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ORIGINAL ARTICLE

Roles of Il-36 in the Pathogenesis of Inflammatory Bowel Disease in a Sample of Iraqi Patients

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ABSTRACT

Background: IBD (Inflammatory bowel disease) is a chronic inflammatory condition that affects the intestines., in which cytokines are thought to have a role in the etiology and pathophysiology. IBD is divided into two types: Ulcerative Colitis (UC), and Crohn's Disease (CD).

Aim of the study: The current study examined the level of, IL-36 in the blood of sixty IBD Iraqi patients (30 CD, 30 UC, and 30 HC). The concentrations were correlated with Age, age at onset, body mass index (BMI), cigarette-smoking status, disease duration, gender, symptoms, and extra-intestinal features.

Result: The findings revealed that IL-36 level for UC & CD patients have been significantly higher than the healthy control). However, there was a significant difference in IL-36 levels between UC and CD patients p. When UC and CD patients were divided into subgroups based on certain features, it was discovered that IL-36 was significantly increased in CD patients with disease duration >10 year compared to UC patients). In addition, It was in BMI underweight, CD patients significantly increased compared, to UC patient. while in CD patient there was a significant difference between patients with BMI underweight compared to patients with BMI obese). In the case of, non-smoker patients there was a significant increase in CD patients compared to UC).

Conclusion: the findings suggest that IL-36 has a role in the etiology & pathophysiology of IBD.

Keywords: IBD, CD, UC, HC, IL-36, Disease duration, Body mass index, cigarette-Smoking, healthy control, Crohn's Disease, Ulcerative Colitis, and Inflammatory Bowel Disease.

INTRODUCTION

Inflammatory Bowel Disease “IBD”, which comprises Ulcerative colitis (UC) & Crohn's Disease (CD), is an idiopathic intestinal inflammatory condition with idiopathic etiology and pathophysiology, that is thought to be an autoimmune disease brought on by a combination of causes (heredity, environment, and infection) [1-4]. UC is distinguished with a persistent pattern of inflammation in mucosa, and submucosa of colon, most commonly affecting the rectum and eventually affecting the whole colon. While the inflammation in CD can affect any portion of the digestive system, the terminal ileum, colon, and perianal area are the most common regions involved in a dis-continuous pattern. Superficial inflammatory changes restricted to the mucosa, as well as crypt inflammation and abscess, are all characteristics of UC. Whereas, Submucosal thickening; Transmural inflammation & Chronic granulomas, characterize CD [5] . Statistical data show that IBD incidence, has been increasing steadily, in developed nations and rapidly increasing in emerging countries, during the last few decades. In general, IBD is associated with excessive sugar, high fat, high pressure of life and serious pollution [6]. Inflammatory responses in the gastrointestinal mucosa are triggered by their interactions [7]. Immune cells have been demonstrated to release active compounds that are linked to the initiation and maintenance of inflammation, as well as causing damage to gut tissue. Excessive immune cell infiltration into the colonic mucosa has also been reported in IBD patients [8] . Furthermore, the pathophysiology of UC and CD has been related to altered cytokine regulation. [9, 10].

Interleukin (IL)-36 is one of these cytokines. The IL-36 subfamily is part of the (IL-1 superfamily), which had 4 isoforms: (IL-36alpha), (IL-36beta), (IL-36gamma) & (IL-36 receptor) (IL-36R) antagonist (IL-36Ra/IL36RN). IL-36α, IL-36 -β, IL-36 -γ promote infiltration of immune cell & inflammatory pathways via (IL-36R) activation, wherewas (IL-36Ra) acts as such an anti-inflammatory cytokine through blocking (IL-36R) signaling [11, 12]. IL-36, isoforms were found in a wide range of cell types & tissue, and their ultimate effects decided by a careful balance among the cellular targets, their concentrations, or the disease's stage and context [12-14] . As a result, IL-36 has been linked to a variety of inflammatory disorders, including psoriasis, arthritis, inflammatory bowel disease, joint disease, pulmonary and renal injuries, and even cancer. [15].

The present study is the first of its kind that aims to assess the serum levels of IL-36 in Iraqi patients with IBD, also the association of IL-36 with gender, disease duration, cigarette-smokinn and body mass index..

