Frequency of Antineutrophil Cytoplasmic Antibodies (ANCA) in Some Autoimmune Diseases

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Abstract

Anti-Neutrophil Cytoplasmic Antibodies (ANCA) are a heterogeneous group of autoantibodies with a broad spectrum of clinically associated diseases. The diagnostic value is established for Proteinase 3 (PR3)-ANCA as well as Myeloperoxidase (MPO)-ANCA. To estimate the frequency of anti-neutrophile cytoplasmic antibodies (ANCA) in sera from a group of Iraqi patients with some autoimmune diseases compared with a healthy control group. Serum samples were collected from one hundred patient, ٤٧ males and 53 females; with age range of 16-70 years; 20 specimens from patients with systemic lupus erythematosus (SLE), 30 from patients with ulcerative colitis (UC), and 50 from patients with rheumatoid arthritis (RA). A group of 40 apparently healthy blood donors was included as controls. ANCA were checked using enzyme-linked immunosorbent assay (ELISA). Positive ANCA was detected in sera of ١٨ (18%) patients with autoimmune disorders. Anti-PR3 was detected in 6 (12%) patients with RA, and in 4(13.4%) patients with UC. Anti-MPO was detected in 3(6%) patients with RA and in 5(16.6%) patients with UC. All serum samples of patients with SLE showed negative ANCA. There were no ANCAs detected in sera from healthy individuals. Mean of serum anti-PR3 (U/ml) among the studied groups was 2.057 in RA, 2.209 in SLE, and 2.283 in UC, and 1.739 in control group. Statistical analysis revealed that differences in the anti-PR3 between RA, UC and controls were highly significant ($P < 0.01$), whereas just significant with SLE ($P< 0.05$). Mean of serum of anti-MPO (U/ml) among the studied groups was 0.711 in RA, 0.695 in SLE, and 1.170 in UC, and 0.652 in control group. Statistical analysis revealed that the differences in the anti-MPO between RA and SLE, controls were non significant ($P > 0.05$), whereas highly significance with UC ($P< 0.01$).It was concluded that ANCA markers might play a role in the inflammatory process and they are important factors for the clinical course, and prognosis in the patients with autoimmunity. However, ANCA in autoimmune disorders must be interpreted cautiously with particular attention paid to laboratory technique, the size, age and genetic background of the populations studied.

Key words: Rheumatoid Arthritis, Systemic Lupus Erythematosus, Ulcerative Colitis, Anti-neutrophile Cytoplasmic Antibodies (ANCA).

