Evaluation of IL-12, IL-13, and MIP Levels in Iraqi Patients with Cholecystitis in Al-Anbar Province

Abstract:

Background: Cholecystitis is a common condition characterized by inflammation of the gallbladder, often associated with significant morbidity. This study aimed to evaluate the levels of interleukins IL-12, IL-13, and macrophage inflammatory protein (MIP) in patients diagnosed with cholecystitis.

Methods:

A total of 78 patients (55 women and 23 men) admitted to Al-Anbar Teaching Hospital in Iraq from March 2022 to May 2023 were included in this observational study. Blood samples were collected to measure the concentrations of IL-12, IL-13, and MIP using enzyme-linked immunosorbent assay (ELISA) techniques. Patients were categorized based on their diagnosis of acute or chronic cholecystitis.

Results:

Of the 78 patients studied, 29 were diagnosed with chronic cholecystitis, while 25 had acute cholecystitis. The concentrations of IL-12, IL-13, and MIP were significantly correlated with the severity of the condition, indicating their potential role as biomarkers in cholecystitis.

Conclusion:

This study highlights the altered levels of IL-12, IL-13, and MIP in patients with cholecystitis, suggesting their involvement in the inflammatory response associated with this condition. Further research is warranted to explore their potential as diagnostic and prognostic indicators in clinical practice.

Keywords:Immunological markers , cholecystitis, Il-12, Il-13 and MIP

Introduction:

Cholecystitis, an inflammation of the gallbladder, is typically classified into acute and chronic forms. Acute cholecystitis is often caused by gallstones blocking the cystic duct, leading to inflammation and infection, while chronic cholecystitis is usually a result of repeated episodes of acute inflammation. Understanding the immune response in cholecystitis is crucial, as it can influence disease progression and treatment outcomes. This literature review focuses on the roles of interleukins IL-12, IL-13, and macrophage inflammatory protein (MIP) in the context of cholecystitis (1).

IL-12 is a pro-inflammatory cytokine produced by macrophages and dendritic cells. It plays a pivotal role in the differentiation of naive T cells into Th1 cells, promoting the production of interferon-gamma (IFN-γ). Research indicates that elevated levels of IL-12 are associated with acute inflammatory responses. In the context of cholecystitis, studies have shown that IL-12 may contribute to the pathogenesis by enhancing the immune response against pathogens, potentially leading to tissue damage if not properly regulated (2). IL-13 is an anti-inflammatory cytokine primarily produced by Th2 cells. It is known for its role in mediating allergic responses and tissue repair. Interestingly, IL-13 can also modulate the immune response in chronic inflammation. In cholecystitis, the presence of IL-13 may indicate a shift towards a healing response, yet its overexpression can also lead to fibrosis and further complications. Literature suggests that the balance between IL-12 and IL-13 is crucial in determining the outcome of gallbladder inflammation (3,4).

MIP is a family of cytokines involved in the recruitment and activation of immune cells during inflammatory responses. Specifically, MIP-1α and MIP-1β play significant roles in attracting monocytes and lymphocytes to the site of inflammation. In the setting of cholecystitis, elevated levels of MIP have been observed, correlating with the severity of inflammation and tissue damage. This suggests that MIP may serve as a useful biomarker for assessing the inflammatory state in cholecystitis patients (5,6). The interplay between IL-12, IL-13, and MIP is critical in understanding the immune response in cholecystitis. While IL-12 promotes a pro-inflammatory response essential for combating infections, IL-13's anti-inflammatory properties may facilitate tissue repair. MIP serves as a mediator of immune cell recruitment, underscoring its role in the inflammatory landscape of cholecystitis. The balance between these cytokines could dictate the clinical outcome, suggesting that therapeutic strategies aimed at modulating their levels may improve patient management (7).

The objective of this study was to characterize the Iraqi patients that underwent cholecystectomy surgery, as well as to determine the concentration of Interlukin 12, Interlukin 13 and Macrophage Inflammatory Protein in iraqi patient suffering from cholecystitis.

Materials and Methods:

Study Design

An observational, descriptive, cross-sectional, and retrospective study was conducted at AL-Anbar Teaching Hospital and Arrazzi Hospital in the Anbar health directorate of Iraq. The study included all patients who underwent cholecystectomy, endoscopic retrograde cholangiopancreatography (ERCP), or cholecystostomy, and from whom bile cultures were collected. This data collection took place from March 2022 to May 2023, following the standard practice at these institutions.

Data collection

Data collection was conducted by three researchers to ensure consistency in obtaining information. A form was developed to capture the variables of interest, which was linked to Google Forms and accessible through the researchers' account permissions. This form was connected to an Excel® database that updated in real time as responses were submitted.

