The Role of Interleukine-33 in Inflammatory Bowel Disease

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ABSTRACT

Background: The inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are heterogenous chronic inflammatory disorders of the gastrointestinal tract. The most widely accepted etiopathogenic hypothesis for these disorders suggests an immune mediated process.

Objective: This study was performed to evaluate the role of interleukine-33 in pathogenesis of inflammatory bowel disease and to correlate their levels with the disease activity and/or severity.

Methods: Fifty five subjects with inflammatory bowel disease (41 ulcerative colitis patients and 14 Crohn's disease patients) their ages range from 16-65 years and 25 apparently healthy volunteers their ages and sexes were matched with the patients were participated in this study. Blood samples were collected from all patients and controls to assess serum concentrations of interleukine-33 by enzyme-linked immune-sorbent assay.

Results: The present data revealed that there was significant elevation (P<0.05) in serum levels of interleukine-33 in ulcerative colitis and Crohn's disease patients as compared with control group. Concerning the correlation between serum levels of interleukine-33 with clinical parameters, the current study revealed a positive correlation between serum interleukine -33 with the disease activity in ulcerative colitis (P=0.41). As regards to disease location, interleukine-33 was significantly higher (P<0.001) in ulcerative colitis patients with pan-colitis (E3) as compared to those patients with proctitis disease (E1) and CD of the colon (E2).

Conclusion: This study indicated that interleukine-33 might be a crucial mediator in the pathogenesis of inflammatory bowel disease.

Key words: Inflammatory bowel disease, IL-33, Ulcerative colitis.

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Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory disorder of the gastrointestinal tract that includes two entities, namely Crohn's disease (CD) and ulcerative colitis (UC). (IBD) refers to two highly related debilitating diseases of the digestive tract with similar clinical, pathological, and epidemiological features [1].

The exact cause of these diseases is unknown, but the latest researches suggest that they may be caused by a malfunction in the body's immune system. IBD has become one of the most common chronic inflammatory conditions only after rheumatoid arthritis, with millions of patients all over the world[2].Complex interactions between different cell types, effectors of both innate and adaptive immunity, regulate the inflammatory status within the intestinal mucosa. Pro- and anti-inflammatory cytokines represent key players in shaping this network and maintaining the communication among various cell types; balance among these mediators appears to be critical for gut immune homeostasis. In fact, a broad wealth of evidence demonstrates the importance of cytokine dysregulation in the onset of inflammatory conditions of the gastrointestinal tract. In particular, IBD, is characterized by a significant dysregulation of cytokine production, with, in general, an overabundance of pro-inflammatory mediators [3]. The IL-1 family of cytokines is constantly expanding, and, recently, new members have been identified and studied, such as IL-1F11, IL-1F6/8/9, IL-1F7, and IL1F10, respectively known as IL-33, IL-36, IL-37, and IL-38 [4]. To date, of these novel members, IL-33 is the best characterized in terms of function and biological effects since its initial description in 2005 [5]. However, controversy still exists as to its precise role in intestinal disorders, particularly in the development of IBD. Aims of the study were to investigate the role of IL-33 in the pathogenesis of IBD and to study the correlation between IL-33 and the severity of the disease.

Methods. Fifty five patients with IBD (41 UC patients and 14 CD patients) were enrolled in this study, their age range from 16 to 65 years. They were seeking treatment in the gastroenterology out patients clinic at Baghdad Teaching Hospital in Medical city and AL Kadmyia Teaching hospital in Baghdad from December 2012 to June 2013. The diagnosis of each case was established by clinical examination done by a specialist gastroenterology and confirmed by colonoscopy and biopsy investigations. The patients were divided into three groups: Group I consisted of 15 recently diagnosed UC patients (before treatment), while group II consisted of 26 patients on treatment and group III included14 patients with CD. All the cases had no complain of other chronic or systemic diseases.

Apparently twenty five healthy volunteers whose ages and gender were matched with patients group were considered as control (Negative finding endoscopy) . All of them received no treatment with no complaint of other chronic or systemic diseases; their age range was (18 to 64) years. Blood samples were collected from all patients and controls to assess serum concentrations of IL-33 by enzyme-linked immune-sorbent assay.

The ethical committee of College of Medicine/Al-Nahrain University approved this study, and all samples were obtained with informed consent in accordance with the Baghdad teaching hospital declaration and AL Kadmyia teaching hospital.

Statistical Analysis: Frequency distribution for selected variables was done first. Among the outcome quantitative variables were described by mean, standard deviation (SD), standard error (SE) and tested for statistical significance by t-test. The association between 2 categorical variables was assessed by Chi-square test. P value less than the 0.05 level of significance was considered statistically significant.

