Detection of Anti-Lactoferrin and Anti-Lysozyme in Ulcerative Colitis Patients

Abbas Jassim Mohammed (BSc, HD)[1]

Abstract

Background: Ulcerative colitis is an autoimmune inflammatory disease. Perinuclear anti-neutrophil cytoplasmic antibodies have been described in patients with inflammatory bowel disease, mainly ulcerative colitis, their role in pathogenesis and diagnostic value are still contentious.

Objective: This study targeted at evaluating the incidence of auto antibodies against lactoferrin and lysozyme which belongs to perinuclear antineutrophil cytoplasmic antibodies and measurement the levels of C-reactive protein ,Study the relationship between anti-lactorferrin and anti-lysozyme antibodies , Study the association between anti-lactorferrin, anti-lysozyme antibodies and C-reactive protein concentration among ulcerative colitis patients.

Patients and Methods: Fifty patients with ulcerative colitis be present at Al-Kadhimiya Teaching Hospital in Baghdad-Iraq and 25 healthy subjects were selected as the control group. Enzyme linked immune sorbent assay technique was used to detect the anti-lactoferrin and lysozyme antibody in sera of patients and healthy control groups and C- reactive protein was measured by qualitative and semi quantitative agglutination methods.

Results: The results of study have showed the male were more affected with UC than female with ratio (1.2:1). The study showed statistically important variances for anti-lactoferrin and lysozyme antibodies between patients and control groups. The present study shown that the (30%) and (38%) of ulcerative colitis patients were seropositive for anti-lactoferrin and lysozyme antibodies, respectively.

Conclusion: The prevalence of anti-lactoferrin and anti-lysozyme antibodies were similar to that reported in previous studies and their presence was associated with severity of ulcerative colitis .

Keywords: Ulcerative colitis, Lactoferrin, LysozymeC-reactive protein,Disease severity, ELISA.

Corresponding Author: abbasjassim79@outlook.com

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[1] Diyala Health Governorate -Diyala- Iraq.

Introduction

Ulcerative colitis (UC) a form of disease belonging to the so-called inflammatory bowel diseases (IBDs), is characterized by incessant inflammation of the intestinal lamina propria, starting from the rectum and involving the whole colonic mucosa. The course of UC is typically changeable; it is a chronic disease characterized by spontaneous transmittals and declines[1]. At present, its pathogenesis is still uncertain, but evidence

suggests that the disease occurs in genetically susceptible subjects, and it is trigged by environmental factors, which lead to an exaggerated and uncontrolled immune response[2].

The aetiology of ulcerative colitis is unknown although there is an plenty of theories and data involving genetic predisposition, immunologic alterations, infectious agents and other environmental factors[3]. Most of the clinical manifestations are associated to inflammation including symptoms of pain, diarrhea, hemorrhage and fever. As yet there is no intervention that prevents the disease nor there is a specific therapy that removes the causative factor, other than maybe surgical resection. Instead, medical therapy is directed at decreasing acute inflammation and treating its manifestations and impediments.

Even if the etiology and possible pathogenetic role of antineutrophil cytoplasmic antibodies (ANCAs) is unclear, they have been confirmed to be of importance in the diagnosis and controlling of patients with systemic vasculitides and Wegener's granulomatosis[6]. With a standard indirect immunofluorescence test, it has been found that ANCAs give two distinct patterns on ethanol-fixed human neutrophils- a cytoplasmic pattern (C-ANCA) or a perinuclear pattern (P-ANCA)[7].

The specific of active Wegener's granulomatosis is C-ANCA[8], whereas P-ANCA associates with systemic vasculitis and inflammatory bowel disease (IBD)[9].

A potential study was directed on the main following groups:

Patients group:A total of 50 patients with UC were studied under consultation(27men, 23women) and their ages ranged (25-65 years). The patients were categorized according to disease severity depending on Montreal classification into mild, moderate and severe[26].

Control group: The control group involved of 25 healthy subjects (14 men, 11 women) without any history of gastrointestinal or other diseases.

The range age was (30-60 years), the period of sample collection was from (July 2011-September 2011). A structure interview using a standard questionnaire was managed by interviewers with patients at their visit to Al-Kadhmiya Teaching Hospital in Baghdad-Iraq and entered the unit of the digestive system disease for endoscopy. The diagnostic conditions were consistent with previous studies of UC[28].

