

## Estimation of Calprotectin, IL-17, and IL-23 levels in the Blood of Iraqi Patients with Crohn's Disease

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### ABSTRACT

**Background:** Crohn's disease (CD) is a common disease of the gastrointestinal tract. It is cause heavy inflammation of the digestive system, and it can hit any area of the digestive tract. Cytokines are very important in the progression and reduction of disease severity so it is important to know about them.

**Objective:** The aim of this study is to measure levels of IL-23, IL-17, and calprotectin in serum of Iraqi patients with Crohn's disease, and also measure the levels of inflammation markers CRP, ESR, and WBCs and the reasons for their elevation in blood or serum of patients.

**Materials and Methods:** In the current study, 30 Iraqi patients with Crohn's disease and 30 Iraqi control were included. From November 2021 to April 2022 blood samples were collected, samples were collected in the Gastro-Enterology and Hepatology Hospital in Baghdad, Iraq. The biomarkers used to assess disease activity were C-reactive protein (CRP), complete blood count (CBC), and erythrocyte sedimentation rate (ESR). Calprotectin, IL-23, and IL-17 were measured by the sandwich enzyme-linked immune-sorbent assay technology.

**Results:** Higher incidence of Crohn's disease was noticed in males compared to females, and a higher incidence was found in patients with medium economic situation. The mean age for Crohn's disease diagnosis was  $22.96 \pm 1.89$ . Abnormal levels of CRP, ESR, WBCs were noticed and also high levels of IL-23, IL-17, and calprotectin were found in serum of Crohn's disease patients.

**Conclusion:** Elevation of cytokines and inflammatory markers in the blood depends on the severity of the inflammation.

**Keywords:** Calprotectin, Crohn's disease, Digestive system, IL-17, IL-23.

### INTRODUCTION

Crohn's disease (CD) is considered a type of inflammatory disease. It is recognized by fistulas, heavy inflammation or abscesses. CD can hit any area of the gastrointestinal tract <sup>(1)</sup>.

Some environmental factors and genes have been related with CD progression. Symptoms of the disease appear on the patient include diarrhea, abdominal pain, blood with feces, and loss of weight. The prevalence of CD in the United States population in 2009 were eighty-five per hundred thousand in children and one hundred and nineteen to two hundred and forty-one per hundred thousand in adults and it is increasing. Also, different studies showed the highest prevalence in Europe was 322 per 100,000 person-year and the number is going up in developing countries <sup>(2,3)</sup>. The name of this disease was given after the gastroenterologist Burrill Bernard Crohn who was born in June 13, 1884, in the City of New York with two colleagues at Mount Sinai Hospital in New York <sup>(4)</sup>.

It is important to know about cytokines, which are small proteins that play important roles in cell signaling and regulate immune response and they are produced by a broad range of cells in response to stimuli, such as interleukins (IL), because the cytokines network is

### MATERIALS AND METHODS

associated with intestine inflammation and to put the basis for establishing some of the biological treatments that are used for inflammatory bowel disease (IBD) therapy <sup>(5,6)</sup>.

Also, calprotectin (a protein found in cytosol of the neutrophils, has a competitive effect with bacteria on zinc, so it kills the bacteria and thus it has antimicrobial properties) it has role in intestine inflammation <sup>(7)</sup>.

Despite all the cytokines are involved in IBD progression, many studies showed that IL-23 and IL-17 have been involved in CD pathogenesis because they have a role in intestinal inflammation. Naïve T cells play role in IBD development, they can be differentiated into Th1, Th2, and Th17 cells. The expression of IL-17 and interleukin 23 receptor from Th17, leads to the produce of interleukin-23 by many immune cells, which in return increases the expression of IL-17. Continuous production of IL-23 leads to a pathogenic model of Th17, which produce multiple cytokines <sup>(5-8)</sup>.

At this time, cytokines of CD are still a research tool. The aim of the current study is to estimate the inflammation markers such as erythrocytes sedimentation rate (ESR), C-reactive protein (CRP), white blood cells (WBCs), IL-23, IL-17, and calprotectin in the blood of Iraqi patients with CD.

Blood samples of 30 cases with a recognized diagnosis of CD based on endoscopic imaging, typical clinical symptoms, and histopathological criteria, and also 30 blood samples of healthy individuals, with ages ranging from 13-45 years were collected. Blood samples were collected from Gastro-Enterology and Hepatology Hospital in Baghdad, Iraq.

Laboratory tests were performed on all samples to assess disease activity including C-reactive protein by using the ichromα CRP test (ichromα, Germany), erythrocytes sedimentation rate by using ESR fast detector (HINOTEK, China), complete blood count (CBC) by using hematology analyzer Diagon-Cell 60 (Diagon, Hungary), also measuring of IL-23, IL-17, serum calprotectin by using sandwich enzyme-linked immunosorbent assay (SUNLONG, China).

Also, demographic data of the patients were studied including: gender, age, and economic situation.

**Ethics approval**

**An approval of this study was obtained from the University of Baghdad Academic and Ethical**

**Committee. Informed consent of all the patients was taken. This study was carried out in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for studies involving humans.**

**Statistical Analysis**

The Statistical Analysis system- SAS (2012) program was used to detect the effect of different factors on study parameters. Quantitative data were presented as mean and standard error (SE) and were compared by independent t-test and Mann-Whitney U Test. Qualitative data were presented as numbers and percentages and were compared by Chi-square test. P<0.05 was considered significant.

