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Investigation of IL-23, IL-17 and Calprotectin Levels in Blood of Iraqi Patients with Ulcerative Colitis

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Abstract : Ulcerative colitis (UC) is a type of inflammatory disease of the colon and it causes heavy inflammation. Knowing about cytokines is very important due to their role in the progression of the disease and in decreasing its severity. The current study aims to estimate calprotectin, IL-17, and IL-23 in the blood of Iraqi patients with ulcerative colitis. Also measuring levels of inflammation markers including ESR, WBCs, and CRP, and the reason for its height in serum or blood of ulcerative colitis patients. In this study, 30 blood samples from Iraqi patients with UC and 30 blood samples from Iraqi healthy individuals as control have been collected from 2021/11 to 2022/4, the samples were collected from Medical City Hospitals, Gastro-Enterology and Hepatology Hospital in Baghdad, Iraq. Calprotectin, IL-23, and IL-17 were measured by the enzyme-linked immune-Sorbent assay technology (sandwich-ELISA method). The Biomarkers used in clinical practice to assess disease activity are complete blood count (CBC), c-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). The current study results are the incidence of ulcerative colitis between males and females is not statically significant, the mean age of diagnostic of ulcerative colitis is 33.93 ±2.59, and it has been noticed a high incidence of ulcerative colitis in Iraqi people who have medium economic status. It was concluded significant differences in levels of calprotectin, IL-17, and IL-23 compared to the control group. Also, higher levels of ESR, WBCs, and CRP in ulcerative colitis compared to the control group.

Keywords: Colon; ulcerative colitis; calprotectin; cytokines; IL-23, IL-17.

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Introduction

Ulcerative colitis (UC) is a disease that causes a lifetime inflammation of the colon, and it is considered one type of inflammatory bowel disease. Patients who got ulcerative colitis undergo symptoms like bloody stool, abdominal pain, and diarrhea. Some patients develop Acute Severe Ulcerative Colitis (ASUC), especially in developing countries which is a life-threatening condition (1). In 1859 Samuel Wilks was the first one who describes ulcerative colitis and compared to Crohn's disease it shows the affected people with ulcerative colitis are more than Crohn's disease. Every year there are 7.6–245 cases per 100,000 reported with ulcerative colitis (2). North America, the United Kingdom, Northern Europe, and Australia reported the highest cases of this disease (3). Intense ulcerative colitis lead to other diseases like colorectal cancer (4).

The cytokines are proteins that are secreted from different cells, these cytokines are important in communications between cells and play important role in UC pathogenicity. Cytokines including interleukins (IL), chemokines, and growth factors (5,6).

In the case of UC, the immune cells release IL-23 when the antigens enter the intestine layers. IL-23 stimulates T helper type 17 cells to produce IL-17, furthermore, IL-17 one of its jobs is to recruit neutrophils to the site of infection (7,8,9).

Calprotectin is located in granules of polynuclear neutrophils, it is a zinc and calcium-binding heterodimer of 36.5 kDs. It forms 5% of total proteins and 60% of cytosolic proteins in macrophages and monocytes cells. It is also expressed in a low concentration from epithelial cells. It has antimicrobial properties used as a biomarker not only for UC but also for many diseases such as kidney infection and early-stage of lung inflammation (10).

The cause of UC may be an interaction between genes such as a mutation in IL23R result overacting of the IL23R–Th17 pathway and environmental factors such as changes in intestinal microflora and food, which mean that the disease multifactorial syndrome (11,12).

The cytokines have been suggested they are the main cause of UC so it has been a research tool. The aim of this study is to investigate the levels of IL-23, IL-17, and serum calprotectin in the blood of UC Iraqi patients. As well as levels of C-reactive protein (CRP), Erythrocytes sedimentation rate (ESR), White blood cells (WBCs).

Materials and methods

Blood samples of thirty Iraqi patients have UC with a proven diagnosis based on consultants at the clinical, radiology, and endoscopy, also thirty blood samples from Iraqi healthy individuals as control have been collected and samples collected from 2021/11 to 2022/4 with ages ranging between 11-65. All samples were collected from Gastro-Enterology and Hepatology Teaching Hospital in Baghdad, Iraq. The patients were characterized according to the following parameters: age, gender, economic status, laboratory findings (white blood cell count; WBC, c-reactive protein; CRP, erythrocyte sedimentation rate; ESR)

Serum calprotectin, IL-17, and IL-23 were measured by using the sandwich enzyme-linked immunosorbent assay (Sunlog, China). ESR Fast Detector is used to measure Erythrocytes sedimentation rate, the ichromα CRP test is used to measure C-reactive protein, and hematology analyzer Diagon-Cell 60 is used to measure complete blood count (CBC).

Statistical analysis

The Statistical Analysis System-SAS (2012) program was used to detect the effect of difference factors in study parameters. Analysis of Variation-ANOVA was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability.