Patients and Methods

Three serological tests were done in this study including anti-lactoferrin antibodies, anti-lysozyme antibodies which were detected by Enzyme linked immune sorbent assay technique and C-reactive protein which was detected by semi quantitative and qualitative method of agglutination by making serial dilution for patient serum.

1-Enzyme Linked Immune Sorbent Assay kit for detection of auto antibodies against lactoferrin (IMMUCHEM-Belgium).

2-Enzyme Linked Immune Sorbent Assay kit for detection of antibodies against lysozyme (IMMUCHEM-Belgium).

3-Qualitative and semi quantitative method for detection

C-reactive protein by serial dilution (SPINREACT).

ELISA test for detection of anti-lactoferrin and lysozyme antibodies to ulcerative colitis patients serum. Qualitative and semi quantitative method for detection of

C-reactive protein to ulcerative colitis patients serum.

Statistical analysis

The suitable statistical methods were used in order to analyze and assess the results, they included the following: Chi-square (x2). Student test (t-test).

Note: The comparison of significant (P-value) in any test were:

S= significant difference (P<0.05)

HS= highly significant difference (P<0.01)

NS= non significant difference (P>0.05)

Results

Analysis of Anti-lactoferrin antibody results:

The anti-LF autoantibodies concentration ranged between (0.461-26.707u/ml) with mean (5.4036) of UC patients.

By using ELISA method, only 15 cases, 30% (15/50) of UC patient group were detected with the presence of anti-lactoferrin antibody, while control group showed 100% negative or within normal level results Ttable (1).

The results shown that 70% of the patients were negative for anti-lactoferrin antibody. The commonness of anti-lactoferrin antibody was not low and made the prevalence difference between UC and control group was significant P=0.006.

Table (1): Percentage of anti-lactoferrin antibodies in study groups**.**

Anti-lactoferrin Abs / Study group	Positive ≥10u/ml		Negative <10u/ml		Total
	No.	%	No.	%	
UC patients	15	30	35	70	50
Healthy control	0	0	25	100	25
X^2=7.636	df=1		P<0.006		

When the disease severity was taken into consideration as a factor affecting the occurrence of these antibodies, the antibodies increased as the severity or activity of disease increased Table (2).

Table (2): Percentage of anti-LF antibodies in patients according to the disease severity.

UC patient group	Total No.	Positive ≥10u/ml	Negative <10u/ml
Mild No.(%)	19	1(6.6)	18(51.5)
Moderate No.(%)	13	5(33.4)	8(22.8)
Severe No.(%)	18	9(60)	9(25.7)
Total	50	15(100%)	35(100%)
X^2=6.3	df=3	P<0.05	

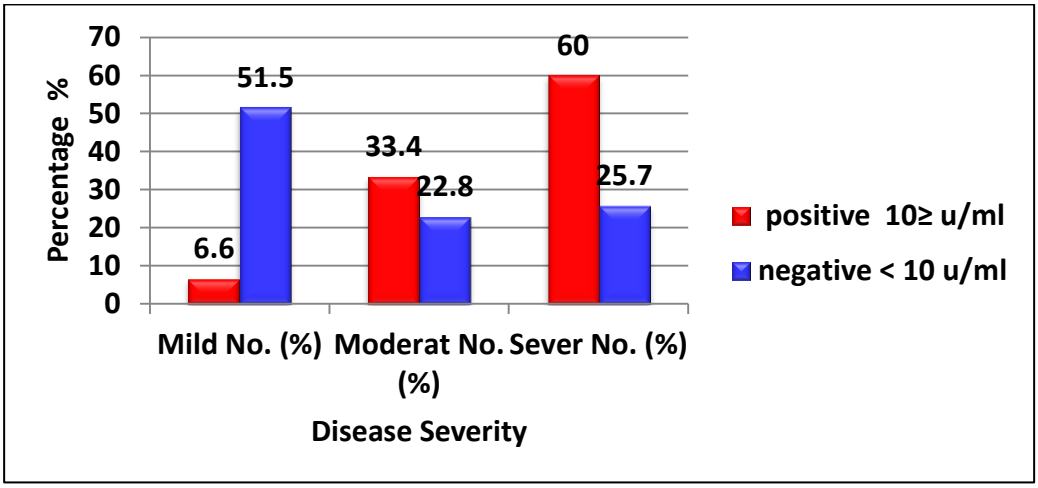

Figure (1): Percentage of anti lactoferrin antibodies in UC patient according to disease severity.

