although TGF-β1 is a multifunctional cytokine that may regulate biliary tract inflammation, an increased level of TGF-β1 promotes gallbladder fibrosis [20] and inflammation of gallbladder in the cholesterol formation process gall stones in human beings [13]. TGF-β1 may be a genetic risk factor for gallstone development, based on its function as previously reported According to earlier research, TGF-β1 is up-regulated in gallstone illnesses and influences the transition and activities of gallbladder cells [20].

This study revealed positive significant correlation among the tested cytokines (Table 5). It has been documented that both IL-23 and IL-17 form a new axis through Th17 cells, which play an important role in autoimmunity and chronic inflammation [21]. IL-17 production is increased in response to the recently described cytokine IL-23 [22]. Additionally, IL-37 protein can be upregulated by inflammatory stimuli and cytokines, such as TGF-β1 [23]. IL-37 was positively correlated with the secreted levels of IL-10 and TGF-β1 [19].

ROC analysis revealed that TGF-β1 had the highest discriminatory ability between disease and control cases and in differentiation between acute and chronic cases (Figures 1, 2).

The crosstalk between pro-inflammatory and anti-inflammatory interleukins is a complex and dynamic process that influences immune responses and immune homeostasis(24). One aspect of the crosstalk between pro-inflammatory and anti-inflammatory interleukins involves negative feedback loops which prevent harmful responses. Pro-inflammatory interleukins can stimulate the production of anti-inflammatory interleukins to limit excessive inflammation. For instance, high expression of IL-1β and IL-6 are linked to reduced IL-10 production and vice versa as a mechanism

to suppress the pro-inflammatory response and prevent tissue damage [25]. This study analyzed the ratios between anti and proinflammatory cytokines to determine the appropriateness of the immune response in patients with cholecystitis. The study revealed that the all of the ratios were more than one (except for IL37 over IL-17 and IL-23) which may reflect that the amount of the anti-inflammatory cytokines were higher than the amount of pro-inflammatory cytokines which indicate stronger systemic anti-inflammatory response. The TGF-β1 over IL-17 and IL-23 ratios had the highest ability in discrimination between patients and control.

CONCLUSION

High incidence rate of chronic cholecystitis was found. The F/M ratio was 2.28:1 with odds ratio 1.06 might reflect the impact of sex on cholecystitis.

The presence of significant increase in cytokine level in healthy subject in comparison to acute and chronic cholecystitis may reflect the inappropriate immune response in diseased patients.

CONFLICT OF INTEREST

Non declared

AUTHOR'S CONTRIBUTIONS

M.S. and A.A analyzed and interpreted the patient data, A.A. supervised data collection an analysis. All authors have reviewed the manuscript.

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Ethics approval

This study was approved by council of University of Kerbala/college of science/ Department of Biology. (CRN :006CSE).

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