MATERIAL AND METHOD

Throughout, (October 2021-January2022). After obtaining approval from “the Iraqi Ministry of Health and Environment's Ethics Committee”. The study includes sixty IBD patients (30 CD, 30 UC) and thirty HC was conducted. For diagnosis and treatment, patients visited Baghdad Teaching Hospital's outpatient gastrointestinal clinics, as well as the “Gastroenterology and Hepatology Teaching Hospital”. Clinic experts made the diagnosis using: standard clinical, endoscopic, radiological, and histological criteria. Patients suffer from Indeterminate Colitis, or another autoimmune illness had been not allowed to participate. The following parameters were used to categorize the patients: Age, age at onset, cigarette-smoking status, disease duration, gender, BMI,

symptoms and extra-intestinal feature, as shown in Table-1. In the control group, healthy blood donors exhibited a negative anti-pathogen antibody profile in their serum. (Central Blood Bank, Baghdad).

Each participant had five milliliters of venous blood taken into a plain tube. At room temperature (18-25ºC), For 30 minutes, the blood was allowed to coagulate. In a chilled centrifuge (4 ºC), The samples were centrifuged about 3000 rpm for 10 min. The serum was kept in the freezer (20 °C) until the lab assessed the IL-36 level

Determination of Il-36 Serum Levels: Human IL-36 levels in the blood were measured using commercial ELISA kits, (MY BIO SOURCE, USA) and following the manufacturer's instructions. A micro-plate reader was used to detect absorbance at a wavelength of 405 nm (HumaReader HS, USA). Using an EXCEL sheet, a standard curve has been created & shown “measured absorbance, versus the concentration, of serially diluted standards”. The cytokine levels were calculated using the curve-fitting equation.

Statistical Analysis: The data was tabulated in a datasheet created using IBM SPSS version 25.0, which was utilized to conduct the statistical analysis. The number and percentage of categorical variables were reported, and significant differences were determined using Pearson's Chi-square test. Continuous variables were presented by mean and standard deviation and

significant differences were assessed using the analysis of variance (ANOVA) test followed by least significant difference (LSD) test and multiple ranges Duncan test. using analysis of variance (ANOVA) test followed by least significant difference (LSD) test and multiple ranges Duncan test. The area under the curve (AUC), 95 percent confidence interval (CI), cut-off value, sensitivity, and specificity were calculated using ROC curve analysis. Statistical significance was defined as a probability value (p) ≤ 0.05.

RESULTS

"Baseline characteristics of populations studied ": Regarding the features listed in Table-1, the mean age of patients with UC (29.9±7.2) years and CD (32.1±12.1) years, while in HC (27.4±4.6) years with no significant difference. The age at onset in UC (23.0±6.1) was lower than CD (25.7±9.6). The percentage of male and female were equal in each group. When patients and control divided according to cigarette-smoking status, the percentage of smoker was 20% and non-smoker was 80% in UC and CD patients, while in HC the smoker was 40% and non-smoker was 60%. As for disease duration 30% of patients with disease duration <5 year, 50% 5-10 year and 20% with duration > 10 year. When patients and their HC divided according to BMI, the percentage of UC patients was (10% underweight ,46.7% healthy weight, 26.7% overweight and 16.7% obese), in CD patients (20% underweight, 46.7% healthy weight, 16.7% overweight and 16.7% obese), while in HC (0%underweight, 46.6% healthy weight,13.3% overweight and 13.3% obese).

Table 1: The characteristics of patients with" Ulcerative Colitis" and "Crohn's Disease", as well as Healthy control, were compared.

Feature		UC (no.30)	CD (no.30)	HC (no.30)	P value
Age "year"		29.9±7.2	32±12.1	27.4±4.6	0.119
Age at onset		23.0±6.1	25.7±9.6	NA	0.198
Gender	Male	15(50)	15(50)	15(50)	1.000
	Female	15(50)	15(50)	15(50)	
Cigarette-smoking	Smoker	6(20)	6(20)	12(40)	0.129
	Non-smoker	24(80)	24(80)	18(60)	
Disease duration (year)	<5	9(30)	16(53.3)	NA	0.169
	5-10	15(50)	9(30)	NA	
	>10	6(20)	5(16.7)	NA	
Body mass index	Under weight	3(10)	6(20)	0	0.115
	Healthy weight	14(46.7)	14(46.7)	22(73.3)	
	Overweight	8(26.7)	5(16.7)	4(13.3)	
	Obese	5(16.7)	5(16.7)	4(13.3)	

* The data is presented as (Mean ±Standard Deviation), or an absolute value with a percentage in parenthesis. CD: Crohn's Disease, UC: Ulcerative Colitis, HC: Healthy Control P: Fisher's exact test probability, ANOVA "analysis of variance", LSD "Least Significant Difference", or Pearson's Chi-squared, NA: Does not apply.

