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Assessment of Serum Interleukin-6 as a Reliable Biomarker for Disease Activity in Ulcerative Colitis

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Abstract

Background: **A chronic idiopathic inflammatory bowel disease of the colon, ulcerative colitis produces varied degrees of mucosal inflammation that extends from the rectum to the more proximal colon. Genetics, environmental factors, autoimmune, and gut microbiota are the main risk factors for ulcerative colitis. The diagnosis of Ulcerative Colitis is made using a combination of histology, endoscopic results, and clinical presentation. In addition to confirming the diagnosis of UC. There is significant evidence that UC patients' blood and inflamed mucosa have higher levels of interleukin-6.** ***Aim:*** **To establish new non-invasive alternatives for monitoring the disease activity in patients with ulcerative colitis.** ***Subjects and Methods:*** **Analytical cross-sectional study was conducted among 42 patients with known or newly diagnosed UC who was presented to the out-patient clinic or admit at the Suez Canal University hospital, Ismailia, Egypt. The diagnosis of UC was based on clinical, endoscopic, and histological criteria.** ***Results:*** **our study demonstrated that more than half of patients (52.4%) had moderately active UC, (28.6%) had severely active and about (14.3%) had mild activity when patients assessed clinically by P Mayo score with significant correlation between the circulating IL-6 level and UC activity in the studied patients (*P* value <0.001), the mean value of IL-6 was double higher in patients with moderate-severe activity compared to those with remission/mild activity (153 vs. 76 pg/ml).** ***Conclusion:*** **Our study concluded that IL-6 can be used as a significant biomarker for both disease activity, severity and extension by colonoscopy in patients with ulcerative colitis.**

Keywords: Inflammatory Bowel Disease, Disease Activity, Severity, Patient, Colonoscopy.

Introduction

The term "inflammatory bowel diseases" (IBD) refers to a group of chronic intestinal disorders that include Crohn's disease (CD) and ulcerative colitis (UC). These diseases are distinguished by their immune-mediated etiology and clinical relapsing course [(1)]. Although the precise cause of IBD is unknown, it is generally accepted that dysregulated enteric immune response, which is induced by reduced intestinal permeability, intestinal microbiota, and environmental factors in genetically predisposed individuals, contributes to the start of the disease [(2)].

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It is currently understood that several pro-inflammatory cytokines are crucial to the pathophysiology of IBD [3]. These cytokines influence the intestinal immune system by upregulating the expression of adhesion factors on endothelial cells, which permits phagocyte and lymphocyte transmigration to inflammatory sites [4].

A pleiotropic proinflammatory cytokine, IL-6 is essential for the etiology of IBD and explained several immunological responses that occurred as the disease progressed. Remarkably, IL-6 levels were discovered to be elevated years prior to an ulcerative colitis diagnosis [5, 6]. Soluble IL-6 receptor (sIL-6R) of IL-6 is primarily responsible for the proinflammatory effects of IL-6. The soluble IL-6R (sIL-6R) binds to IL-6, and the resulting IL-6–sIL-6R complex binds to the gp130 surface protein to trigger the production of pro-inflammatory cytokines by intestinal lamina propria T cells and prevents their planned cell death (apoptosis) [3].

Monoclonal antibodies intended to inhibit IL6R signaling may prove useful in the future for the treatment of IBD [7]. While the endoscopic examination remains the most dependable method for determining the activity of the IBD disease [8], it has a number of drawbacks such as more expensiveness, time-consuming, and even dangerous consequences. As a result, attempts are made at non-invasive endoscopic alternatives. According to published research, circulating IL-6 [9], intestinal mucosal IL-6 levels [10], and salivary IL-6 levels [11] have all been positively correlated with disease activity and can predict an individual's reaction to immunosuppressive treatment [12, 13]. Nonetheless, there is still disagreement on this matter [14, 15].