Influence of gender on anti-LF autoantibodies percentage in UC patients when gender was taken into concern the males showed more positive results 9/27(33.3%) of anti-LF autoantibodies than females 6/23(26.1%) in studied group. These difference were statistically not significant (P>0.05) as seen in Table (3).

Table (3): Distribution of anti-LF antibodies in UC patients according to gender.

Gender \ Results	Positive ≥10u/ml		Negative <10u/ml		Total
	No.	%	No.	%	
Males	9	60	18	51.4	27
Females	6	40	17	48.6	23
Total	15	100	35	100	50
X^2=1.28	df=1		P>0.05		

Analysis of anti-lysozyme antibody results

The anti-lysozyme autoantibodies concentration ranged between (0.491-23.383u/ml) with mean (6.5169) of UC patients. Among the UC patients, there were 19 cases 38% of patients found positive for anti-lysozyme antibodies. The occurrence of anti-lysozyme antibodies positive in UC patients were significantly higher than that of control (P<0.001).

Table (4): Percentage of anti-lysozyme antibodies in study groups.

Study groups \ Anti-lysozyme Abs	Positive ≥10u/ml		Negative <10u/ml		Total
	No.	%	No.	%	
UC patients	19	38	31	62	50
Healthy control	0	0	25	100	25
X^2=10.431	df=1		P<0.001		

Anti-lysozyme antibodies showed to be established in patients with severe state compared with other states as reported in Table (5).

Table (5): Percentage of anti-lysozyme antibodies in patients according to the disease severity.

UC patient group	Total No.	Positive ≥10u/ml	Negative <10u/ml
Mild No.(%)	19	1(5.3)	18(58.1)
Moderate No.(%)	13	5(26.3)	8(25.8)
Severe No.(%)	18	13(68.4)	5(16.1)
Total	50	19(100%)	31(100%)
X^2=4.33	df=2	P<0.05	

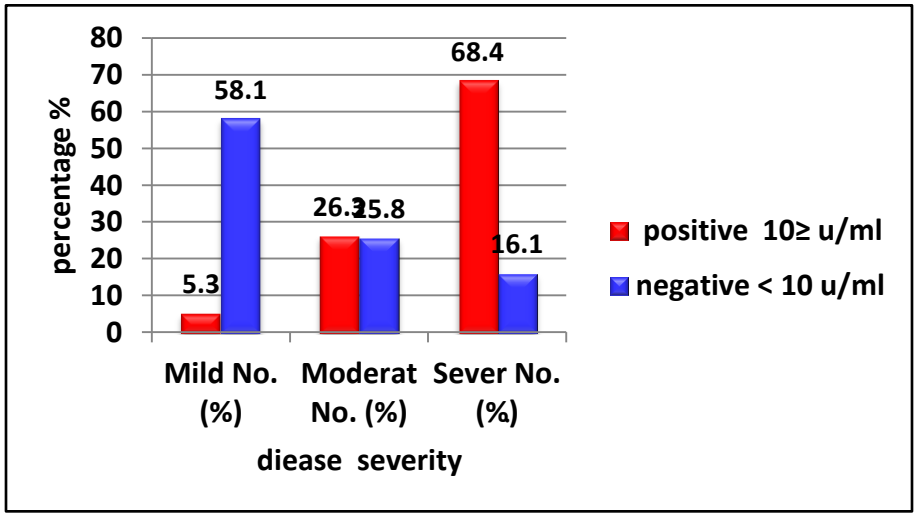

Figure (2) : Percentage of anti lysozyme antibodies in UC patient according to disease severity.

Regarding the gender of 27 male patients 11(57.9%) were seropositive for anti-lysozyme autoantibodies in comparison to female 8(42.1%) Table (6)**.**

Table (6): Distribution of anti-lysozyme antibodies in UC patients according to gender.

Results / Gender	Positive ≥10u/ml		Negative <10u/ml		Total
	No.	%	No.	%	
Males	11	57.9	16	51.6	27
Females	8	42.1	15	48.4	23
Total	19	100	31	100	50
X^2=1.4	df=1	P>0.05			

Analysis of C-reactive protein results

The C-reactive protein concentration ranged between (6-384mg/L) with mean (73.08) of UC patients. As shown in table (7), CRP results showed that most of patients (92%) were positive CRP test compared with the control group (0%). These differences were significant at P<0.05.

