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Role of Interleukin - 1 alpha (IL-1α) and Regulatory T (Treg) Cells In The Pathogenesis of Inflammatory Bowel Disease

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Abstract:
Background: *Continuous effort has been paid to deeply understanding the pathophysiology of Inflammatory bowel diseases (IBD).It has been postulated that multiple factors such as genetic susceptibility, immunological and environmental factors are involved and generate a dysregulated response of the mucosal immune system toward intraluminal antigens. Accumulating evidence indicates that discrepancy between the number of T regulatory cells (Treg) in peripheral blood and in inflamed colonic mucosa has a central role and Interleukin (IL)-1 is a key mediator in the pathogenesis of IBD.*
Objective: *The aim of this study was to determine the role of Interleukin- 1 alpha (IL-1α) and T regulatory (Treg) cells in the pathogenesis of IBD.*
Study Design: *This study was conducted in internal medicine Department of El- Hussein university hospital, AL-Azhar University. In this study, 50 subjects were selected and divided into two groups; Group A (n=30) were patients with IBD and Group B (n=20) were healthy control group. The two groups were subjected to the following; full history taking, clinical evaluation, Abdominal ultrasonography, CBC with differential count, Liver function tests, renal function tests, C-reactive protein , ESR and serum level of both Treg cells and IL-1α . IL-1α was detected in serum by ELISA and (Treg) (FoxP3) cells by flow cytometry techniques. Colonoscopy with multiple biopsies and histopathological assessment was performed to group A only.*
Results: *Our results showed that the serum level of Treg cells was significantly decreased in Group A (3.16±2.26) in comparison to control group B (9.14±0.639) (P<0.001). moreover ,the serum level of Treg cells was significantly decreased in moderate to severe disease activity (1.755± 0.556) as compared with patients with mild disease activity (5.980±1.616) (P<0.001) . The present study also revealed that, the serum level of IL-1α was significantly increased in patients Group A (11.74±3.24) in comparison to control Group B (2.84±0.877) (P<0.001). Additionally, the level IL-1α was significantly higher in moderate to sever disease activity (13.750 ± 1.602) than patients with mild disease activity (7.720±1.289) (P<0.001).*
Conclusion: *The level of Treg cells and Interleukin- 1 alpha have an important role in the pathogenesis and propagation of the inflammatory response in IBD and had a dynamic changes in both remission and active disease stages. These results suggested that Treg cells and Interleukin- 1 alpha can be used as a diagnostic markers and a potential therapeutic targets for IBD.*
Keywords: *inflammatory bowel disease (IBD) . Interlukin 1 alpha (IL1 α). T regulatory cell (Treg).*

Introduction

Inflammatory bowel disease, an umbrella term for both Crohn's disease and ulcerative colitis, is thought to be caused by barrier disruption leading to a change in the intestinal flora and a consequent aberrant activation of the mucosal immune system [1]. Both ulcerative colitis and Crohn's disease result from interrelated genetic and environmental factors that are channeled through an abnormality in mucosal immune function [2]. Despite all the advances in understanding the pathophysiology of IBD, it's exact cause remain unknown. The most widely accepted hypothesis is that overly aggressive acquired (T-cell) immune response to a subset to commensal enteric bacteria develops in genetically susceptible hosts, and environmental factor precipitate the onset or reactivation of the disease [3]. Current etiologic theories concerning IBD focus on environmental triggers, genetic factors, and immunoregulatory defects and microbial exposure [4].Treg cells originate from thymus, being called natural Treg cells. These cells move to periphery to exert their roles [5]. In the periphery, these cells can be emigrated from thymus or differentiated in the local places [6].Thus, Treg cells can be classified into natural (nTreg) and induced Treg (iTreg) cells [7]. Accumulating evidence indicates that the imbalance of immunity, including a discrepancy between the number of Tregs in peripheral blood and in inflamed colonic mucosa, an excess of pro-inflammatory stimuli and an altered function of immunoregulatory cells has a central role in the pathogenesis of IBD [8].T regulatory (Treg) cells are critical for maintaining immune homeostasis and establishing tolerance to foreign, non-pathogenic antigens including those found in commensal bacteria and food [9]. Interleukin 1 is a polypeptide cytokine produced by various tissue cells' and has a variety of biological properties. It is akey mediator that is released by monocyte macrophages in inflammatory and immunological responses. Interleukin 1 acts locally by releasing prostaglandins, thromboxane, and platelet activating factor from the inflammatory cells, and systemically as a circulating hormone, it induces fever and the production of acute phase reactants by the liver [10]. The original members of the IL-1 super family are IL-1α, IL-1β, and the IL-1 Receptor antagonist (IL-1RA). IL-1α and -β are pro-inflammatory cytokines involved in immune defense against infection. Both IL-1α and IL-1β are produced by macrophages, monocytes, fibroblasts, and dendritic cells. They form an important part of the inflammatory response of the body against infection [11]. peripheral blood mononuclear cells obtained from patients with Crohn's disease were shown to produce in vitro high quantities of interleukin 1 compared with normal control cells [12]. Moreover, enhanced production of interleukin 1-beta was shown in colonic mononuclear cells isolated from patients with inflammatory bowel disease [13].