Patients and methods

Study Populations, area and data collection: Analytical cross-sectional study was conducted among 42 patients with known or newly diagnosed UC who was presented to the out-patient clinic or admit at the Suez Canal University hospital, Ismailia, Egypt, over the period from February 2023 to August 2023. The diagnosis of UC was based on clinical, endoscopic, and histological criteria. A structured interview-based questionnaire was used. Data collected from each patient included: socio-demographic characteristics general symptoms, GIT symptoms and Extra-intestinal manifestation. Disease activity was assessed using Partial Mayo score (PMS), CBC and ESR levels. In addition, Assessment of IL-6 levels using human IL-6 ELISA kits. Blood samples were withdrawn from patients under complete aseptic conditions; tube was allowed to clot for 30 min. then centrifuged at 3,000 RPM for 15min., and serum was stored at -80°C until IL-6 analysis by using the ELISA kits. Samples and standards were added together with second antibody labeled with biotin and ELISA solutions and put at 37°C for 60 min. to react. Then, the plate was washed five times. Chromogen solution A and B were added, Incubated for 10 minutes at 37 °C for color development. After that a stop solution was added and finally absorbance (OD) was measured and calculated to estimate IL-6 level.

Results

Patients' ages ranged from 18 to 65 years old. Males were more predominant (66.7%) than females (33.3%) (Table 1).

Table (1): The demographic data of the studied patients (n = 42)		
Demographic data	No.	%
Gender		
Male	28	66.7
Female	14	33.3
Age (year)		
Min. – Max.	18.0 – 65.0	
Mean ± SD.	40.74 ± 13.33	
Median (IQR)	40.0 (33.0 – 50.0)	
Residence		
Rural	10	23.8
Urban	32	76.2
Marital status		
Married	28	66.7
Single	11	26.2
Divorced/Widow	3	7.2
IQR: Inter quartile range SD: Standard deviation		

Table (1): shows socio-démographique data of the studied patients with (66.7%) were males and (33.3%) were females. The mean age of the study participants was 40.74 years.

With (11.9%) of patients were active smokers and (7.1%) were diabetic and hypertensive, with no evidence of autoimmune diseases (Table 2).

Table (2):Distribution of smoking and chronic illnesses among the studied cases (n = 42)		
	No.	%
Active smokers	5	11.9
Cardiac disease		
Hypertension	3	7.1
Ischemic heart disease	1	2.4
Valvular heart disease	1	2.4
Endocrinal disease		
Diabetes Mellitus	3	7.1
Hypothyroidism	1	2.4
Diabetes Mellitus, Hyperthyroidism	1	2.4
Liver disease (CLD)	3	7.1
Renal disease (CKD)	1	2.4
Neurological disease (history of intracranial hemorrhage)	1	2.4
Other autoimmune disease	0	0.0

Table (2): showing that only (11.9%) of patients were active smokers, (7.1%) were diabetic and hypertensive, with no evidence of autoimmune diseases.

more than half of patients (52.4%) had moderately active UC, (28.6%) had severely active and about (14.3%) had mild activity when patients assessed clinically by P Mayo score. On assessment of disease extension by Montreal score (based on colonoscopy), (47.6%) of population had extensive colitis (E3), (35.7%) had left sided colitis (E2) and only (16.7%) of patients had proctitis (Table 3).

Table (3): Distribution of the studied cases according to Partial (P) Mayo score for clinical assessment of UC activity and Montreal score for UC extension assessment by colonoscopy (n = 42)

	No.	%
P Mayo score		
Remission	2	4.8
Mild activity	6	14.3
Moderate activity	22	52.4
Severe activity	12	28.6
Montreal score		
E1 (UC proctitis)	7	16.7
E2 (Left-sided colitis)	15	35.7
E3 (Pancotitis)	20	47.6

Table (3): shows more than half of patients (52.4%) had moderately active UC, (28.6%) had severely active and about (14.3%) had mild activity when patients assessed clinically by P Mayo score. On assessment of disease extension by Montreal score (based on colonoscopy), (47.6%) of population had extensive colitis (E3), (35.7%) had left sided colitis (E2) and only (16.7%) of patients had proctitis.