Results. The current results revealed no statistical

significant differences among the three studied groups according to age, as clearly shown in table 1, there were no significant differences (p>0.05) in the mean of age between healthy controls and UC, and between healthy control and CD group. In addition, this results also showed no statistical significant difference between the two groups of patients (p>0.05).

Regarding smoking habit the current data showed that there was statistical significant differences among the study groups (p<0.05), the higher percentage of smoking was found in CD patients (35.71%), while (24.39%) found in UC patients group. Furthermore; (2.44%) of UC patients had positive family history, whereas (7.14%) of CD patients showed positive family history, as shown in table 2.

Table 3 showed that half of patients with CD (50%) had penetrating behavior followed by 29% with inflammatory behavior and 21% with stenosing behavior. The anatomical location of the two diseases was clearly observed in table 4. For UC, 46% of patients with UC disease were located in left side of colon (E2), followed by 29% of patients with pan-colitis (E3) and 24% had proctitis disease (E1). For CD, 64% of patients with CD located in the colon (L2) followed by 28% of patients with ileocolonic (L3) and 7% had ileal disease (L1).

Distribution of UC patients according to disease activity were presented in table (5), about 39% of patients were grade 2, 29% were grade 1, 17% were grade 3 and the rest 15% were grade 4 of disease activity. With respect to treatment, results in table 5, indicate that 63% of UC patients were on treatment and the remaining 37% were as newly diagnosed patients.

Table 6, revealed a significant elevation (p<0.01) in the level of serum IL-33 in UC patients (28.47 ±2.97 pg/ml) as compared with healthy control group (15.43 ±1.95 pg/ml). Also, there was statistically significant increase (p<0.05) in the level of serum IL-33 in CD patients (30.92 ±6.27pg/ml) as compared with healthy group (15.43 ± 1.95 pg/ml). In contrast, there was no statistical significant difference (p>0.05) in the serum level of IL-33 between the two groups of patients (UC and CD).

Findings in tables 7 were showed that there were no significant differences in levels of IL-33 between smokers and non-smokers UC patients and CD patients, (p>0.05).

Regarding the serum levels of IL-33 according to disease behavior, location of disease and age at diagnosis in CD patients were summarized in tables (8 and 9). The present results were pointed out to that there was no significant difference (p>0.05) in serum levels of IL-33 in CD patients according to disease behavior and nor according to location of disease.

As anticipated, the current study revealed positive linear correlation between serum IL-33 and the activity of the disease (P<0.001). Table 10 showed that the serum level of IL-33in UC patients increased with advanced grade.

As regards to the location of disease table (11) showed that serum level of IL-33 was significantly higher (P<0.001) in patients with E3 (44.85±5.91) as compared to those patients with E1 and E2 (14.69±1.96 and 25.37±3.52 respectively).

Table 1: Descriptive characteristics of age in the studied groups.

	Study groups		
Age (years)	**Healthy control**	**UC**	**CD**
Maximum	64.00	65.00	60.00
Minimum	18.00	16.00	23.00
Mean	38.72	35.24	37.71
Median	39.00	32.00	38.00
Standard Error of Mean	2.28	2.09	3.04
p value	0.522^{NS}		
Control vs UC	0.275^{NS}		
Control vs CD	0.809^{NS}		
UC vs CD	0.523^{NS}		

NS= not significant difference (p>0.05).

Table 2: Distribution of smoking and family history in the studied groups.

		Study groups						P value
		Healthy control		UC		CD		
		Count	**%**	**Count**	**%**	**Count**	**%**	
Smoking	**No**	24	96.00%	31	75.61%	9	64.29%	0.036*
	Yes	1	4.00%	10	24.39%	5	35.71%	
	Total	25	100.00%	41	100.00%	14	100.00%	
Family history	**No**	25	100.00%	40	97.56%	13	92.86%	0.391^{NS}
	Yes	0	0.00%	1	2.44%	1	7.14%	
	Total	25	100.00%	41	100.00%	14	100.00%	

* = Significant difference (p≤0.05), NS= not significant difference (p>0.05).

Table 3: Frequency of CD according to disease behavior.

		CD	
		Count	**%**
Disease behavior	Inflammatory	4	29.00%
	Stenosing	3	21.43%
	Penetrating	7	50.00%
	Total	14	100.00%

Table 4: Frequency of UC patients and CD patients according to disease location.

		Study groups			
		UC		CD	
		Count	**%**	**Count**	**%**
Location of UC disease	E1	10	24.39%		
	E2	19	46.34%		
	E3	12	29.27%		
	Total	41	100.00%		
Location of CD disease	L1			1	7.14%
	L2			9	64.29%
	L3			4	28.57%
	Total			14	100.00%

Table 5: Distribution of UC patients according to disease activity.