Subjects & Methods

In our study, fifty (50) subjects were selected from Outpatients clinic and Inpatients of internal medicine Department of El- Hussein university hospital, AL-Azhar University during the period from June 2013 to October 2015. This study has been performed in accordance with the ethical standards and all persons gave their informed consent prior to their inclusion in the study. The subjects were divided into two groups; Group A (n=30) (IBD group) and Group B (n=20) (healthy control). Inclusion criteria of Group A included; 30 patients with IBD diagnosed clinically and by colonoscopy then confirmed by histopathology. Exclusion criteria of this group included; Patients with history of autoimmune disease (RA, SLE , etc"), Patients with history of systemic chronic infections (lung abscess, pyelonephritis, etc"), Patients with history of malignancies, Patients with history of COPD, patients with complicated diabetes mellitus, and history of Smoking (as smoking triggers an immune-inflammatory response and associated with increased levels of inflammatory markers). The two groups were subjected to the followings; Full clinical examination, Full investigation included; Liver function tests (ALT, AST, and Serum Albumin), Kidney function tests (Creatinine, Blood

Urea),Complete blood count, Abdominal ultrasonography, C-reactive protein and ESR .Colonoscopy and histopathological assessment was done in group A Only. Assessment of disease activity in ulcerative colitis patients was done in correlation to mayo score disease activity index[14]. Assessment of disease activity in patients with Crohn's disease according to Crohn's disease ctivity index [15] and Crohn's disease endoscopic index of severity [16] and consequently patients with IBD were divided to mild, moderate and severe disease activity. Both groups were tested for serum level of interleukin -1α and Treg cells . IL-1α was detected in patients' serum by ELISA and Treg cell (CD3-CD4-CD25-FOXP3) was detected in patients' serum by flow cytometry techniques.

Statistical Methodology

Data was analyzed on an IBM personal computer using statistical package for science(SPSS) software computer program version 18. Description of all data in the form mean (M) and standard deviation (SD) for all quantitative variables was done. Frequency and percentage for all qualitative variables was calculated .comparison between quantitative variables was done using t-test to compare two groups and ANOVA(analysis of variance) to compare more than two groups. comparison of qualitative variables was done using chi-square test. Correlation coefficient also was done to find linear relation between different variables using r-test or sperman correlation coefficient. Significant level measured according to P value (propability), P>0.05 is insignificant, and P<0.05 is significant.

Results

The present study included 50 cases divided into two groups; Group A (n=30) were patients with IBD 6 female (20%) and 24 male (80%) and Group B (n=20) were healthy control group 12 male (60%) and 8 female (40%) with statistically significant difference (p<0.001) (table. 1). The age ranged from 18 to 65 years , The mean age of patients group A was (36.73 ±13.3) and the mean age of control group was (36.2±12.020) without statistically significance (p < 0.886) (table .2). In Group A 18 (60%) patients had UC while 12 (40%) had CD and 20 patients with IBD (66.66%) had moderate to sever disease activity 18 male and 2 female while 10 patients (33.33%) had mild disease activity 6 male and 4 female. The control group (Group B) included (20) normal persons 12 male (60%) and 8 female (40%) . As regard to inflammatory markers, our results discovered that there was highly statistically significant difference between patients groups A and control group B, in relation to C_reactive protein (CRP) level (P<0.001) with mean of CRP in group A (13.76±4.83) and group B (5.100±1.61) (table. 3) (Fig.1). Our study found that the serum level of IL-1α was significantly increased in patients group A (11.74±3.24) in comparison to control group B (2.84±0.877) with statistically significant difference (P<0.001) (table.4) (Fig.2). in addition to that IL-1α was significantly increased in moderate to sever disease activity (13.750 ± 1.602) more than patients with mild disease activity (7.720±1.289) with statistically significant difference (P<0.001) (table .5). Also Our study established that the level of Treg cells were significantly decreased in patients group A (3.16±2.26) in comparison to control group B (9.14±0.639) with statistically significant difference (P<0.001) (table .6) (Fig.3) . moreover , the level of Treg cells were significantly decreasd in moderate to sever disease activity (1.755± 0.556) as compared to patients with mild disease activity (5.980±1.616) with statistically significant difference (P<0.001) (table.7).