The circulating IL-6 levels had significant correlation with disease activity in patients with UC with (*P* value <0.001), the mean value of IL-6 was double higher in patients with moderate-severe activity compared to those with remission/mild activity (153 vs. 76 pg/ml) (Table 4).

Table (4): Relation between IL-6 LEVEL and P Mayo score (Remission and mild activity vs. Moderate and severe activity) and disease extension by colonoscopy (n = 42)

	N	IL-6 LEVEL			*P*-value
		Min. – Max.	Mean ± SD.	Median	
P Mayo score					
Remission + mild activity	8	43.70 – 96.10	75.85 ± 16.99	80.70	<0.001*
Moderate + severe activity	34	70.90 – 350.0	152.94 ± 90.16	111.30	
Colonoscopy					
E1	7	43.70 – 347.0	236.44 ± 118.73	273.70	0.034*
E2	15	60.80 – 123.5	96.45 ± 18.15	96.10	
E3	20	70.90 – 350.0	135.24 ± 81.33	110.45	

SD: Standard deviation U: Mann Whitney test
p: p value for comparing between different categories
*: Statistically significant at p ≤ 0.05

Table (4): Showed significant correlation between the circulating IL-6 level and UC activity in the studied patients (P value <0.001), the mean value of IL-6 was double

higher in patients with moderate-severe activity compared to those with remission/mild activity (153 vs. 76 pg/ml). Additionally, the relation between IL-6 level and disease extension by colonoscopy were statistically significant with *P*-value (0.034). with cut point of circulating IL-6 level according was (87.8) pg/ml with sensitivity (88.24%) and specificity (75%) (Table 5).

Table (5):IL-6 Level to discriminate moderate/severe vs remission/mild activity

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
IL-6 Level	0.915	<0.001*	0.826 – 1.0	>87.8	88.24	75.0	93.7	60.0

AUC: Area Under a Curve p value: Probability value
CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value
*: Statistically significant at p ≤ 0.05

Table (5): shows that cut point of circulating IL-6 level according to ROC curve was (87.8) pg/ml with sensitivity (88.24%) and specificity (75%).

IL-6 level was (43.7 – 71.2) pg/ml in patients with remission while patients with mild, moderate and severe activity was (60.8 – 96.1) pg/ml, (70.9 – 347) pg/ml and (77.6 – 350) pg/ml respectively (Figure 1).