Serum Levels of Il-36: In UC and CD patients, serum levels of IL-36 were significantly higher than in HC (306±143 and 384± 164 vs. 74 ± 8 pg/ml) respectively, (P <0.001), as in (Fig-1). However, CD patients had greater levels of IL-36 than UC patients, with a significant difference (P = 0.018).

IL-36's predictive value was discovered using ROC curve analysis. Cytokines took up in UC, a significant (AUC = 1.000), (95% CI = 1.000 -1.000), (p< 0.001), (Sensitivity = 96.9%), (Specificity = 100%), (Cut-off value = 96.9 pg/ml), while in CD (AUC = 0.999),(95% CI = 0.995-1.000),(p < 0.001), (Sensitivity = 96.7%), (Specificity = 96.7%), (Cut-off value = 93.0 pg/ml). As shown in the (Table- 2) and (Fig-2) and (Fig- 3).

*UC for "Ulcerative Colitis"; CD for" Crohn's Disease"; AUC for" area under the curve"; P for probability; & significant P is bolded.

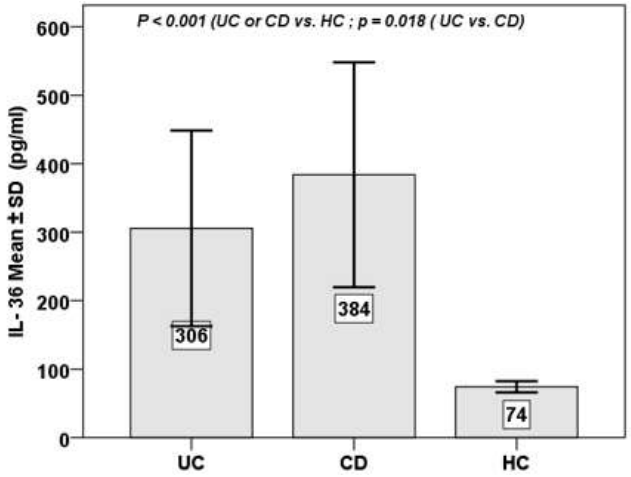

Figure-1: Mean± SD. IL-36 serum levels in Ulcerative Colitis (UC) and Crohn's Disease (CD) patients and HC group.

Table-2: IL-36 ROC curve study in patients with "Ulcerative Colitis" &" Crohn's Disease".

Group	(AUC)	(95% CI)	P value	Cut off value	Sensitivity; %	Specificity; %
UC	1.000	1.000-1.000	< 0.001	96.9	100	100
CD	0.999	0.995-1.000	< 0.001	93.0	96.7	96.7

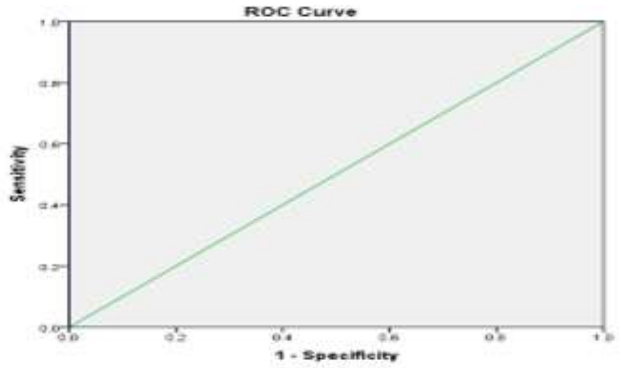

Figure-2: The ROC (receiver operating characteristic) curve for IL-36 levels, in Ulcerative Colitis (UC) patients, the area under the curve (AUC) is shown.

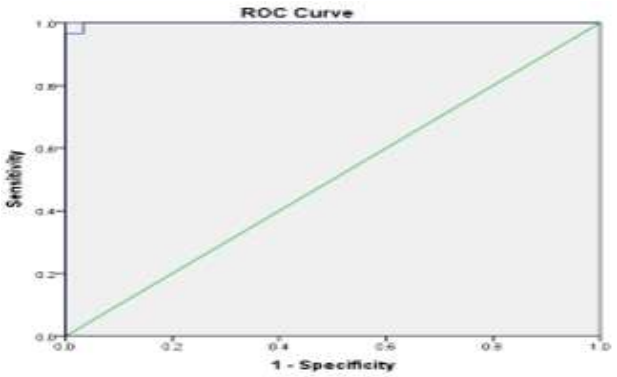

Figure-3: The ROC (receiver operating characteristic) curve for IL-36 levels, in Crohns Disease (CD) patients, the area under the curve (AUC) is shown.