Table (1) Sex Distribution in patient and control groups

Sex		Groups	
		patients	control
female	N	6	8
	%	20.00	40.00
male	N%	24	12
	%	80.00	60
total	N	30	20
Chi-square	%		
	X^2	6.660	
	P value	p<0.001	

There was statistically significant difference between the two groups in relation to sex distribution (p<0.001).

Table (2) Age distribution in patient and control groups

	Age		T-test	
	Patients	Control	t	P-value
Range	23 - 65	23 - 65	0.145	0.886
Mean±SD	36.733 ± 13.232	36.200 ± 12.020		

There was no statistically significant difference between the two groups in relation to age distribution with p=0.886.

Table (3) CRP level Distribution in patient and control groups

	CRP		T-test	
	Patients	control	T	P-value
Range	7-25	2-8	8.441	<0.001
Mean±SD	13.767±4.384	5.100±1.619		

There was highly statistically significant difference between patients groups A and control group B, in relation to Creactive protein (CRP) level (P<0.001).

Figure (1) CRP level Distribution in patient and control groups

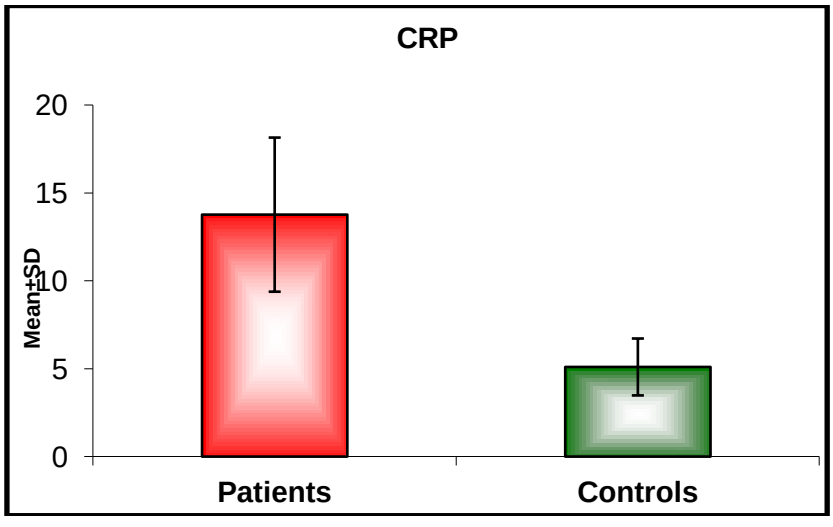

Table (4) I L1alpha level Distribution in patient and control groups

	IL1- alpha		T-test	
	patients	controls	T	p-value
Range	6.2-16	1.5-4.8	11.927	<0.001
Mean±SD	11.740±3.249	2.840±0.877		

The level of IL-1α was significantly increased in patients group A (11.74±3.24) in comparison to control group B (2.84±0.877) with statistically significant difference (P<0.001).

Figure (2) I L1 alpha level Distribution in patients and control groups

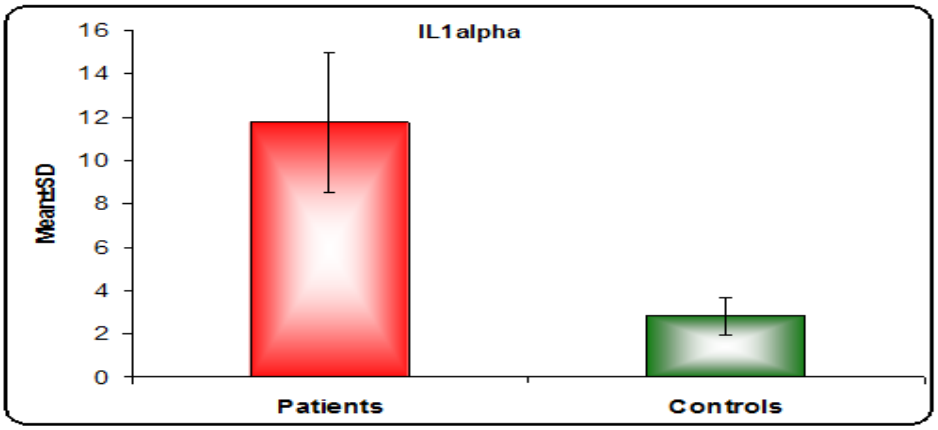

Table (5) IL1 alpha in mild,moderate and sever disease activity

	Mild	Moderate to sever	control	ANOVA			Tukey´s test	
	Mean±SD	Mean±SD	Mean±SD	F	p-value	P1	P2	P3
IL-1α	7.720±1.289	13.750±1.602	2.840±0.877	357.875	<0.001	<0.001	<0.001	<0.001

IL-1α was significantly increased in moderate to sever disease activity more than patients with mild disease activity with statistically significant difference (P<0.001).