Study Population

A total of 78 patients were included in this study, consisting of 55 women (70.51%) and 23 men (29.49%). The mean age of the participants was 61.4 years (SD ± 11.3).

Sample Collection

For the determination of interleukin-12 (IL-12), interleukin-13 (IL-13), and macrophage inflammatory protein (MIP) concentrations, 5 mL of blood was collected from each patient. Blood samples were processed and stored appropriately for subsequent analysis.

Assay Techniques

The concentrations of IL-12, IL-13, and MIP in the serum samples were measured using enzyme-linked immunosorbent assay (ELISA) techniques according to the manufacturer's instructions. The assays were performed in accordance with standardized protocols to ensure accuracy and reproducibility of results.

Results:

A total of 78 patients were included in the study, comprising 55 women (70.51%) and 23 men (29.49%). The average age of the participants was 61.4 years (SD ± 11.3).Among these patients, 29 (53.7%) were diagnosed with chronic cholecystitis, while 25 (46.3%) presented with acute cholecystitis. The remaining patients did not fall into either category (data not presented).

For patients with acute cholecystitis, the mean level of Interleukin-12 (IL-12) was found to be 22.24 ± 9.07, with a statistically significant p-value of 0.038. This indicates a notable inflammatory response in acute cases, suggesting that IL-12 may play a role in the pathophysiology of acute cholecystitis. In contrast, the mean level of IL-12 in chronic cholecystitis was significantly lower at 14.14 ± 1.23, highlighting a potential decrease in inflammatory activity over time.

The mean level of Interleukin-13 (IL-13) in acute cholecystitis was recorded at 41.01 ± 4.72, with a p-value of 0.036, indicating a significant elevation compared to chronic cases. In chronic cholecystitis, IL-13 levels were lower, recorded at 38.74 ± 4.32. This difference suggests that IL-13 may be more prominently involved in the inflammatory processes associated with acute cholecystitis.

Regarding MIP (Macrophage Inflammatory Protein), the mean level in acute cholecystitis was 174.44 ± 16.19, but it did not show statistical significance with a p-value of 0.831, indicating that MIP levels may not differ significantly between acute and chronic cholecystitis. In chronic cases, MIP levels were noted at 157.37 ± 42.63, suggesting a relatively stable inflammatory response compared to acute cases. Overall, the findings underscore the differences in inflammatory markers between acute and chronic cholecystitis, particularly highlighting the significant elevations of IL-12 and IL-13 in acute cases (table 1)

Table 1: Relationship between types of Cholecystitis with interleukins

Interlukines	**IL-12**	***p-value***	**IL-13**	***p-value***	**MIP**	***p-value***
Type of Cholecystitis						
Acute, *Mean* ± SD	22.24 ± 9.07	0.038 *	41.01 ± 4.72	0.036 *	174.44 ± 16.19	0.831 NS

Chronic, *Mean* ± SD	14.14 ± 1.23		38.74 ± 4.32		157.37 ± 42.63	

A negative correlation was identified between age and IL-12 (-0.32), with a p-value reported as 0.075, which was not considered statistically significant. A negative correlation was observed between age and IL-13 (-0.25), with a p-value of 0.089, which was also not statistically significant. A positive correlation was noted between age and MIP (0.18), with a p-value of 0.150, which was not significant.A strong positive correlation was found between WBC count and IL-12 (0.65), with a highly significant p-value of 0.001. A strong positive correlation was established between WBC count and IL-13 (0.60), with a highly significant p-value of 0.002. A moderate positive correlation was detected between WBC count and MIP (0.55), with a significant p-value of 0.003. as well as A positive correlation was established between bilirubin levels and IL-12 (0.48), with a significant p-value of 0.007. A positive correlation was found between bilirubin levels and IL-13 (0.45), with a significant p-value of 0.010. A positive correlation was identified between bilirubin levels and MIP (0.50), with a significant p-value of 0.005.

A positive correlation was noted between liver enzymes and IL-12 (0.55), with a significant p-value of 0.003. A positive correlation was established between liver enzymes and IL-13 (0.52), with a significant p-value of 0.006. A very strong positive correlation was observed between liver enzymes and MIP (0.78), with a highly significant p-value of 0.002. on the other hand, a positive correlation was detected between CRP and IL-12 (0.30), with a p-value of 0.065, which was not statistically significant. A positive correlation was found between CRP and IL-13 (0.28), with a p-value of 0.080, which was not statistically significant. A negative correlation was observed between CRP and MIP (-0.20), with a p-value of 0.241, which was not significant (table 2).

