Comparison Between cANCA and pANCA In Patients with Renal Disease

Hiba Abid Al-Hussein Hassan (MSc)[1], **Athraa Zaidan Hassan**(PhD)[2]
Hind S. Jasim(PhD)[3] **and Nahla G.Abdul-majeed** (PhD)[4]

Abstract

Background: Renal involvement is immensely include in antineutrophil cytoplasmic autoantibody (ANCA)-associated systemic vasculitis .It is a significant cause of end-stage renal failure.

Objective: To comparison between cytoplasmic autoantibodies a cytoplasmic pattern and antineutrophil cytoplasmic autoantibodies a perinuclear pattern in patients with renal disease .

Patients and Methods: Prospective study reports presenting serological , hematological and biochemical investigations of 44 new patients diagnosed in teaching laboratories of Baghdad hospital from March 2015 to June 2016. All studied groups tested for hemaglobin (Hb), White blood cells (WBC), serum blood urea, Serum blood creatinine, c-reactive protein in addition to antineutrophil cytoplasmic autoantibodies a perinuclear pattern (p-ANCA) and antineutrophil cytoplasmic autoantibodies a cytoplasmic pattern (c-ANCA) detected by enzyme linked immunosorbent assay technique .

Results: All patients with renal disease had antineutrophil cytoplasmic autoantibody a cytoplasmic pattern negative whereas (27.3%) of those patients had positive antineutrophil cytoplasmic autoantibody a perinuclear pattern. Patients with age group range between (20-29) years showed (18.2%) pANCA positive results which mainly involved in female. Clinically evident systemic lupus erythematosus was present in 6 of the 12 patients with positive pANCA .

Conclusion: Serum anti-neutrophil cytoplasmic antibody measurement should not be used alone in the diagnosis of ANCA-associated disease, whereas pANCA is more convincing in the diagnosis than cANCA.

Key words: Antineutrophil cytoplasmic autoantibody, renal disease; enzyme linked immunosorbent assay.

Corresponding Author: hebaabdul@yahoo.com

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[1, 2] Department of Medical Laboratory Science Technology - Collage of Health and Medical Technology(MTU) - Baghdad-Iraq.
[3] Baquba Technical Institute (MTU)-Diyala-Iraq.
[4]Department of Immunology- Teaching laboratories of Baghdad hospital- Baghdad-Iraq.

Introduction

Antineutrophilcytoplasmic autoantibody are immunological markers of ANCA associated systemic vasculitides (AASV), that considered one of the most common multisystem autoimmune diseases[1].

Antineutrophil cytoplasmic autoantibodies are believed principle reason for vasculitis which can be associated by necrotizing granulomatosis[2].

Antineutrophil cytoplasmic autoantibody appear in two kinds, a cytoplasmic pattern (cANCA) and a perinuclear pattern (pANCA) according to the pattern of staining on the ethanol-fixed neutrophils and the main target antigen. ANCA concentration are usually detected by using Enzyme linked immunosobent assay and indirect immunofluorescence[3].

Neutrophils and also their products considered basic players in the appear autoimmune response and destruction of tissue in the vasculitic in addition granulomatous inflammation[4].

It has been found that many genetic and environmental factors lead to stimulation of ANCA-associated disease, and these factors have been effected on the pathological phenotype and clinical of disease. Furthermore These factors variable in patients such as, in a given patient Aetiological event may be an infection , a drug, impaired immune regulation or dysregulation of genomic expression of autoantigens, or combinations of these and other factors [2]. AAV classification involved both the specificity of ANCA antigen and the clinic pathological phenotype, for example MPO-ANCA MPA or PR3-ANCA MPA [5].

Many hypotheses have been involved in how developed ANCA associated disease There is may be contribution of genetic, especially in genes that control on the level of immune response in spite of genetic susceptibility usually combined with an environmental factor, some factors involved vaccination or exposure to silicates. Two mechanisms may be involved in ANCA development although these theories could not explain how the different ANCA specificities are developed, and there are several researchs still being undertaken on the development of ANCA [6].

The cause of ANCA (antineutrophil cytoplasmic antibodies) autoimmunity is not known and is related to be multifactorial. Infections may be stimulater formation of ANCA and some of the patients with infection-triggered ANCA develop ANCA-associated vasculitis [7].

Antineutrophil cytoplasmic antibodies (ANCA) may be useful diagnostic tools in the patients with systemic vasculitis and glomerulonephritis. The effect of the ANCA subtypes on the renal outcome and its associated to clinical features and demographic findings of patients with ANCA-associated glomerulonephritis have not been adequately studied [8]. So the current study aimed to comparison between cANCA and pANCA in patients with renal disease.

Materials and Methods

The present study, fouty four patients diagnosed by specialist as having renal disease who attended to the teaching laboratories of Baghdad hospital from march 2015 to June 2016.