Table.(6) T.reg cell level Distribution in patients and control groups

	Treg		T-test	
	patients	control	T	p-value
Range	1-9	8.1-10	11.489	<0.001
Mean±SD	3.163±2.262	9.145±0.639		

Treg cells were significantly decreased in patients group A in comparison to control group B with statistically significant difference (P<0.001).

Figure (3) T.reg cell level Distribution in patients and control groups

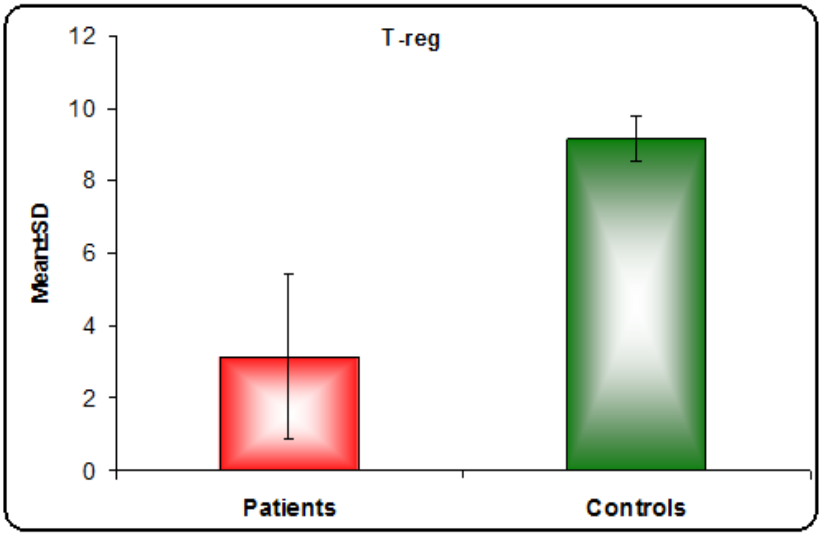

Table (7) Treg cell in mild,moderate and sever disease activity.

	mild	Moderate to sever	control	ANOVA		Tukey´test		
	Mean±SD	Mean±SD	Mean±SD	F	p-value	P1	P2	P3
Treg	5.980±1.616	1.755±0.556	9.145±0.639	347.022	<0.001	<0.001	<0.001	<0.001

The level of Treg cells were significantly decreasd in moderate to sever disease activity as compared to patients with mild disease activity with statistically significant difference (P-value<0.001

Discussion

Despite all the advances in understanding the pathophysiology of IBD, it is exact cause remain unknown. The most widely accepted hypothesis is that overly aggressive acquired (T-cell) immune response to a subset to commensal enteric bacteria develops in genetically susceptible hosts, and environmental factor precipitate the onset or reactivation of the disease. The intestinal mucosal tissues pose a unique challenge for the maintenance of immune homeostasis. Representing the largest mucosal surface area in the body, these tissues are in direct contact with the external environment and must simultaneously maintain tolerance to commensal bacteria and food, and the ability to eliminate pathogens [3]. Interleukin1 has been implicated as a major mediator in inflammatory and immunological responses [10]. Since immune as well as other tissue injury mechanisms may play a part in the pathogenesis of inflammatory bowel disease, the role of interleukin 1 as a key mediator may be of importance [17]. These observations suggest that IL-1 is one of the critical mediators of intestinal inflammation in IBD. The present study demonstrated that the serum level of IL1-α was elevated in IBD patients (11.740±3.249) as compared to healthy control (2.840±0.877) (p<0.001) , similarly it was significantly elevated among those with moderate to sever disease activity (13.750±1.602) as compared to patients with mild disease activity (7.720±1.289) (p<0.001). from these results we can concluded that the level of IL-1 α was increased in patients with IBD and The levels correlated with the degree of inflammation . the same results obtained by Satsangi et a1 [18] who found enhanced spontaneous interleukin 1 production by blood mononuclear Cells in Crohn's disease and increased Lipopolytsaccharaide stimulated production Of blood mononuclear interleukin 1 in Crohn's Disease and in ulcerative colitis. furthermore Our results was compatible with the study of Periklis Vounotrypidis et al [19] who investigated the associations of the proinflammatory cytokine I L-1 in treated

