Evaluation of the IL-23 serum level in inflammatory bowel disease for Iraqi patients

Ali Hussein Al-Sultany[1], Ghasoun mohammed-ali wadai[2]

[2,1]College of Biotechnology,University of Al-Qadisiah,Iraq

Corresponding author email ali.alsultany988@gmail.com ghasoun.wadai@qu.edu.iq

Abstract

Background: Inflammatory bowel disease (IBD) is a long-term inflammatory condition believed to be related to cytokines serum level, especially IL23, which plays a critical role in inflammation progression and regulates the activation and immune function

Objective: Determination of serum IL-23 level IBD in Iraqi patients.

Materials and Methods: Thirty-seven patients with CD and twenty-three patients with UC were among the fifty patients with IBD diagnoses in this study. These patients were then compared to a group of 50 healthy individuals serving as controls.IL23 level was measured using an ELISA kit according to the company's instructions.

Result: The findings indicated a significantly increased serum IL23 level in patients with CD and UC, compared to healthy individuals (10.44 ± 3.26 and 9.20 ± 3.27 vs. 4.01 ± 1.01; p = 0.001). Between patients with CD and UC, there was no statistically significant difference in the cytokine levels (p = 0.458).

Conclusion: This study demonstrates that serum IL23 levels are elevated in people diagnosed with inflammatory bowel disease (CD and UC) compared to healthy individuals. The results revealed that IL23 has a role in disease progression in people with CD and UC.

Keywords: *Inflammatory bowel disease, Crohn's disease, Ulcerative colitis, IL-23.*

1. Introduction

IBD can be classified into two primary clinical forms: CD and UC, each of which has unique clinical characteristics [1,2The immune system also contributes to the development of IBD. Immune cells can be split into natural immune cells (macrophages, dendritic cells, neutrophils, NK, as well as innate lymphocytes) as well as acquired immune cells that are important in the immune response of IBD [3]. An important part of the onset, development, and diagnosis of IBD is the abrupt immune response in the intestinal mucosa. A common feature of all IBD patients is T cell activation [4,5]. People with IBD experience many

changes as the disease develops and progresses, and cytokine mechanisms may be responsible for the patient's clinical changes [6].

The pathophysiology of IBD is primarily mediated by IL-23 [7].IL-23 is a heterologous inflammatory cytokine [8]. IL-23 is a member of the IL-12 family of cytokines and is composed of two units: IL12p40 and IL23p19 [9]. IL-23 is produced mediated by APCs in the gut, secretion cells, and respiratory cells [10,11]. IL-23 regulates the activation and immune function of several cell types in the gut by activating IL-23R, which is expressed on NK, Th17, ILCs as well as intraepithelial lymphocytes (IEL). These cytokines, in turn, inhibit the stimulation of Treg [12]. When microorganisms activate APCs, they release IL-23, which then interacts with the heterodimeric IL-23 protein (IL23R) and produces inflammatory mediators (IL-17, IL-22, GM-CSF, TNF) using activation of JAK-STAT signaling mechanisms[13].due to the role of IL-23 in inflammation prorgress,we design this research to study the role of IL-23 serum level in IBD development

2 . Materials and Methods

2.1 Subjects

A case-control research was conducted between September and January 2024, involving 50 patients with IBD (23 with UC and 27 with CD), as well as 50 healthy individuals. Approval was obtained from the Ethics Committee of the Iraqi Ministry of Health. Verbal consent was obtained from each patient as well as healthy controls. The patients were selected from the Digestive and Liver Center in Hilla, Babil Governorate, and the ages of the patients ranged from (14 - 50 years). Patients are diagnosed based on clinical, endoscopic, and radiological criteria as well as biopsies [14]. Exclusions from the trial include people with major medical conditions, those who have undergone past immunosuppressive therapy, and those who have other visible illnesses including hepatitis, cancer, or HIV.

2 .2 Collection of samples

Each participant provided four milliliters of fresh venous blood, which was then placed in a gel tube and centrifuged at 2600 rpm for 12 min .Subsequently, we gathered and preserved the serum at around 20°C until we utilized and quantified human IL-23. The serum IL23 concentration was measured using a commercially available ELISA kit for human IL23 (Sunlong Biotech, China) following the instructions provided by the manufacturer.

2.3 Statistical analysis

The data was input, encoded, and analyzed using the Statistical Package for the Social Sciences (SPSS) software, especially version 26. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to verify that the data distribution was normal. The association between category variables was evaluated using the chi-square test. Using the receiver operating characteristic (ROC), we compute the area under the curve (AUC), sensitivity, specificity, and cut-off point. P-values were considered statistically significant if they were less than 0.05.

3. Results

The present study comprised a cohort of 50 individuals diagnosed with IBD, with 23 individuals having UC and 27 individuals having CD. We also included a control group of 50 healthy participants. matching between the patient and control group based on age and gender, revealing no statistically significant disparities.

A assessment was conducted to determine the levels of IL-23 in patients with IBD and healthy control subjects. The findings of this comparison are presented in table (1). The average levels of IL-23 in patients with CD, patients with UC, and the healthy control group were 10.44± 3.26, 9.20± 3.27, and 4.01 ± 1.01, respectively. Both patients with IBD had higher average levels than the healthy control group, and this difference was statistically significant (P = 0.001). However, the mean count was a non-significant difference between the patient groups themselves (P= 0.458).

Table (1): IL-23 level in patients with IBD and healthy control.