**RESULTS**

**Crohn’s disease according to gender**

Table 1 shows no significant differences in demographic data between Crohn’s disease patients and the control group.

**Table 1: Comparison of samples study according to demographic data of the patients and control**

Group	No	Male No. (%)	Female No. (%)	P-value	Good No. (%)	Medium No. (%)	Poor No. (%)	P-value	Mean ± SE Age (year)
Crohn’s disease	30	18 (60.00%)	12 (40.00%)	0.060	9 (30.00%)	14 (46.67%)	7 (23.33%)	0.281	22.96 ±1.89
Control	30	16 (53.33%)	14 (46.67%)		5 (16.67%)	20 (66.67%)	5 (16.67%)		27.63 ±1.30

### Crohn's disease according to CRP, ESR, and WBCs

There was a significant difference in the rate of ESR, WBCs, and CRP between the control group and CD patients and as shown in table 2 and table 3.

**Table 2: Comparison between Crohn's disease patients and Control in CRP, and ESR**

Group	Mean rank	
	CRP (mg/l)	ESR (mm/h)
Crohn's disease	44.33	43.32
Control	16.67	17.68
Mann-Whitney U Test	35	65.500
P-value	0.0001	0.0001

**Table 3: Comparison between Crohn's disease patients and Control in WBCs**

Group	No	Mean ± SE
		WBCs ( $\times 10^9/l$ )
Crohn's disease	30	8.77 ±0.72
Control	30	6.35 ±0.19
P-value		0.002

### Crohn's disease and IL-23, IL-17, calprotectin in serum

As shown in table 4 there was a significant difference in IL-23, IL-17, and serum calprotectin between the control group and CD patients.

**Table 4: Comparison between control and CD patients in IL-23, IL17, and calprotectin in serum**

Group	Mean ± SE		
	IL-23 (pg/ml)	IL-17 (pg/ml)	Calprotectin in Serum (ng/ml)
Crohn's	9.76 ±0.78	35.03 ±1.34	34.83 ±2.30
Control	4.02 ±0.31	21.39 ±1.18	12.99 ±0.91
P-value	0.0001	0.0001	0.0001

### DISCUSSION

The current study results show that IL-23 and IL-17 play role in the progression of Crohn's disease. Both cytokines were elevated in CD patients. This may indicate that interleukin-23 and interleukin-17 participate in a common pathway of inflammation in Crohn's disease patients. This is consistent with what was found by other studies<sup>(9,10)</sup>.

Dendritic cells produce IL-12 and IL-23. These cytokines are important for the maintenance of Th1 and Th17 responses. IL-17 is a pro-inflammatory cytokine secreted from Th17 cells. Naïve T cells play role in Crohn's disease development, they can be differentiated into Th1, Th2, and Th17 cells<sup>(5-8)</sup>. Also, abnormal levels of ESR, WBCs, and CRP have been noticed in CD patients compared to the control group; perhaps the reason for inflammatory conditions is microbial invasion to the intestine such as fungal, viral, or bacterial invasion, and intestine cells damage. This is in agreement with what was found by some studies<sup>(11-12)</sup>.

CRP is a protein secreted by the liver in response to inflammatory conditions that increases following IL-6 secretion by some immune cells. CRP has a physiological role, it can activate the complement system by binding to phosphocholine that is present on the surface of dying or dead cells, and some types of bacteria through C1q<sup>(13)</sup>.

The current study shows no significant differences of Crohn's disease with gender, but the results show the incidence of males is higher than in females in Iraqi patients. Perhaps this is related to the nature of the male living system where the males have a higher risk of getting Crohn's disease because of their habits of drinking alcohol, smoking, not monitoring their diet, and also work pressure<sup>(14,15)</sup>. These results contradict some studies in Western society, which say that there is male and female equality in Crohn's disease incidence because in Western society they have gender equality unlike the society and culture of Iraq, as females drink alcohol, smoke, and do what males do. These results are inconsistent with these studies<sup>(16-17)</sup>.

In the aspect of economic situation, the current study showed that incidence of Crohn's disease was higher in people of medium economic status, it might be the reason that most of the Iraqi people have medium economic situation, and they have the ability to buy canned meat that contains preservatives, food that contains additives, and food that contains a high percentage of fat, these types of food can cause a higher risk of CD. This is consistent with what was found by another study<sup>(18)</sup>.

In contrary, people having a good economic situation have the ability to buy fresh meat and fresh food that has the ability to risk reduction of CD<sup>(19)</sup>.

Most of the patients in this study were young, the highest incidence of young people may be due to the frequent eating of ready-made food and fast food in addition to drinking alcohol and smoking increasingly at this age. This is in agreement with several studies<sup>(20,21)</sup>.

### CONCLUSION

The conclusion of this study is the relationship of gender and the economic situation with the incidence of

Crohn's disease. Levels of inflammation markers, and levels of IL-23, IL-17, and serum calprotectin were higher with Crohn's disease Iraqi patients. However, more studies about cytokines and inflammation markers are needed.

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