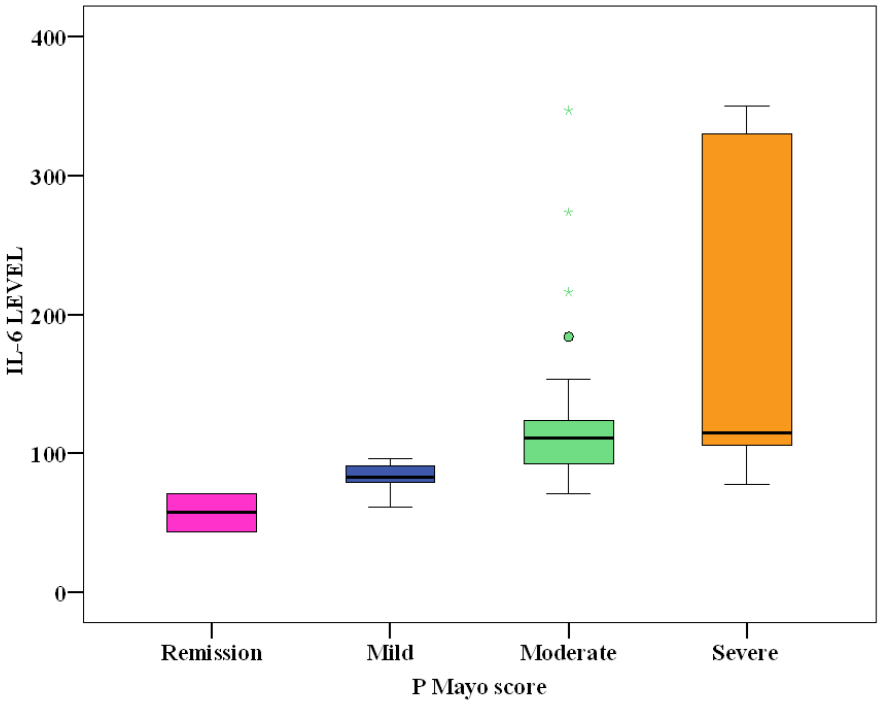

Figure (1): Relation between IL-6 LEVEL and P Mayo score (n = 42)

Figure (1): shows that patients in remission IL-6 level was (43.7 – 71.2) pg/ml while patients with mild, moderate and severe activity was (60.8 – 96.1) pg/ml, (70.9 – 347) pg/ml and (77.6 – 350) pg/ml respectively.

The relation between IL-6 level and disease extension by colonoscopy were statistically significant with *P*-value (0.034) (Table 4). IL-6 level in different disease extension, were (43.7 – 347) pg/ml, (60.8 – 123.5) pg/ml and (70.9 – 350) pg/ml in proctitis (E1), left sided colitis (E2) and pancolitis (E3) respectively (Figure 2).

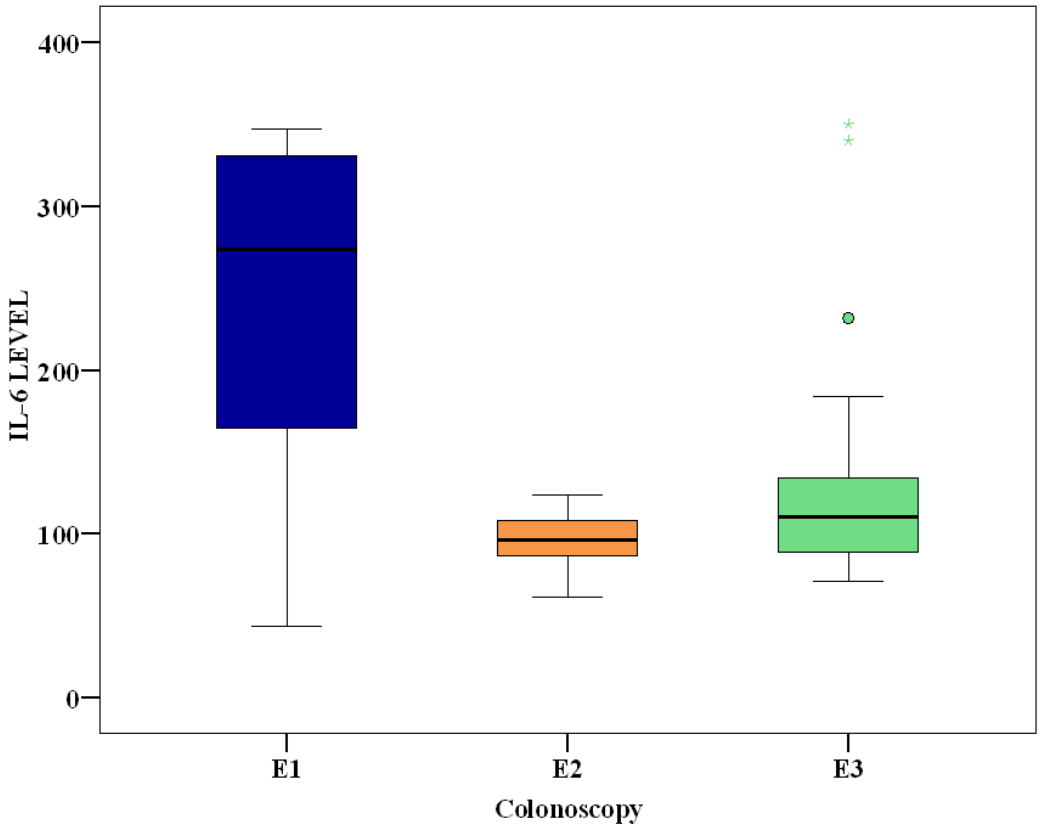

Figure (2): Relation between IL-6 LEVEL with colonoscopy (n = 42)