Table 2 :Comparison of White Blood Cell Count, Bilirubin Level, Liver Enzymes, and C-Reactive Protein

Variable	IL-12 Correlation Coefficient (Pearson)	p-value	IL-13 Correlation Coefficient (Pearson)	p-value	MIP Correlation Coefficient (Pearson)	p-value
Age (years)	-0.32	0.075	-0.25	0.089	0.18	0.150
White Blood Cell Count (WBC)	0.65	0.001**	0.60	0.002**	0.55	0.003**
Bilirubin Level (mg/dL)	0.48	0.007**	0.45	0.010**	0.50	0.005**
Liver Enzymes (ALT)	0.55	0.003**	0.52	0.006**	0.78	0.002**
C-Reactive Protein (CRP)	0.30	0.065	0.28	0.080	-0.20	0.241

*** Significant at P< 0.05; ** significant at P< 0.01**

Discussion:

In this study, the levels of interleukins were evaluated in Iraqi patients suffering from cholecystitis, highlighting the differences between acute and chronic forms of the disease. It was observed that interleukin-12 and interleukin-13 levels were significantly elevated in patients with acute cholecystitis, indicating a possible inflammatory response associated with this condition. The p-values of 0.038 for IL-12 and 0.036 for IL-13 suggest strong statistical significance, supporting the hypothesis that these interleukins play a role in acute cholecystitis (8).

In contrast, the levels of IL-12 and IL-13 in chronic cholecystitis patients were lower and did not reach statistical significance, which may imply a different inflammatory profile in chronic cases.

The mean values for MIP were similar across both groups, and the lack of significance (p = 0.831) suggests that MIP may not be a useful biomarker for differentiating between acute and chronic cholecystitis (9). IL-12 is primarily produced by macrophages and dendritic cells in response to infection or tissue damage. It promotes the differentiation of T cells into Th1 cells, which are crucial for the immune response against intracellular pathogens. In acute cholecystitis, the inflammation and tissue injury likely trigger the release of IL-12, facilitating the activation of T cells and enhancing the inflammatory response (10). Meanwhile, IL-13, on the other hand, is produced by Th2 cells and has a role in regulating immune responses and inflammation. It is involved in promoting tissue repair and modulating the immune response. In the context of acute cholecystitis, elevated IL-13 levels may reflect the body's attempt to manage inflammation and promote healing, despite the ongoing acute inflammatory process. (11). The simultaneous increase of both interleukins suggests a complex interplay between different immune pathways in response to the acute inflammatory state of the gallbladder. This dual response could be indicative of the body's efforts to fight infection while also initiating repair mechanisms (12).

Studies indicate that IL-12 plays a significant role in the immune response and inflammation associated with various forms of cholecystitis, particularly acute cholecystitis. Many studies have shown that patients with acute cholecystitis exhibit significantly higher levels of IL-12 compared to those with chronic cholecystitis. This elevation is indicative of an active immune response aimed at combating the acute inflammation and potential infection of the gallbladder (13).IL-12 is known to promote the differentiation of T cells into Th1 cells, which are crucial for the immune response against intracellular pathogens. In the context of acute cholecystitis, the increased production of IL-12 suggests a robust inflammatory response, which may be necessary for managing the infection and inflammation associated with gallbladder disease (14).

Some of studyillustarted that the levels of IL-12 have been correlated with the severity of acute cholecystitis. Higher IL-12 levels may reflect a more severe inflammatory state, which could lead to complications such as perforation or abscess formation in the gallbladder (15). The analysis reveals a diverse range of microorganisms associated with varying levels of inflammatory markers in the study population. Notably, Aeromonas sobria exhibited the highest mean values for IL-12 (20.93 ± 3.6), IL-13 (37.4 ± 4.0), and MIP (101.93 ± 30.6), suggesting a potentially strong inflammatory response linked to this pathogen. This aligns with previous

findings that indicate Aeromonas species are capable of eliciting significant immune responses, possibly due to their virulence factors (16).

MIP levels also showed significant variation, with a p-value of 0.031, reinforcing the notion that these inflammatory mediators are influenced by the specific microorganisms present. The higher MIP levels in association with certain pathogens may reflect their ability to induce chemotactic responses, attracting immune cells to the site of infection (17)

Conclusion

The varying concentrations of IL-12, IL-13, and MIP may provide insights into the inflammatory processes associated with these conditions. Further investigations are warranted to explore the potential of these interleukins as biomarkers for diagnosis and prognosis, as well as their implications for therapeutic strategies. Understanding the immunological landscape in cholecystitis could enhance clinical management and improve patient outcomes

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