الخلاصة

الأجسام السايتوبلازمية المضادة للخلايا المتعادلة هي مجموعة مختلفة من الاجسام الذاتية ذات طيف واسع من الامراض المرتبطة سريريا .تم اجراء هذه الدراسة للفترة من تشرين الثاني ٢٠٠٦ لغاية شباط ٢٠٠٧ لغرض تقدير نسبة تكرار الاجسام السايتوبلازمية المضادة للخلايا المتعادلة (ANCA) في أمصال مجموعة من المرضى العراقيين المصابين ببعض الأمراض ذاتية المناعة مقارنة بالمجموعة الضابطة للأصحاء . تم جمع ١٠٠ عينة مصل من المرضى (٤٧ ذكور و ٥٣ أناث بمعدل عمري ١٦-٧٠ سنة) ، ٢٠ عينة من المصابين بداء الذئاب الحماموي المجموعي ، و٣٠ من المصابين بالتهاب القولون التقرحي ، و٥٠ عينة من المصابين بالتهاب المفاصل الرثوي . جمعت ٤٠ عينة مصل من بعض المتبرعين الأصحاء في مصرف الدم كمجموعة ضابطة . تم التحري عن وجود ANCA باستخدام تقنية المقايسة المناعية الإنزيمية (ELISA) . تم تشخيص هذه الاجسام المضادة في 18 (18%) من كل المرضى . ظهر anti-PR3 في ٦ (١٢%) من المصابين بالتهاب المفاصل الرثوي وفي ٤(١٣.٤%) في مرضى التهاب القولون . ظهر anti-MPO في ٣ (٦%) من مرضى التهاب المفاصل الرثوي ، و 5(16.6%) من مرضى التها ب القولون. كانت النتائج سالبة في داء الذئاب الحماموي المجموعي. لم تظهر هذه الاجسام في المجموعة الضابطة . ظهر من خلال هذه الدراسة ان متوسط anti-PR3 (وحدة / سم ^{٣}) بين المجاميع التي درست كان 2.057 في التهاب المفاصلالرثوي ، 2.209 في داء الذئاب الحماموي المجموعي ، و 2.283 في التهاب القولون التقرحي ، و1.739 في المجموعة الضابطة . أظهر التحليل الإحصائي بان الاختلاف في anti-PR3 بين التهاب المفاصل والتهاب القولون التقرحي مقارنة بالمجموعة الضابطة كان معنويا بشكل عال ($P<$ ٠.٠١) ، في حين كان الاختلاف معنويا فقط في داء الذئاب ($P<$ ٠.٠٥) . ظهر كذلك ان متوسط anti-MPO (وحدة / سم ^{٣}) كان 0.711 في التهاب المفاصل ، و 0.695 في داءالذئاب الحماموي ، و 1.170 في التهاب القولون التقرحي ، و 0.652 في المجموعة الضابطة . ظهر كذلك ان الاختلاف في النتائج بين التهاب المفاصل وداء الذئاب الحماموي ومجموعة السيطرة كان غير معنوي ($P<$ ٠.٠٥) في حين كان الاختلاف معنويا بشكل عال مع التهاب القولون التقرحي ($P<$ ٠.٠١) . يستنتج من هذه الدراسة بان الأجسام السايتوبلازمية المضادة للخلايا المتعادلة (ANCA) قد تلعب دورا في العملية الالتهابية وتحديد المسار السريري ومصير المرضى المصابين بامراض المناعة الذاتية . ولكن وجود هذه الاجسام المضادة يجب ان يشخص بحذر في امراض المناعة الذاتية مع تركيز الانتباه على تقنية الفحص و عدد واعمار المرضى وخلفيتهم الوراثية.

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Received : 3 / 3 / 2009
Accepted : 26/ 9 / 2009

Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are a heterogeneous group of autoantibodies with a broad spectrum of clinically associated diseases. Antineutrophil cytoplasmic antibodies (ANCA) have specificity for constituents of neutrophil granules[1] . There are two different subtypes of ANCA identifiable by indirect immunofluorescence method. One subtype is an antibody against myeloperoxidase (MPO), which stains in a perinuclear pattern (P-ANCA) indirect immunofluorescence (IIF) using a neutrophil substrate, and the other subtype is an antibody against proteinase-3(PR-3), which stains in a diffuse granular cytoplasmic pattern (C-ANCA) by IIF[2]. Antineutrophil cytoplasmic antibodies (ANCA) are mostly known as a useful diagnostic tool in patients with small-vessel vasculitis. With the accumulating knowledge of these autoantibodies, however, it becomes clear that the role of ANCA may not be only limited to a diagnosis of such disorders[3]. The current review addresses, in addition to classical diagnostic associations, other diseases connected with ANCA positivity, both in adults and in children[4]. The etiology of ANCA remains unknown, but still, the importance of both genetic and environmental factors is undoubted. The role of infection and chemicals in the etiology of ANCA-associated diseases is stressed in particular. A pathogenetic role of ANCA is suggested because of clinical observations based on the correlation of the vasculitis activity and the titer of ANCA[5]. Many experiments show the effects of ANCA in various steps of an inflammatory process, particularly on leukocyte microbicidal activity and oxidative burst. Recent findings are analyzed in the experimental field and they are correlated with clinical significance [6]. Many *in vitro* studies show that those ANCA have phlogistic potential, particularly at the interface of neutrophils and endothelial cells. A limited number of studies in experimental animals support their pathogenetic role. However, ANCA alone are not sufficient, as based on clinical and experimental data, and other, probably exogenous factors, seem necessary for disease induction and reactivation [7]. Data concerning the investigation of pathological and/or diagnostic role of ANCA in autoimmune disorders from Iraq are scarce. This study was designed to evaluate the frequency of ANCA in serum samples from a group of Iraqi patients with certain autoimmune disorders compared with apparently healthy controls.