Endoscopy grade	Newly diagnosed UC patients		UC patients on treatment		Total
	Count	%	Count	%	
Grade 1	5	33.33%	7	26.92%	12
Grade 2	2	13.33%	14	53.85%	16
Grade 3	5	33.33%	2	7.69%	7
Grade 4	3	20.00%	3	11.54%	6
Total	15	100.00%	26	100.00%	41

Table 6: Descriptive serum IL-33 level characteristics in the studied groups.

IL-33	Study groups		
	Healthy control	**UC**	**CD**
Mean	15.43	28.47	30.92
Median	12.50	19.80	17.37
Standard Error of Mean	1.95	2.97	6.27
Control vs UC	0.005*		
Control vs CD	0.010*		
UC vsCD	0.654^{NS}		

* = Significant difference (p≤0.05), NS= not significant difference (p>0.05).

Table 7: Serum IL-33 levels according to smoking in UC and CD patients.

		Smoking		P value
		No	Yes	
UC	IL-33	27.30±3.40	32.08±6.29	0.244 NS
CD	IL-33	26.87±8.57	38.23±8.54	0.065 NS

NS= not significant difference (p>0.05).

Table 8: Serum IL33 levels according disease behavior in CD patients.

	Disease behavior			
	Inflammatory	Stenosing	Penetrating	P value
IL-33	24.46±8.70	15.27±1.37	41.32±10.44	0.234 NS

NS= not significant difference (p>0.05).

Table 9: Serum IL33 levels according to Location of disease in CD patients.

	Location of disease CD			
	L1	L2	L3	P value
IL-33	13.40	28.31±6.81	41.19±15.90	0.526 NS

NS= not significant difference (p>0.05).

Table 10: Serum IL33 levels according to disease activity in UC disease patients.

	disease activity				P value
	Grade 1	Grade 2	Grade 3	Grade 4	
IL-33	18.72±2.09	27.46±4.57	33.89±9.07	44.31±9.3	0.041*

* = Significant difference (p≤0.05).

Table 11: Serum IL33 levels according to Location of disease in UC disease patients.

	Location of disease UC			
	E1	E2	E3	P value
IL-33	14.69±1.96	25.37±3.52	44.85±5.91	0.001*

* = Significant difference (p≤0.05).

Discussion. Fifty five patients with IBD were enrolled in the current study, a diagnosis of UC was more common than CD (74.5% and 25.4%, respectively) this result was consistent with other result reported by Porter *et al.*, [6], who found that UC more common than CD among IBD. According to gender, the current results denoted a slight predominance of males among UC (56.10%) than a female (43.90%) which is comparable with other Iraqi study conducted by Mahdi [7]. For CD patients this study found that the proportion of females and males were equal (50%), but in other study conducted by Zahir *et al.*, noticed that (54.6%) of CD patients were male and (45.4%) were female [8]. Furthermore; as regards to the location of disease, the current result revealed that 24% of UC patients had E1, 46%of patients with E2 and 29% had E3.For CD,7%of patients with CD had L1, while 64% of patients hadL2 and 28% of patients with L3. The present results are at variance with other results reported by Beltran and colleagues who observed that 28% of UC patients haveE1,14%had E2 and 57% withE3, where as the percentages of disease localization for CD were 28% had L1, 57% had L2 and 14% had L3 [9].

According to disease behavior, our data showed that half of patients with CD 50% had penetrating behavior, followed by 29% with inflammatory behavior and 21% with stenosing behavior. In contrast Díaz-Jiménezet al., pointed out to that 96.1% of CD patients were within inflammatory behavior and 3.9% with stenosing behavior, while there were no patients with penetrating behavior 0.0% in their stud[10].The discrepancies observed between various studies could be caused, in part, by the influence of sample size and patient selection.

Recently, the IL-33 has been recognized as crucial to the homeostasis of the epithelial inflammatory response, included within the intestinal epithelium .

The present work found a significant increase in serum level of IL-33 in UC and CD patients when compared to controls, these findings were in accordance with the observations of the previous researchers [9,11,12]. Pastorelli and associates showed that serum IL-33 levels were elevated in UC patients compared with controls[11].

On the other hand, other studies [9,13]. Denoted that serum concentrations of IL-33 were low or did not differ between UC patients and healthy controls. However, although Carriere and co-workers demonstrated expression of IL-33 in endothelial cells of CD intestine [14], subsequent studies have failed to demonstrate a significant role for IL-33 in CD [11, 15,16].

The differences observed between various studies could be caused, in part, by the influence of ethnicity and racial background, moreover, differences in methodology, sample size and patient selection could also have served as a source of discrepancy. It has been proposed that in UC, IL-33 may be released by injured epithelial cells to induce pro-inflammatory cytokines production (i.e. IL-1, IL-6, TNF-α, IL-5 and IL-13) through activation of signal transducer 2 ligand (ST2L) in mast cells, macrophages, eosinophils and neutrophils [17].

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