Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 26s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Relationship Between Circulating Cytokines And Crohn'S Disease Development In Patients From Baghdad City

Khaleel I. Rashid[1], Eman S, Mahmood[2], Majeed J. Naef[3], Sarah K.yunis[4]

[1]phD, Al-Farabi University* Science and Technology center

Email ID: khilil.abrahim@alfarabiuc.edu.iq

[2]phD, Al-Farabi University* Science and Technology center

[3]MsC, Al-Farabi University* Science and Technology center

[4]Al-Farabi University* Science and Technology center

[5]Senior teacher of the Department of Plant Protection and Quarantine of the Karakalpakstan Institute of Agriculture and Agrotechnologies, Doctor of Philosophy in Agriculture (PhD)

[6]Assistant teacher of the Department of Plant Protection and Quarantine, Karakalpakstan Institute of Agriculture and Agrotechnologies, Candidate of Agricultural Sciences

Cite this paper as: Shamuratova Nagima Genjemuratovna, (2025) Relationship Between Circulating Cytokines And Crohn'S Disease Development In Patients From Baghdad City. *Journal of Neonatal Surgery*, 14 (26s), 878-882.

ABSTRACT

This study was conducted in Baghdad city to evaluate the relationship between the circulatory cytokines association with crohn's disease. The cytokines levels and activity were studied on five cytokines; lL-23, IL-12, IL-6 and INF-γ, TNF-α. Circulating cytokines values were compared according to the disease activity in active and queisnt phases.

Results showed that IL-23 significantly the higher level as compared to other cytokines in CD patients, followed by INF-γ. The lower value was found in IL-2 as compared to others. In the other hand, cytok9nes activity in active and queicent phases in the CD patients IL23 showed the high value, where as, TMF-α was recorded the moderate value, and the lower value was recorded to IL-12 as compared to the active and quiescent phases. The differences were not statistically significant.

The study showed the association of some circulating cytokines and Crohn's disease in patients, further studies are needed to determine the effects of the cytokine IL-23 and INF- γ in management of CD and IBD diseases.

1. INTRODUCTION

Crohn's disease (CD) is an autoimmune disease that involves at least the intestinal mucosal immune system, when invaded by food or bacterial antigens. Crohn's disease is a relapsing systemic inflammatory disease which affecting the gastrointestinal tract with extraintestinal manifestations and associated immune disorders [1] .

Crohn's disease and ulcerative colitis (UC) are the two main components of inflammatory bowel disease (IBD). CD is mainly affecting the gastrointestinal tract and presents with abdominal pain, fever and clinical signs of bowel obstruction or diarrhea with blood or mucus [2].

In inflammatory bowel disease; Crohn's disease and ulcrative colitis, plasma and tissue concentrations of proinflammatory cytokines; tumer necrosis factor TNF, IN-Y, interleukin (IL)- 1β, IL-6 and IL-8 are increased. Interleukin-6 (IL-6) is a key mediator cytokinine of the immune response and a regulator of many pathological and physiological processes [3].

The upregulated proinflammatory cytokines include; Tumer necrosis factor TNFα, IFN-Y, IL-6 and IL-1. In contrast, inflammation-resolving cytokines such as IL-10 and TGF β,are also downregulated. Among key inflammatory cytokines involved in CD, IL-6 is a significant contributor to the inflammation and pathogenicity. Tumer necrosis factor is proinflammatory cytokines that lays an important role in pathogenesis of crohn's disease [4].

In crohn's disease there are healthy parts of the intestine mixed in between inflamed areas. Alcerative colitis is continuous inflammation in the colon, UC only affects the inner most lining of the colon whereas CD can occur in the all layers of the bowel walls. Ulcers form in place that the inflammation killed the cells lining the colon, the ulcers bleed and produce pus. UC is an inflammatory bowel disease (IBD) which is the general name for diseases that cause inflammation in the small intestine and colon [5].

TNF-α is a mediator of intestinal inflammatory processes, thus being one of the main cytokines involved in the pathogenesis of IBD, its levels are pre-Crohn's disease sent in the serum of IBD patients when measured [6].

IL-12 heterodimer was found to be increased in lamina propria mononuclear cells in CD but not in UC. IL-27 increased levels heterodimer have been reported in individuals with IBD [7].

The pathogenesis of IBD includes both genetic and environmental factors such as, smoking, unhealthy diet patterns, and major depression may contribute to its development [8]. Recently, increasing knowledge indicated that inflammation particularly cytokines is included in development of IBD [9].