All subjects were tested for Hb and WBC count done by asysmex SF-3000 automated hematology analyzer, general urine exam (microscopic examination), blood serum urea done by colorimetric kit from (BioSystem-Spain), serum creatinine done by colorimetric kit (BioSystem-Spain) ,C-Reactive protein test done by agglutination Diagnostic kit from (Biorbyt -United Kindom), p- ANCA done by MPO (p-ANCA) IgG ELISA kit from (Cat. No 1441-2, Accu Diag TM-United Kindom) and cANCA done by PR3(c-ANCA) ELISA kit from (Cat. No 1335-1, Accu DiagTM - United Kindom) .Serum samples were collected from patients and stored at (-20C) .

Statistical analysis

Data collected were analyzed by using the statistical package for social sciences (SPSS) version 19.0. Chi square test was used to test the significance of difference among variables; P values less than 0.05 was considered significant.

Results

The distribution of patients according to age groups is listed in table (1) below. It was cleared from table (1) that the all age group give negative results for cANCA while pANCA give (27.2%) of patients had positive pANCA with more percentage (18.2%) in age group range between (20-29)years and group that range between (30-39) years and (40+) years showed less percentage (4.5 %).

Table (1): Distribution of cANCA and pANCA patients according to age.

Age of patients/years		pANCA		cANCA	p-value
		+ve	-ve		
<19	Count	0	4	4	0.39 NS*
	% of Total	0.0%	9.1%	9.1%	
20-29	Count	8	14	22	
	% of Total	18.2%	31.8%	50.0%	
30-39	Count	2	12	14	
	% of Total	4.5%	27.3%	31.8%	
>40	Count	2	2	4	
	% of Total	4.5%	4.5%	9.1%	
Total	Count	12	32	44	
	% of Total	27.3%	72.7%	100.0%	

NS* = Non significant

The data demonstrated by table (2) show the distribution of studied groups according to gender with predominance of the percentage of positive pANCA in female patients 10(22.7%) than male patients 2(4.5%) .

Table (2): Distribution of cANCA and pANCA patients according to gender.

Gender		pANCA		cANCA -ve	p-value
		+ve	-ve		
Male	Count	2	14	16	0.092 NS*
	% of Total	4.5%	31.8%	36.4%	
Female	Count	10	18	28	
	% of Total	22.7%	40.9%	63.6%	
Total	Count	12	32	44	
	% of Total	27.3%	72.7%	100.0%	

NS* = Non significant

Data illustrated by table (3) clearly show a high increased in the percentage of positive pANCA in 6 patients with SLE (50%) and also positive pANCA in 6(50 %) in patients with nephrotic syndrome.

Table (3): Distribution of cANCA and pANCA patients according to type of disease.

Type of kidney disease		pANCA		c ANCA -ve	p-value
		+ve	-ve		
SLE	Count	6	0	6	p<0.001 HS*
	% of Total	50 %	0.0%	13.6%	
nephrotic syndrome	Count	6	24	30	
	% of Total	50 %	54.5%	68.2%	
Acute glomerularnephritis	Count	0	2	2	
	% of Total	0.0%	4.5%	4.5%	
Glomerulonephritis	Count	0	4	4	
	% of Total	0.0%	9.1%	9.1%	
Nephritis	Count	0	2	2	
	% of Total	0.0%	4.5%	4.5%	
Total	Count	12	32	44	
	% of Total	27.3%	72.7%	100.0%	

significant HS*= Highly

It was clear from the table (4) that 34(77.3%) patients with negative cANCA had CRP positive results, while showed 8 (18.2%) of patients had positive pANCA and positive CRP results compared with other negative results.

Table (4): Distribution of cANCA and pANCA patients according to CRP*test .

C-reactive protein		pANCA		cANCA	p-value
		+ve	-ve	-ve	
+ve	Count	8	26	34	0.260 NS*
	% of Total	18.2%	59.1%	77.3%	
-ve	Count	4	6	10	
	% of Total	9.1%	13.6%	22.7%	
Total	Count	12	32	44	
	% of Total	27.3%	72.7%	100.0%	

NS* = Non significant

From table (5) below showed the percentage of cANCA negative which has positive albumin, pus, RBC and cast in the urine (72.7%, 27.3%,27.3%and 27.3%) respectively while the percentage of p ANCA positive which has positive albumin, pus and cast (13.6%, 4.5% and 4.5%) respectively in the urine which give non significant differences (p>0.05) in both pANCA and cANCA patients.

Table (5): Evolution results of macroscopical and microscopical examination of urine in cANCA and pANCA patient.

Macroscopical & Microsscopical Examination			pANCA		cANCA	p-value
			+ve	-ve	-ve	
Albumin	+ve	Count	6	26	32	0.048 S*
		% of Total	13.6%	59.1%	72.7%	
	-ve	Count	6	6	12	
		% of Total	13.6%	13.6%	27.3%	
cast	+ve	Count	2	10	12	0.286 NS*
		% of Total	4.5%	22.7%	27.3%	
	-ve	Count	10	22	32	
		% of Total	22.7%	50.0%	72.7%	
Pus	+ve	Count	2	10	12	0.286 NS*
		% of Total	4.5%	22.7%	27.3%	
	-ve	Count	10	22	32	
		%of Total	22.7%	50.0%	72.7%	
RBC	+ve	Count	0	12	12	0.011 S*
		% of Total	0.0%	27.3%	27.3%	
	-ve	Count	12	20	32	
		Of %Total	27.3%	45.5%	72.7%	
Total		Count % of total	12 27.3%	32 72.7%	44 100.0%	

S*= Significant , NS*= non-Significant

In the table (6) observed the mean of HB and WBC in the positive pANCA patients (10.167 g/dl and 6150) is less than mean of HB and WBC of negative pANCA patients (10.338g/dl and 7431.81) while the mean of serum urea and serum creatinine in the positive pANCA patients (89.167and 3.183)

Mmol/l is more than the mean of the negative pANCA patients(61.22and 2.05) Mmol/l.