IL-36 Levels and characteristics of patients: Serum IL-36 levels were initially measured in control subjects who were divided by gender, cigarette smoking status, and BMI. In the Healthy control

group, there's no statistically significant difference in IL-36 levels among males and females, smokers and nonsmokers, or healthy weight, overweight, or obese. AS in Table-2.

However, subdividing UC and CD patients based on the parameters listed in Table-1 indicated some substantial differences. There were no statistically significant differences between male and female patients. While, IL-36 Serum level in patient with disease duration > 10 year, was substantially higher in CD patients than in UC patients (485 ± 149 vs. 290 ± 159 pg/ml; p = 0.039), and IL-36 significantly increased in CD patients with BMI underweight compared to UC patients (493±147 vs. 250±104 pg/ml; p = 0.005), while in CD patients there was a significant difference between CD patients with BMI underweight compared to obese (493±147 vs. 235±147 pg/ml; p = 0.001). When comparing non-smoker UC patients to non-smoker CD patients, it was likewise considerably higher in CD (394±160vs. 300±132 pg/ml; p = 0.005), as shown in Table-3

Table 3: Serum IL-36 levels in control (HC) participants were dispersed according to gender, cigarette smoking, and BMI.

Characteristics		IL-36 Mean±SD (pg/ml)	P
Gender	Male (N=15)	67±8	0.938
	Female (N=15)	72±8	
Cigarette-smoking	Smoker (N= 12)	75±8	0.965
	Non-smoker (N=18)	73±8	
Body mass index	Under weight	NA	0.930
	Healthy weight	74±8	
	Overweight	77±12	
	Obese	71±9	

Table 4: The levels of IL-36 in patients with ulcerative colitis and Crohn's disease were distributed according to various features.

Features		IL-36 mean±SD (pg/ml		P
		UC (No.30)	CD (No.30)	
Gender	Male	294±148	367±180	0.121
	Female	317±142	401±151	0.067
	P	0.612	0.459	
Cigarette-smoking	Smoker	349±190	320±184	0.229
	Non-smoker	300±132	394±160	0.005
	P	0.405	0.178	
Body mass index	Under weight	250±104	493±147	0.005
	Healthy weight	333±153	385±160	0.266
	Overweight	298±137	372±147	0.310
	Obese	264±148	235±147	0.726
	P	0.724	0.653	
Disease duration (year)	< 5	296±74	329±156	0.608
	5-10	318±173	425±164	0.100
	>10	290±159	485±149	0.039
	P	0.111	0.053	

*P: ANOVA" (analysis of variance", LSD "Least Significant Difference", NA: Dose not apply, the letter" P" has been bolded to emphasize its significance, UC: Ulcerative Colitis, CD: Crohns Disease