The aim of this study is to evaluate the association between inflammatory Bowel disease (IBD), Crohn's disease (CD) and cytokines

Methodology

This study was conducted in Baghdad City at the period from 1 November 2024 to 1 September 2025 to assay the circulating cytokines levels and Crohn's disease development in 40 patients and 40 as control. Blood samples for routine laboratory tests and cytoknine analyses were obtained at baseline, serum and plasma aliquotes for cytokine measurements were ready.

I and 10 mlx 1n addition, Blood samples of age and sex matched controls were obtained for this experiment, and the control samples were used for the comparison of cytokine values.

Serum levels of Il-6, IL-12, IL-23, were measured using an enzyme linked 3x1 bottle and 6x1 bottle duplicate. Statistical analysis was done for all parameters to compare the results using P.value test.

2. MATERIALS REQUIRED AND SUPPLIED

The following material were required for this experiment:

Absorbent paper, distilled water, pipettes and pipette tips, ELIZA reader for measuring absorbance at 450 nm, and incubator at constant temperature.

The following materials were supplied:

1- Microelisa stripplate; 48 well kit and 96 well kit

2- Standards; S1- S6, red, pink, blue, green, yellow and white color respectively.

3- Sample diluent; blue color, 3ml x1 bottle and 6ml x1 bottle

4- HRP-Conjugate reagent; red color 5ml xl bottle and 10 ml x 1 bottle

5- 20 x wash solution; white color, 15 ml x 1 bottle, and 25 ml x 1 bottle

6- Stop solution; yellow color, 3ml x 1 bottle and 6 ml x 1 bottle

7- Chromogen solution A; purple color 3 ml x 1 bottle and 6 ml x 1 bottle

8- Chromogen B black or brown color; 3ml x 1 bottle and 6 ml x 1 bottle

9- Closure plate membranes; 2x pieces for 48 well kit and 96 well kit

10-Manual 1x paper for both 48 and 96 well kit

Sample Collection

Collection of serum, plasma, and whole blood as described bellow;

Serum: Collect and centrifuge at 3000 rpm for approximately 20 minutes, collect the supernatant carefully, and assay immediately.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant, Standard ()centrifuge the plasma at 3000 rpm for approximately 20 minutes, collect the supernatant carefully, and assay immediately.

Whole blood: Collect whole blood using EDTA or heparin as an anticoagulant, and using ultrasonication, Centrifuge the whole blood at 3000 rpm for approximately 20 minutes. Collect the supernatant carefully, and assay immediately.

Assay procedure

Step 1: Check the plate and equipment with no problem, allow the lables and the color of the covers of the vails and make sure no mistake.

Step 2: Allow the plate, all reagents and samples to come at roon temperature (18 C^{o} - 25 C^{o}) naturally before starting assay, without using hot water bath to heat the plate and samples.

Step 3: Remove the plate from the foil pouch. Return the unused strips to the foil pouch with the desiccant pack and reseal.

Step 4: Set Blank wells, set standard wells, set sample wells.

Step 5: Add 50 ul Standard (S1, S2, S3, S4, S5, S6) to corresponding Standard wells.

Add 50 ul sample to every sample well

Step 6: Add 100 ul HRP-Conjugate Reagent to every well except Blank well

Step 7: Cover the plate with a closure plate membrane and incubate for 60 minute at 37

Co.

Step 8: Wash all wells (including all Blank wells) 4 times.

Step 9: Add 50 ul Chromogen solution A to every well

Step 10: Add 50 ul Chromogen solution B to every well (protect chromogen solution B from light).

Step 11: Mix gently and incubate the plate for 15 minutes at 37C^{o} (protect the p0late from light)

Step 12: Add 50 ul Stop Solution to every well.

Step 13: Read the optical Density OD at 450 nm using an ELISA Reader within 15 minutes after adding Stop Solution.

Assay of circulating cytokine level

Serum level of IL-23, IL-12, IL-6 and INF-y were measured using enzyme linked immunosorbent assay (ELISA; Bioscience San Diego, USA)according to the manufacturer s instructions. The plasma TNF-a level was analyzed using quantitative immunoassays (Quantikine; R&D systems, Abingdon, UK). The sensitivity of the assay was 15 pg/ml for IL-23, 4 mg/ml for IL-12 and INF-y, 2 mg/ml for IL-6and 0.55 pg/ml for TNF-a. When the values of the assay were under the lower limit of detection, the circulating cytokine levels were considered to be null. The measurement of each assay was done in duplicate.

Statistical analysis

P-value was used with the parameteric data and clinical characteristics among the groups were compared. All of the statistica analyses were performed using the software package (SAS institute, Car, NC, USA). P-value < 0.05 was depended to be statistically significant.

Calculation of Results

1- Average the duplicate readings for each standard and sample to subtract average optical density of the blank (OD0).