Materials and Methods

This prospective controlled study was conducted during the period from November 2006 to February 2007. Blood samples were collected from one hundred patients with autoimmune disorders; 20 specimens from patients with systemic lupus erythematosus (SLE), 30 from patients with ulcerative colitis (UC), and 50 from patients with rheumatoid arthritis (RA). The study patients were admitted with approved and documented autoimmune diseases. A questionnaire form was formulated that involved name, age, gender, clinical history, disease type, disease duration, family history, residence, socio-economic status, general health condition, smoking, drinking, and any possible previous therapies. Patient specimens were gathered from Baghdad Teaching Hospital, and Gastrointestinal and Liver Diseases Center. A group of 40 apparently healthy blood donors was included as a control from National Bank for Blood Transfusion. Blood specimens from patients and healthy controls were properly conveyed to the location of processing and testing at the Department of Immunology, Central Laboratories for Public Health. The serum specimens were obtained and distributed in Eppendrof vials and saved in deep freezing at -20 C° until testing.

Anti-PR3 testing

Anti-PR3 (ELISA Anti-PR3 Kit, Biomaghreb, Tunisia) is an indirect solid phase enzyme immunometric assay. It is designed for the quantitative measurement of IgG class autoantibodies directed against proteinase 3 (PR3). The microplate is coated with highly purified proteinase 3(PR3). When autoantibodies exist, it will bind to the PR3 and form a complex. By adding the enzyme conjugate solution and its substrate, an enzymatic color reaction occur which can be read later. Four parameters fit with ling-long coordinates for optical density were used to calculate and convert the optical density readings. Cut off value for the studied parameters were normal when anti-PR3 Ab < 5 U/ml, and elevated when anti-PR3 Ab > 5 U/ml.

Anti-MPO testing

Anti-MPO (ELISA Anti-MPO Kit, Biomaghreb, Tunisia). It is designed for the quantitative measurement of IgG class autoantibodies directed against myeloperoxidase (MPO). In case of the presence of autoantibodies to MPO, there will be a combination with the highly purified myeloperoxidase that coat the microplate, which in turn form an immune complex and amplified when add the enzyme conjugate

solution and its substrate resulting in an enzymatic reaction that produce color. Four parameters fit with ling-long coordinates for optical density were used to calculate and convert the optical density readings. Cut off value for the studied parameters were normal when anti-MPO Ab < 5 U/ml, and elevated when anti-MPO Ab > 5 U/ml.

Statistical analysis

Descriptive statistics was used for frequencies and percentages. Inferential statistics was used in order to accept or reject the statistical hypotheses they include: Binomial test for testing the difference between two ratios related to binary nominal responding with pointed their *P*-values; Chi-square test for testing independency between the two categories factors in the contingency table with pointed their *P*-values, and ANOVA test for less significant differences (LSD).

Results

One hundred patients were included in this study, 47 (47%) males and 53 (53%) females. Distribution of patients among age groups showed that 29 (29%) patients were in 16-30 yrs age group, 53 (53%) patients were in 31-50 yrs age group, and 18 (18%) patients were in 51-70 yrs age group. Frequency of anti-PR3 and anti-MPO antibodies in sera from patients and apparently healthy controls was shown in table1. Positive ANCA was detected in sera of 18 (18%) patients with all studied autoimmune disorders. Anti-PR3 was detected in 6 (12%) patients with RA, and in 4(13.4%) patients with UC. Anti-MPO was detected only in 5(16.6%) patients with UC. Total serum samples of patients with SLE showed negative ANCA. There were no ANCAs detected in sera from healthy individuals

Table 1: Frequency of anti-PR3 and anti-MPO antibodies in sera from patients and apparently healthy controls

Studied groups	No.	Positive Anti-PR3 Abs		Positive Anti-MPO Abs		Total
		No.	%	No.	%	
Control	40	-	-	-	-	-
RA[1]	50	6	12%	3	6%	9
SLE[2]	20	-	-	-	-	-
UC	30	4	13.4%	5	16.6%	9
Total	140					18

[1:]RA: Rheumatoid arthritis, [2]SLE: Systemic lupus erythematosus, [3]UC: Ulcerative colitis .