Results and discussion

Ulcerative colitis according to sex

The current study shows no significant differences in the incidence of UC between males and females as shown in table 1, which means UC can hit males and females an equal rate, these results are in an agreement with some studies in the Latin West including Iceland, Norway, Canada, the U.K, United States, Switzerland, and Australia (13,14). The reason for the equality in incidence between males and females is maybe the common habit of smoking in males, which

smoking has been shown as a protective factor for the development of UC (15).

Table (1): Distribution of samples study according to sex in ulcerative colitis patients, and the control groups.

Group	No	Male No. (%)	Female No. (%)	P-value
Ulcerative colitis	30	16 (53.33%)	14 (46.67%)	0.207 NS
Control	30	16 (53.33%)	14 (46.67%)	0.207 NS
P-value	--	0.392 NS	0.392 NS	---
* (P≤0.05), NS: Non-Significant.				

Ulcerative colitis according to the economic status

Compared with the poor and good economic status higher incidence of UC is shown in medium economic status as demonstrated in table 2. It perhaps, fresh meat and fresh food can be afforded by people who have good economic status, this type of food can decrease the risk of getting UC, people who have poor economic status do not have the ability to buy fresh food or fast food they eat what they have, and also most of the Iraqi people have medium economic status which this layer can afford to buy food that contains additives, food that contains a high percentage of fat, and canned meat that contains preservatives, and these types of food can increase the risk of getting UC, these results have agreement with (16,17).

Table (2): Distribution of samples study according to economic status in ulcerative colitis patients, and the control groups.

Group	No	Good No. (%)	Medium No. (%)	Poor No. (%)	P-value
Ulcerative colitis	30	7 (23.33%)	16 (53.33%)	7 (23.33%)	0.0073 **
Control	30	5 (16.67%)	20 (66.67%)	5 (16.67%)	0.0001 **
P-value	--	0.219 NS	0.0255 *	0.219 NS	---
* (P≤0.05), ** (P≤0.01).					

Ulcerative colitis according to age group

Table 3 shows the mean age of incidence is 33.93 ±2.59. Drinking alcohol, eating fast food, and ready-made food is common in young people, these factors it may have effect on the incidence of UC, and this agreement with this study (18).

Table (3): Comparison between ulcerative colitis patients, and the control groups in age

Group	Mean ± SE Age (year)
Ulcerative colitis	33.93 ±2.59
Control	27.63 ±1.30

Ulcerative colitis according to WBCs, ESR, and CRP

Ulcerative colitis has been established as an inflammatory disease so it is normal to see inflammation markers such as ESR, and CRP in abnormal ranges compared to healthy individuals. Maybe the reason for the high levels of CRP, and ESR is the destruction of the intestine wall, fungal and bacterial invasion, these results have in agreement with (19,20). An increase in the number of white blood cells can be observed compared to healthy people, and this may be due to the recruitment of neutrophils which is one type of white blood cells to the site of infection as in table 4 (21,22).

Table (4): Comparison between ulcerative colitis patients, and the control groups according to inflammatory biomarkers.

Group	Mean ± SE		
	WBCs ($\times 10^9$/l)	ESR (mm/h)	CRP (mg/l)
Ulcerative colitis	9.02 ±0.52	20.10 ±3.85	17.14 ±3.83
Control	6.35 ±0.19	5.20 ±0.23	4.18 ±0.32
P-value	0.0007	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).			

Ulcerative colitis and calprotectin in serum, IL-17, and IL-23

There are significant differences between ulcerative colitis and the control groups in calprotectin in serum, IL-17, and IL-23 as shown in table (5).

Cytokines like IL-23 and IL-17 probably show a role in UC progression. IL-23 can be secreted from many cells upon stimuli. The secretion of IL-23 can cause T helper type 17 cells to secret IL-17. The recruitment of neutrophils to the site of infection can be caused by many cytokines one of them is IL-17, due to neutrophils having an antibacterial effect, this is in agreement with what was found by (23, 24, 25, 26, 27). So in patients who have UC, we see higher IL-23, IL-17, and calprotectin in serum compared to healthy individuals.

Table (5): Comparison between ulcerative colitis patients, and the control groups in calprotectin in serum, IL-17, IL-23.

Group	Mean ± SE		
	Calprotectin in Serum (ng/ml)	IL-17 (pg/ml)	IL-23 (pg/ml)
Ulcerative	31.01±3.22	32.18 ±1.08	6.68 ±0.38
Control	12.99 ±0.91	21.39 ±1.18	4.02 ±0.31
P-value	0.0001	0.0001	0.0001
Means having with the different letters in same column differed significantly. ** (P≤0.01).			

Conclusion

Relationships in sex and economic state with the incidence of ulcerative colitis in Iraq, levels of IL-17, IL-23, and calprotectin in serum, and levels of inflammation markers are the conclusion of the current study. Further studies about inflammation markers and cytokines are needed.

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