Concentration	Blank	S1	S2	S3	S4	S5	S6	
Mean OD (450 nm)	OD0	OD1	OD2	OD3	OD4	OD5	OD^	

2- Use the professional curve fitting to make the standard curve (usually the standard curve is linear, quadratic and cubic curve) and calculate the level of the analyte.

3- If the higher OD value of the samples are higher than the highest OD of the standards, must dilute the sample with equal or double volume of sample diluent and repeat the assay again.**Result and Finding**

The parameters in Table (1) showed the clinical characteristics of the patients at baseline and controls. The patients were 40 divided into 27 male and 13 female with a mean of 29.2 as compared to the control 26 male and 14 female with a mean of 29,4.

Table (1): Parameters comparison between crohns disease patients and healthy

Parameters	**Patient (n=40)**	**Control (n=40)**
Age range **(Mean ± SD) years**	10-65 29.2	10-67 29.4
Sex	27 male 13 female	26 male 14 female
Duration of disease **(year+_ SD) years**	1-10 year (5.5 year)	

Site of involvement:		
Small intestine	7	
Small and large intestine	28	
Large intestine	5	
Smoking	19	17

The site of involvement; is small intestine, small and large intestine, and large intestine only which include 7, 28 and 5 of patients respectively. Smoking case include 19 patients and 17 for control. Results showed table (2) the comparison of the circulating cytokine levels between CD patients at baseline and control subjects.

Results in table (2) showed that IL-23 significantly showed the high level 115, as compared with other cytokines in the CD Patients followed by INF-y which recorded 99 and IL-6 recorded 75 and TNF-x recorded 60. The lower value was found in IL-12 which recorded 32 as compared to others.

Table (2): Comparison of Cytokines levels between crohn's disease patients and control (healthy)

Cytokines levels	**Patients**	**Control**	**p. value**
IL-23 (pg/m)	115 ± 25 90 – 140	8 ± 2.5 0 - 13.0	0.001
IL-12 (pg/m)	32 ± 10 30 - 45	14 ± 6 2 – 20	0.6
INF-y(pg/m)	99 ± 30 88 - 140	20 ± 5 5 – 19	0.007
IL-6 (pg/m)	75 ± 25 48 – 100	12 ± 7.0 0 – 22	0.003
TNF-X (pg/m)	60 ± 40 78 - 110	10 ± 9.0 4 – 23	0.005
CRP (MG/dl)	20 ± 15 15 - 39	20 ± 15.0 15 – 39	1-5 – 1.0 1 – 3.5

Table (3) showed the cytokines activity in active phase and in quiescent phase, in the CD patients; IL-23, INF-y and IL-6 were recorded the high value 100, 95 and 90 respectively. TNF-x showed moderate value 75 as compared to others. The lowest value 40 was recorded to Il-12.as compared to the active and quiescent phases. The differences were not statistically significant.

Table (3): Activity of cytokines; active phase and quiescent phase

Activity of cytokines	**Active phase**	**Quiescent -hase**	**p. value**
IL– 23 (Pg/m)	**100 ± 18.0**	**80 ± 270**	**0.002**
IL- 12 (Pg/m)	**40 ± 150**	**20 ± 8.5**	**0.2**
INF-Y (Pg/m)	**95 ± 220**	**70 ± 19.0**	**0.007**
IL-6 (Pg/m)	**90 ± 120**	**56 ± 18.0**	**0.005**
TNF-x (Pg/m)	**75 ± 100**	**52 ± 9.0**	**0.001**
CRP (mg/dl)	**33 ± 9.0**	**18 ± 12.0**	**0.001**

3. CONCLUSION

Results showed that IL-23 significantly showed the high level 115, as compared with other cytokines in the CD Patients followed by INF-y which recorded 99 and IL-6 recorded 75 and TNF-x recorded 60. The lower value was found in IL-12 which recorded 32 as compared to others.

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TNF-α has an inflammatory effect on inhibition of some cytokines through their effect of neutralization of TNF-α and blocked of its effect [8]. Close association of the cytokiness lL-23, lL-12, lL-6 with the pathogenesis of CD has been reported by [9], among various cytokines , the research chose TNF-α , INF-γ , IL-6, IL-12 and IL-23and assessed the profile in CD patients. Epidemiological studies have reported that circulating levels of cytokines . such as interleukin and monokine induced by interferon- gamma (MIG) were increase in IBD patients as compared to the control [10].

Other studies showed similar results elevated levels of IL-23 transcription and the increased expression of IL-23 of lamina propria mononuclear cells have been reported [11]. These results are in agreement with other studies in the same side of researches but they used another parameters such as genetic factors and treatment of CD patients [11, 12, 13].

Further researches must be done in the genetic effects of various cytokines on human health.

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