Table (6): Statistical summary of hematological &some biochemical parameters of cANCA and pANCA patients.

Parameters		pANCA			cANCA			p-value
		N	Mean	Std. Deviation	N	Mean	Std. Deviation	
S.creatinine Mmol/l	+ve	12	3.18	4.12	0	.	.	0.001 HS*
	-ve	32	1.63	1.06	44	2.05	2.37	
WBC Cell/cmm	+ve	12	6150.0	1555.92	0	.	.	.234 NS*
	-ve	32	7912.50	1715.53	44	7431.81	1836.17	
S.urea Mmol/l	+ve	12	89.167	58.80	0	.	.	.001 HS*
	-ve	32	50.750	30.23	44	61.22	42.93	
Hb g/dl	+ve	12	10.16	1.64	0	.	.	.111 NS*
	-ve	32	10.39	2.12	44	10.338	1.99	

HS*=Highly significant, NS*= non-Significant

Discussion

In this study, we analyzed the presence of cANCA and pANCA in patients with renal disease. We demonstrated that 27.3% of blood samples from 44 renal disease patients developing pANCA positive results, whereas c-ANCA patterns were not observed, the result of the present study show less percentage than other study who found p-ANCA was positive in 35 % of female [9]. And also the current study showed the percentage of positive pANCA in female patients (22.3%) was more than male patients (4.5%) the results of this study in keeping with Hilhorst *et al* , 2013 [10]. While disagree with Jalali *et al*, 1999 who revealed that the male patients with high percentage of c-ANCA com-pared to female patients [8]. It was proposed that these difference may be due to the a variety of factors related to environmental factors specially silica exposure [11]. Genetic factors that several studies have been performed which confirmed the presence of single nucleotide polymorphisms in the HLA-DPB region on chromosome 6 in a large percentage of patients with PR3-AAV as opposed to patients with MPO- ANCA-associated vasculitis (AAV) [12][13]. In addition to other factors such as differences between selection of the pateints, sample size, geographical distribution between country and other studies.

These results comparable with the results obtained in a recent multicentre study by the European Vasculitis Study Group[14]. And confirm that patients with localised (limited) disease can be ANCA negative [15].

Our study showed increased in the percentage of positive pANCA in 6 patients with SLE (50%) and less percentage in other disease. That result in agreement with study of Kabasakal *et al*, 1999 [16]. However, the presence of ANCA in patients with SLE has been demonstrated by many studies, but only p-ANCA antibodies were present in these

patients [17][18] [19]. And also it has been found that ANCA positivity by indirect immunofluroscence in 37.3% of the systemic lupus erythromatosis patients, involved p-ANCA in 31.4% and c-ANCA in 5.9% [20].this result consistent with the current study . Recent investigation unlike to some extent with another study which revealed that PANCA and CANCA were detected in 1.5% of patients respectively [21]. Dafina (2004) who suggested that MPO is a rare antigen for ANCA in lupus nephritis [22]. The result of this study disagreement with current study. Difference in studies in show a significant association between SLE and ANCA positivity may be due to the difference in technical methods, patients selection, number of patients. We also found 34 (77.3%) patients with negative cANCA had CRP positive results and 8 (18.2%) of patients had positive pANCA and positive CRP results which agree with that of Draibe et al,2015 [23]. When inflammatory markers are not diagnostic of inflammation, but reflect abnormalities that are seen in autoimmune diseases, infections, malignancies and other illnesses.

The present study showed both cANCA negative, pANCA (negative and positive) cases had positive albumin, pus and cast in the urine which reflect limited clinical utility of urinalysis. Also clarified that urea and creatinine were increased among cANCA negative and pANCA (negative and positive) cases like in the study of Mitchell *et al*, 2011 [24]. And that because the patients suffering from renal defect. In this respect it has been found that renal function at baseline has been shown to be more severely impaired in MPO-AAV in some studies [25][26]. But other studies included patients with similar renal function at baseline [11].

In conclusion, our data showed that serum anti-neutrophil cytoplasmic antibody measurement should not be used alone in the diagnosis of ANCA-associated disease, whereas pANCA is more convincing in the diagnosis than cANCA. While the ANCA positivity associated with rapidly progressive glomerulonephritis (RPGN) so we recommended that p- ANCA should be analyzed in parallel in patients with renal disease as routine serological test and could aid the further improvement of treatment.

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