The results shown in table 2 indicate that mean of anti-PR3 antibody levels in sera from patients and healthy controls did not rise above normal cut off value, although there were remarkable differences between patient and control antibody levels**.**

Table 2: Mean of anti-PR3 Ab levels (U/ml) in sera from patients and healthy controls

Studied groups	No.	Mean	SD[4]	Min.	Max.	ANOVA (f-test)	
						P-value	Sig.
Control	40	1.739	0.618	1.20	4.30	0.024	P< 0.05
RA[1]	50	2.057	0.836	0.15	5.70		
SLE[2]	20	2.209	0.637	1.45	3.50		
UC[3]	30	2.283	0.912	1.10	5.20		
Total	140						

[1:] RA: Rheumatoid arthritis, [2] SLE: Systemic lupus erythematosus, [3] UC: Ulcerative colitis, [4] SD: Standard deviation .

In table 3, comparison of significance of differences in anti-PR3 antibody levels among patient and control groups indicates that were highly significant differences between controls and RA and UC. On the other hand, a significant difference only was shown between control and SLE.

Table 3 : Comparison of significance of differences in anti-PR3 Ab levels among patients and control groups

Studied groups		LSD[4] (f-test)	
		P-value	Significance
Control	**RA[1]**	0.048	Highly Sig. (P< 0.01)
	SLE[2]	0.024	Sig. (P< 0.05)
	UC[3]	0.003	Highly Sig. (P< 0.01)
RA	**SLE**	0.449	Non Sig. (P> 0.05)
	UC	0.198	Non Sig. (P> 0.05)
SLE	**UC**	0.735	Non Sig. (P> 0.05)

[1:]RA: Rheumatoid arthritis, [2]SLE: Systemic lupus erythematosus, [3]UC: Ulcerative colitis, [4] LSD: Less significant difference

In table 4, the mean of anti-MPO antibody levels in sera from patients and healthy controls did not rise above normal cut off value, although there were remarkable differences between patient and control antibody levels.

Table 4 :Mean of anti-MPO Ab levels (U/ml) in sera from patients and healthy controls

Studied groups	No.	Mean	SD[4]	Min.	Max.	ANOVA (f-test)	
						P-value	Significance
Control	40	0.652	0.217	0.32	1.18	0.001	Highly sig. (P< 0.01)
RA[1]	50	0.711	0.294	0.47	5.20		
SLE[2]	20	0.695	0.278	0.32	1.50		
UC[3]	30	1.170	1.080	0.45	5.80		
Total	140						

[1:] RA: Rheumatoid arthritis, [2] SLE: Systemic lupus erythematosus, [3] UC: Ulcerative colitis, [4] SD: Standard deviation .

The results in table 5 show a comparison of significance of differences in anti-MPO antibody levels among patient and control groups that indicate non significant differences between controls and RA and SLE. On the other hand, a high significant difference only was shown between control and UC .

Table 5: Comparison of significance of differences in anti-MPO Ab levels among patients and control groups

Studied groups		LSD[4] (f-test)	
		P-value	Significance
Control	RA[1]	0.672	Non Sig. (P> 0.05)
	SLE[2]	0.814	Non Sig. (P> 0.05)
	UC[3]	0.001	Highly Sig. (P< 0.01)
RA	SLE	0.924	Non Sig. (P> 0.05)
	UC	0.003	Highly Sig. (P< 0.01)
SLE	UC	0.014	Sig. (P< 0.05)

[1:]RA: Rheumatoid arthritis, [2]SLE: Systemic lupus erythematosus, [3]UC: Ulcerative colitis, [4] LSD: Less significant difference