DISCUSSION

The findings support the idea that IL-36 plays an important role in the etiology and pathophysiology of UC and CD. In both groups of patients, serum levels of IL-36 were significantly higher. There was also a substantial difference among UC vs CD patients. That might indicate that IL-36 is implicated in a unique inflammatory response mechanism that influences the pathophysiology of the both phenotypes of IBD. Roc analysis revealed the IL-36 had a significant AUC in patients with UC and, CD is augmented. IBD is caused by a mismatch between innate and adaptive immunity. T helper responses, which are characterized by a high concentration of pro-inflammatory cytokines, may be triggered by this imbalance [16] . According to research, new cytokines like IL-36 might be used to detect disease development. IBD patients' inflamed mucosa, notably in UC patients, mRNA expression levels of (IL-36α) and (IL-36γ), but not (IL-36β), were elevated [17] . In patients with active IBD, differentiation proteins of: IL-36α, IL-36β, IL-36γ, & IL-36Ra, macrophages; intestinal epithelial cells; CD8+T cells; and/or DCs, have also been overexpressed [18] . That suggests, IL-36 might be utilized, as an additional possible diagnostic for IBD activity, with variations in (IL-36β) activity being utilized to differentiate between UC and active IBD. Despite, the lack of canonical protease cleavage sites in IL-36α and IL-36γ, several extracellular Neutrophil, were able to efficiently alter the activity of these cytokines by processing them. Multiple proteases were released into the extracellular space by activated neutrophils. Thus, multiple proteases were released into the extracellular space by activated neutrophils, these proteases may be, enzymatically converted, & activate IL-36R ligand if they were present locally [19] . Furthermore, via activating the CXC chemokines such as CXCL1, CXCL2, CXCL3, ect, and also acute- phase proteins, IL-36α and IL-36γ may have a pro-inflammatory role in the pathogenic process of IBD (Nishida et al., 2016). IL-36Ra has been shown to reduce inflammation via blocking receptor ligand binding. Thus, inhibiting IL-36R may have anti-inflammatory properties. Antifibrotic signals may also be produced [18] . Furthermore, IL-36R activation, which drives pro-inflammatory Th1 and Th9 responses, affected T cell differentiation. Simillary, the anti-inflammatory and pro-inflammatory Th9 cells of regulatory T cells are balanced via the IL-36R axis. [19] . IL-36R had been detected on: intestinal epithelial cells, CD4 + T lymphocytes, neutrophils, fibroblasts, in the lamina propria [20, 21] . IN term of function, (IL-36beta) induced rapid (CD4 + T lymphocyte) proliferation, indicating that IL-36R signaling is required for mucosal T cell activation. In experimental (DSS colitis), the level of IL-36alpha and IL-36gamma were raised [21]. The microflora was required for the induction of IL-36alpha, which was not seen in germ-free mice [21] . According to functional studies, revealed that impaired IL-36R signaling increased susceptibility to acute DSS colitis [21] . Within the intestinal mucosa of patients with UC, IL-36α and IL-36γ levels were higher; whereas, IL-36Ra levels were lower, suggesting a potential pathogenic role of IL-36R signalling in UC [18, 22]. IL-36α/γ protein expression is enhanced in the epithelial and lamina propria mononuclear cells (LPCs), in IBD patient' intestinal mucosal lesions, particular UC [17, 23]. The interaction of IL-36 with the gut microbiota has been studied because the epithelial barrier function is disturbed in inflammatory intestinal disorders. Germ-free mice do not generate IL-36 expression in response to DSS-induced injury, according to research [24]. IN addition, IL-36 signaling has been recognized as a significant cause of tissue fibrosis in IBD patients [21]. Higher levels of IL36A and type VI collagen were found in fibro stenotic CD, the stimulation of IL-36R in human fibroblasts led in expression of genes, which control tissue remodeling and fibrosis such as (cytokines & matrix-metalloproteinases), indicating that signaling of (IL-36R) signaling, development of fibrosis. In experimental colitis models, functional studies using an IL-36R neutralizing antibody indicate reduce of tissue inflammation and fibrosis (chronic DSS and TNBS colitis). In rats with chronic colitis, such therapy proved effective in lowering existing fibrosis, even at late stages. As a result, antibodies that block (IL36R) signaling could be useful for treating intestinal fibrosis in patients with IBD. To evaluate this hypothesis, more clinical study is required [25] . The study showed significant increase in IL-36 serum level in CD patient compared to UC patient with disease duration > 10 year. As for BMI, the serum level in CD patient significantly increase in patient with BMI underweight compared to patient with BMI obese, while there was no difference in CD patient. Also, there was a variation between non-smoker UC and non-smoker CD. No previous study targeted disease duration, cigarette-smoker and BMI in UC and CD patient with respect to the level of IL-36. Thus, this study might be the first in Iraq and in the area. IL-36 receptor agonists are elevated during chronic intestinal inflammation in mice and in human IBD, according to several studies [21, 23]. IL-36R signalling not just to cotrol the synthesis for pro-inflammatory cytokines, such as:,TNF-α, IFN-γ, IL-6 & IL-23, however, it could also regulate immune cell trafficking, and trigger the creation of extra factors that might lead to fibrosis. [26]. Blocking IL-36R signaling may help IBD patients in two ways.

During persistent intestinal inflammation, inhibiting IL-36R lowers inflammatory cytokines, for starters and secondly, intestinal fibrosis is the most prevalent complication of IBD, and there are presently no anti-fibrotic medications available. Because (IL-36) increases fibrosis/tissue remodelling mediators like matrix metalloproteinases “MMPs” & TGF-β, in mouse and also human myofibroblasts, in IBD patients, blocking IL-36R signaling may reduce intestinal fibrosis [26, 27].

Finally, the current findings show that IL-36 has a role in UC and CD pathogenesis and etiology. However, a sample size of the study was limited, increasing patients and healthy control will undoubtedly improve our understanding of the link between IL-36 and risk for both IBD phenotype.

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Conflict of Interest: There are no conflicts of interest declared by the authors.

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