Characteristic	**CD n=27**	**UC n=23**	**Control subjects n=50**	***P***
IL-23 levels				
Mean± SD	10.44 ± 3.26	9.20± 3.27	4.01 ± 1.01	0.001 S
Range	0.87 -24.30	1.38-18.54	1.71-7.15	
CD vs UC	0.458 NS			
Different letters denote the significant differences at p< 0.05				

*S: Significant

The diagnostic accuracy of using Interleukin-23 (IL-23) concentrations to differentiate between patients with IBD and healthy control participants was investigated using receiver operating characteristic (ROC) analysis. The findings are displayed in table (2) and figure (1). An AUC value of 0.776 (95% confidence interval [CI], 0.668-0.883, P < 0.001), sensitivity of 78.0%, specificity of

78.0%, PPV of 78.0%, and NPV of 78.0% were obtained for an ideal IL-23 cut-off value of greater than 4.66 mg/dL. According to the current data, IL-23 is regarded as a useful diagnostic marker.

Table (2): Sensitivity and specificity of IL-23 (< 4.66-fold) in IBD disease

Parameters	Cut-off point	Sensitivity %	Sensitivity %	AUC	CI 95%	P-value	Relative risk	IBD patients NO.%
IL-23 level	> 4.66	78	78	0.776	0.668-0.883	<0.001	PPV %	78.0 %
							NPV %	78.0%

* AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value, Sig at p<0.05.

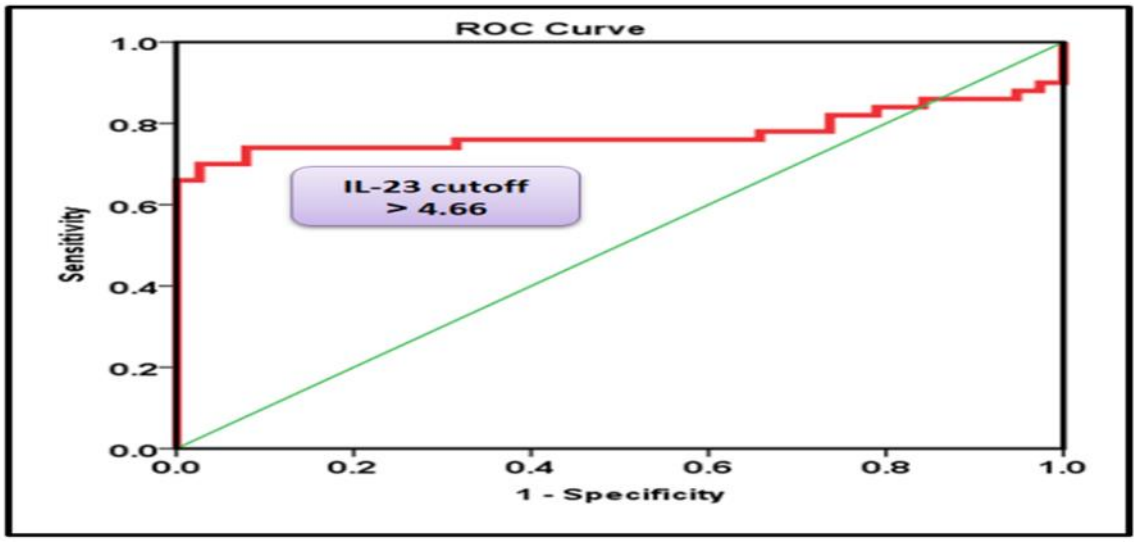

Figure (1) : Receiver operator characteristic curve analysis of IL-23 for the calculation of possible diagnostic cutoff value.

4. Discussion

This study affirms the significant involvement of IL23 in the development of CD and UC. The levels of IL23 were higher in both groups of patients as compared to healthy controls. Furthermore, there were no notable disparities detected between individuals with CD and UC. ROC analyses confirmed that Il23 occupied the AUC region in both species, this study agrees with a study conducted in Iraq, where the IL23 level was high compared to healthy controls, and the p -p-value was (0.001) [15]. Another study conducted in Iraq showed that IL23 levels were high in UC and CD. Nevertheless, the comparison of cytokine levels between the two groups did not reveal any significant difference, as evidenced by a p-value of 0.777 [16]. Similar to the current findings by Andre Geofremou & Markus F. Neurath, where IL23 levels were two times higher in IBD compared to healthy individuals; The CD patient had larger values than the UC patient [17]. Furthermore, IL23 is increased in the serum of both

CD and UC. Genetic, functional, and descriptive investigations conducted in both human models and laboratory animals all converge on one finding: The IL23 pathway is essential in the progression of IBD. Genome-wide association (GWA) studies have established a connection between UC and CD, as well as several genes within the IL23 axis [18,19]. Devinney et al. showed that lamina propria T cells in UC produce IL17 when stimulated by IL23. In addition, disease severity was associated with lower Treg/TH17 rates [20].

Serum IL23 levels correlate with disease severity and duration, suggesting that it could be used as a risk factor for intestinal inflammation [21]. These results suggest that IL23 has a crucial function in the development of intestinal inflammation, making it a necessary target for treatment and a possible indicator of the severity and prognosis of intestinal diseases[22]. Many previous studies have relied on evaluating IL23 expression based on disease severity, especially using scores to indicate the rate of disease progression and stage [23,24] The disease can progress, requiring aggressive treatment to reduce the effect. Suppression of IL10 production is an additive effect of IL23 on immune cells in the intestinal mucosa [25,26].

Conclusion: Available evidence suggests that IL23 plays a crucial role in the development and advancement of CD and UC. However, the current study was limited by a small sample size, and increasing the number of patients will lead to a more thorough understanding of the disease.

5. Reference

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