patients with inflammatory bowel disease (IBD) and the enteropathic seronegative spondylarthritis (eSpA). In this study Thirty four patients with Crohn's disease (CD), 26 with ulcerative colitis (UC) and 14 patients with SpA were participated. IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra) were measured by ELISA. The results showed that Active and inactive CD significantly differ on IL-1α levels (11.2 vs. 3.9 pg/ml; p = 0.034). Active and inactive UC significantly differ on IL-1β (3.7 vs. 2.3 pg/ml; p = 0.054) and IL-1Ra levels (15.9 vs. 12.7 pg/ml; p = 0.023). they concluded that IL-1α is associated with CD activity, while IL-1β and IL-1Ra are associated with UC activity in treated patients with IBD. Therefore The results of the present study suggested that interleukin1α may have an important role in the pathogenesis and propagation of the inflammatory response in ulcerative colitis and in Crohn's disease and its inhibition by specific agents may bear a potential therapeutic benefit. Moreover, it may help as a diagnostic marker in patients with active IBD. Regulatory T cells (Treg) are a subpopulation of T cells including CD4+CD25+ forkhead box P3 (Foxp3) + T cells, Tr1, Th3, and CD8+ Tregs. They serve to suppress the immune system and maintain self-tolerance. Inappropriate T cell response to intestinal microflora has a role in the pathological process of inflammatory bowel diseases [20]. Our data emphasized that the peripheral blood Treg cells was found to be significantly lower in IBD patients (3.16±2.26) as compared to healthy control (9.14±0.639) (p<0.001). moreover, Treg cells was significantly lower in patients with moderate to sever disease activity (1.755±0.556) as compared to patients with mild disease activity (5.980±1.616) (p<0.001) . from these results we can concluded that the level of Treg cells was decreased in patients with IBD and the levels correlated with the degree of inflammation. Our results was similar to the study of Frisullo et al [21] who found that The frequency of circulating Tregs was shown to be lower in patients with relapsing than in remitting autoimmune diseases. The same results obtained by Eastaff-Leung et al [22] who investigated the Treg and Th17 cell numbers in the peripheral blood of Crohn's disease, ulcerative colitis, coeliac disease and control subjects using multicolour, intracellular flow cytometry. A decrease in Treg cell numbers and an increase in Th17 cell numbers was observed in IBD, but not in coeliac disease. This suggested a disturbance in regulatory and effect or cell equilibrium. Furthermore, the excess of Th17 cells and deficiency of Tregs could contribute to the pathologies observed in IBD. Also the study of Maul et al [23] reported that CD4+CD25+ cells were decreased in the peripheral blood of IBD patients. Moreover, Wang et al [8] found the percentage of CD4+Foxp3+ Tregs in peripheral blood to be significantly lower in IBD (both ulcerative colitis and Crohn's disease) patients compared with healthy controls. However, at the mucosal level Maul et al [23] and Wang et al [8] found that the frequency of Tregs were higher in active and inactive IBD samples compared to healthy controls. The results of Wang et al [8] suggested that an insufficient number of Tregs in the peripheral blood may be associated with the recurrence of IBD. Their results and the results of Holmén et al [24] indicated that the increased frequency of CD25+ T cells in inflamed mucosa might be associated with the decreased number of circulating CD4+CD25+ T cells and explaining the lower frequency detected among patients with active IBD . Also our study was consistent with the study of *Suen JL et al* [25] who conducted that the level of Treg cell decrease in prepheral blood in patients with IBD thus Tregs represent a promising strategy for engineering tolerance to self and non-self antigens in chronic inflammatory bowel diseases. As regard to inflammatory markers, Our results showed increased acute phase reactant (ESR, CRP) in IBD and the level was increased with advanced activity, confirming previous studies and suggested as a beneficial tools for assesment and early detection of activity and early detection of drug response in IBD. Our results were compatible with the results of Fagan EA et al [26] who found that in CD,

serum levels of CRP correlate well with disease activity: median CRP is higher in severe CD compared with moderate CD which is on its turn higher than mild CD. For UC, the same trend can be observed, although CRP is overall much lower than in CD. The study by Fagan et al showed that both CRP and ESR correlated well with disease activity but the correlation was better for CRP.

Conclusion

Our study confirmed that the serum level of Treg cells and Interleukin- 1 alpha have an important role in the pathogenesis and propagation of the inflammatory response in IBD and had a dynamic changes in both remission and active disease stages. These results suggested that Treg cells and Interleukin- 1 alpha can be used as a diagnostic markers and a potential therapeutic targets for IBD.

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