Table (7): Percentage of CRP in study groups.

CRP Percentage / Study group	Positive ≥6mg/L		Negative <6mg/L		Total
	No.	%	No.	%	
UC patients	46	92	4	8	50
Control	0	0	25	100	25
X^2=53.667	df=1		P<0.05		

When the disease severity was taken into thought as affecting the incidence of the CRP positivity, it was establish that the seropositivity for the CRP in severe patients (40%) which was more than moderate (25%) and close to mild group (35%). However, the data explained no significant difference (P>0.05) Table (8).

Table (8): Distribution of CRP in UC patients according to the disease severity.

Results / UC patient group	Total No.	Positive ≥6mg/L	Negative <6mg/L
Mild No.(%)	19	14(35)	5(50)
Moderate No.(%)	13	10(25)	3(30)
Severe No.(%)	18	16(40)	2(20)
Total	50	40(100%)	10(100%)
X^2=1.8	df=1	P>0.05	

When gender was taken into consideration, males 22(55%) showed more abnormal CRP than females 18(45%). However, statistical analysis showed no significant differences in Table (9).

Table (9): Distribution of CRP seropositive in study patients according to gender.

Results / Gender	Positive ≥6mg/L		Negative <6mg/L		Total
	No.	%	No.	%	
Males	22	55	6	60	27
Females	18	45	4	40	23
Total	40	100	10	100	50
X^2=1.34	df=1		P>0.05		

Discussion

The correlation of anti lactoferrin and lysozyme antibodies to IBD was first defined in 1990[9]. In most successions, 50-80% of patients with UC are anti lactoferrin and lysozyme antibodies positive[10]. The relationship of anti lactoferrin and lysozyme antibodies to disease activity and genetic susceptibility is less clear[23].

Several other neutrophil components were tested for their reaction with ANCA from patients with IBD by ELISA. Gender distribution in inflammatory bowel disease is dependent on the disease subtype, Crohn's disease or ulcerative colitis. In CD there is a greater prevalence of females, while in UC population-based studies have shown no

significant differences[18]. The present results showed male: female ration (1.2:1) which approved with previous study of Stonnington and his colleagues which displayed that the ratio of male: female was (1.4:1). The incidence of definite chronic UC among men was virtually twice the comparable rate for women[22]. The existent results were also in consistence with the described study which showed male to female ration was (1.2:1) for UC patients [27]. This difference may be due to specimen biases and epidemiologic studies on non-selected populations which may provide healthier figures.

The present study showed low prevalence of anti lactoferrin and anti lysozyme antibodies among patients with UC. These results agreed with similar pattern found in study of Brimnes et al. who tested UC sera with ELISA for antibodies against different neutrophil autoantigens and its result was close to the present study[24].

It was reported that (9-50%) of UC patients were positive for anti-lactoferrin antigens [27]. The frequency of P-ANCA (63.9%) in group UC patients was significantly higher than CD and control healthy groups in study [29]. Present results confirmed results of previous studies which showed that ANCA common in UC and that P-ANCA is main in patient IBD [20].

Experimentally, it has been presented that anti-lactoferrin antibodies can increase both the amount and period of hydroxyl radical formation[24]. Theoretically, one might expect that the presence of such auto antibodies can weaken the immune system[15].

Anti-lactoferrin auto antibodies may also stimulate inflammation by responding the anti-inflammatory effects of LF, exaggerate and delay mucosal inflammation made by numerous altered mechanisms, and the antibiotics may thus have pathogenetic significance equal though their happening does not appear to associate with disease activity[28]. The origin of anti LF auto antibodies is not known. Present results confirmed the presence of antibodies against lysozyme in serum by ELISA.. tests demonstrated that (0-53%) of sera UC patients were positive with anti-lysozyme antigens [11,13]. Antibodies to granulocyte antigen (ANCA) have been detected in serum samples from 50-80% of patients with UC and 10-40% of patients with CD[18].

Recommendations

To study the dominance of cathepsin G, elastase and Bactericidal permeability increasing protein (BPI) among UC patients, Recruiting a huge number of ulcerative colitis patients for measuring auto antibodies against lactoferrin and lysozyme, Using another method indirect immunoflourescent (IIF) to detect ANCA, Study the prevalence of P-ANCA for LF and lysozyme in UC and crohn's disease patients and Study the connection between P-ANCA and disease severity of UC patients for disease monitoring.

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