Figure (2): shows that IL-6 levels in different disease extension proctitis (E1), left sided colitis (E2) and pancolitis (E3) were (43.7 – 347) pg/ml, (60.8 – 123.5) pg/ml and (70.9 – 350) pg/ml respectively.

Discussion

IBDs are chronic diseases with activation and remission periods. It is required to find out noninvasive, easy, inexpensive, and accurate methods to evaluate the activity of the disease, decide therapy, and distinguish the IBD flairs, which have been noted in 20–30% of the patients suffering from IBD [16].

This study is an analytical cross-sectional study that included 42 patients with known or newly diagnosed UC presented to the out-patient clinic or were admitted at the Suez Canal University hospital aiming to assess the significance of circulating IL-6 levels as a biomarker of

clinical activity and severity in patients diagnosed with UC.

Regarding smoking, only (11.9%) of the participants studied were active smokers and it wasn't associated with clinical remission by univariate analysis with (*P*=0.954), which was in agreement with Mavropoulou et al., where (16.6 %) of their population were active smokers and (P=0.21) [17]. And to some extent to Chen et al., a study conducted in Taiwan to assess Smoking and disease outcomes of IBD, the smoking prevalence in UC patients was (20.9%) with no difference observed in the follow-up duration, medication use, and number of ER visits, disease severity, surgery rate, and laboratory data between smokers and non-smokers [18]. That was against the thought that smoking is protective against ulcerative colitis.

With regards to Distribution of the cases studied according to clinical disease activity, (52.4%) presented with moderate disease activity, this was strongly against results of Martinez-Fierro et al., and Mavropoulou et al., where (60.8%) and (69%) of their population, respectively, were in remission [17, 19]**.** that can be accounted for the level of health care provided and population awareness regarding the disease.

In our study, IL-6 content is elevated in plasma from patients with UC and increases with progression of the disease. ELISA was used to evaluate the level of circulating IL-6 in peripheral blood. IL-6 content in plasma from patients with moderate or severe activity (152.94 ± 90.16 pg/ml) was significantly higher than that of the patients with mild activity or remission (75.85 ± 16.99 pg/ml) with (*P*<0.001). Similarly in El-Zayyadi et al., IL-6 was significantly higher in active cases compared with inactive ones (395.43 vs. 133.17 pg/ml; *P*<0.001) [20]. And Li et al., a study that was conducted on 52 patients with UC in Jinzhou, China, IL-6 content in plasma from patients with active UC (110.5±5.1 pg/ml) was significantly higher than that in patients who were in remission (68.4±3.4 pg/ml) [21].

According to our results, circulating IL-6 level is a significant biomarker for disease activity and severity "clinically measured by P mayo score" in patient with ulcerative colitis with (P value <0.001 and 0.006) subsequently. that agrees with the results K. Mitsuyama et al., in which IL-6 level was significantly higher in patients with active UC than that of the healthy controls (P value <0.001). In addition, Serum IL-6 levels were determined during both the active phase and inactive phase after treatment. Comparison of individual levels before and after treatment revealed that the serum IL-6 level during the inactive phase was lower than that during the active phase (P value <0.01) [22]. And it corresponds with a cohort study in Netherlands, where clinical disease indices showed a significant correlation with serum IL-6 levels (P value <0.01) [23]. El-Zayyadi et al., also reported that IL-6 was significantly higher in cases compared with controls (P<0.001) and when it comes to IL-6 levels and disease activity IL-6 had significantly higher values in active UC cases compared with inactive ones (P<0.001) [20].

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