Discussion

Antineutrophil cytoplasmatic antibodies (ANCA) are a group of autoantibodies found in several inflammatory disorders in which they supposedly amplify the inflammatory process [8]. Anti-neutrophil cytoplasmic antibody (ANCA) tests are a routine clinical assay in most of Western World. The ANCA tests are being widely applied with very poor return. Guidelines for more effective usage are proposed [9]. Although substantial studies have been carried out for investigation of autoimmune disorders, data concerning the pathological and/or diagnostic role of ANCA from Iraq are scarce. These studies concerned with investigation of epidemiological and pathological aspects of autoimmune disorders rather than ANCA profiling. In this study, positive ANCA was detected in sera of 18 (18%) patients with autoimmune disorders. The low percentage of positive results detected by ANCA testing could be attributable to the limitations of our study, that's including: some of study patients were referred while they in remission stage rather than active phase of the diseases, the ELISA test was not coupled with indirect immunofluorescence technique (IIFT) due to the hospital policy, ANCA levels were not measured serially by quantitative image analysis, and the clinical relevance of the studied cases might be doubtful. Specific ELISAs for antibodies to PR3 and MPO are commercially available, and should be part of any standardized approach to the testing for ANCA. PR3-ANCA and MPO-ANCA are associated with substantially higher specificities and positive predictive values than the immunofluorescence patterns to which they usually correspond (C- and P-ANCA, respectively) [10] . It was reported that the use of the immunofluorescence method coupled with identification of ANCA sub-specificities by ELISA, is recommended for detection of ANCA in clinically suspected cases of small vessel and other systemic vasculitis [11]. Several studies suggested that ANCA titers correlated with disease activity. Serial measurements of PR3- and MPO-ANCA titers in patients with ANCA-associated vasculitis during remission can help predict relapses, and preemptive increases in immunosuppression following fourfold titer rises reduces the risk of relapses[12] In this study, it was found that in patients with ulcerative colitis, antineutrophil cytoplasmic antibody titers do not correlate with disease activity and there was four patients (13.4%) showed titer elevation for anti-PR3 and five (16.6%) for anti-MPO. In a study conducted in Baghdad on 2004 by Al-Badry et al, approximately the same result was recorded[13]. Several retrospective and prospective studies have suggested that the demonstration of ANCA lacks sensitivity and specificity, but these series have detected ANCA with neutrophil-indirect immunofluorescence alone and have included patients with inactive disease. In an Iraqi study carried out in Baghdad city it was confirmed that ANCA is not useful as a routine test for ulcerative colitis, rheumatoid arthritis and systemic lupus erythematosus and it is not specific for them [14]. The 'International Consensus Statement on Testing and Reporting ANCA' has been developed to optimize the clinical relevance of ANCA testing by the

adoption of standardized testing and reporting procedures. International collaborative efforts continue to focus on improving the tests for ANCA [15]. In ulcerative colitis, several studies reported that high titers of ANCA were particularly found in patients with active disease[16]. Other studies failed to detect a relation between disease activity and ANCA titer [17].

In the present study, the main findings of systemic lupus erythematosus were the total absence of elevation in ANCA above normal levels. Systemic lupus erythematosus known to be diagnosed with elevated antinuclear antibodies (ANA) rather than ANCA. ANCA testing remains unstandardized and there are no references for normal ranges. In sizing up the utility of ANCA testing in clinical practice, it must be borne in mind that most studies of ANCA serologies have been performed at tertiary care centers using research laboratories focused on ANCA testing. Translating the test characteristics from the environments of research laboratories to clinical practice must be done with caution. [18].

Conclusion: ANCA markers might play a role in the inflammatory process and they are important factors for the clinical course, and prognosis in the patients with autoimmunity. However, ANCA in autoimmune disorders must be interpreted cautiously with particular attention paid to laboratory technique , the size , age and genetic background of the populations studied . The results of ANCA testing by ELISA alone rema in controversial . Indirect immunofluorescence test, known as the "Gold Standard" for screening of ANCA, can be further substantiated by ELISA for confirmation and for identifying sub-specificities like anti-Myeloperoxidase (anti-MPO), anti-Proteinase 3 (anti-PR3) and anti-Lactoferrin (anti-LF.).

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