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FREQUENCY OF ANTINEURTOPHIL CYTOPLASMIC ANTIBODY IN GLOMERULONEPHRITIS

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ABSTRACT

Introduction: *Aim of the study was to determine the frequency of antineutrophil cytoplasmic antibody (ANCA) (p-ANCA and c-ANCA) in clinically diagnosed glomerulonephritis. Autoimmune diseases including systemic vasculitis, affect a large number of people in whom the leading cause of morbidity and mortality is glomerulonephritis that is often associated with chronic kidney disease. There are many risk factors for kidney diseases such as chronic inflammation, autoimmune diseases, immunosuppressive therapy, etc. Early phases of renal injury in autoimmune patients are clinically silent. For the detection of nephron damage, histopathological examination is gold standard but detection of antineutrophil cytoplasmic antibody (ANCA) can be used to find out early nephron damage.* ***Materials and Methods:*** *Design was analytical Cross-sectional. The study was conducted at the Department of Immunology, University of Health Sciences, Lahore in a period of November 2008 to october 2009. Study included 64 clinically diagnosed of glomerulonephritis.* ***Results:*** *Levels of ANCA (MPO and PR3) were determined by ELISA technique. Out of which four (6.25%) patients showed positive reaction to myeloperoxidase [MPO] antigen while 1 (1.56%) patient was positive for proteinase-3 [PR3] antigen. In 40-60 years of patients, seropositivity for MPO and PR3 was 14% and 3.6% respectively. p-value for MPO and PR3 was < 0.05 and >0.05 respectively. We concluded that glomerulonephritis is better related with MPO-ANCA than PR3-ANCA.* ***Conclusion:*** *The difference in the levels of MPO-ANCA in different age groups was significant but it was non-significant among different genders. Difference in the levels of PR3-ANCA was not significant for both age and gender.*

Keywords: *Glomerulonephritis, ANCA, Autoimmune diseases.*

INTRODUCTION

Autoimmune diseases occur when physiological tolerance to self-antigens is lost and thereafter, human body can not discriminate between self and non-self tissues.[1] In autoimmunity, production of different auto-antibodies result in a variety of pathological manifestations.[2] They are hallmark of both systemic and organ specific autoimmune diseases.[3] Various studies reported renal involvement in 75%-90% of autoimmune diseases.[4] Chronic glomerulonephritis ultimately results in chronic kidney disease (CKD).[5]

Glomerulonephritis is an inflammatory condition of renal glomeruli which occurs due to deposition of antigen-antibody complexes or as a consequence of autoantibodies, in glomerular capillaries.[6] Glomerulonephritis is classified into primary and secondary glomerular disease.[7] Over the years, glomerulonephritis patients may develop chronic kidney disease (CKD) which is characterised by progressive loss of renal functions. Rapid and progressive loss of kidney functions associated with severe oliguria is known as rapidly progressive glomerulonephritis (RPGN).[8] It may result in acute glomerulonephritis or nephritic syndrome, which ultimately results in renal failure and end stage renal disease.[8] Patients with kidney damage are at high risk for the loss of kidney functions and development of cardiovascular diseases.

Among various autoantibodies, anti-neutrophil cytoplasmic antibody (ANCA) is a useful diagnostic tool for systemic vasculitis and auto-immune glomerulonephritis.[9-12] Polymorphonuclear granulocyte (PMN) contains two important granules, primary or α- granules and secondary granules. Enzymes in these granules are targets for ANCA.[13] ANCA demonstrates two major types of staining patterns. One is p-ANCA that shows a perinuclear staining pattern and myeloperoxidase (MPO) is its common target antigen. Therefore antibody staining results in florescence around nucleus. Another type of ANCA is cytoplasmic pattern (cANCA) that shows a diffuse granular cytoplasmic staining pattern and it is due to neutrophil cytoplasmic antigen which corresponds mostly with proteinase 3 (PR3) antibo-

dies.[14-16] Considering specificity and positive predictive value for the detection of these antibodies, ELISA is a superior technique compared to immunofluorescent method.[17] Determine the levels of antineutrophil cytoplasmic antibodies in the blood of patients with glomerolunephitis.

MATERIALS AND METHODS

A total of 64 samples of glomerulonephritis patients were collected from the Department of Nephrology, Sheikh Zayad Hospital, Lahore. The samples were processed and analysed at the Department of Immunology University of Health Sciences Lahore. It was an analytical cross-sectional study which was performed during the period of October 2008 – September 2009. Glomerulonephritis patients were diagnosed on the basis of kidney biopsy, urine analysis, urinary protein electrophoresis and renal function tests. p-ANCA and c-ANCA were performed by ELISA technique.

Data Analysis:

Data was analysed by SPSS version 16.0. quantitative variables were analyzed by mean ± SD whereas qualitative variables by their frequencies and percentages. Independent *t*-test was applied for comparison between two groups. Chi-square was applied for comparison between qualitative variables. *p* value of < 0.05 was considered as statistically significant.

RESULTS

A total of 64 patients were included in this study. Thirty-seven (57.8%) were males and 27 (42.2%) were females, hence male to female ratio was 1.8:1.

On the basis of age, patients were divided into two groups. Group A consisted of 35 (54.69%) patients and they were from 11 years - 39 years of age. Group B had 29 (45.31%) patients from 40 years - 60 years of age. Age and gender distribution is shown in Fig. 1. Mean values of p-ANCA in Group A was 0.50 ± 0.34 and in Group B it was 3.8 ± 9.37. The comparison of p-ANCA between Group A and Group B was statistically significant ($p<0.05$). Mean values of c-ANCA in Group A was 0.94 ± 0.62 and in Group B it was 1.31 ± 2.15. The comparison of c-ANCA between Group A and Group B was not statistically significant ($p>0.05$) (Table 1).

Patients included in the study had the duration of disease ranging from 1 month to 192 months. On the basis of duration of disease, patients were divided into three groups i.e. less than 12 months, 12 - 60 months and more than 60 months as Group 1, 2 and 3 respectively. In Group 1 mean level of p- ANCA was 2.14 ± 6.71 and c-ANCA was 1.08 ± 1.57. The comparison between p-ANCA and c-ANCA in group 1 was statistically non significant ($p>0.05$) (Table 2). In group 2 mean values of p-ANCA was 0.31 ± 0.09 and c-ANCA was 0.99 ± 0.42. In this group, the difference between p-ANCA and c-ANCA was statistically significant ($p<0.05$) (Table 2). In group 3 mean levels of p-ANCA was 0.45 ± 0.19 and c-ANCA was 1.44 ± 0.53. The comparison between p-ANCA and c-ANCA in this group was also statistically significant ($p<0.05$) (Table 2).

Table 1: *p-ANCA and c-ANCA between the age groups.*

	Mean ± SD		*p*-value
	Group A (n=35)	**Group B (n=29)**	
p-ANCA	0.50 ± 0.34	3.8 ± 9.37	0.03
c-ANCA	0.94 ± 0.62	1.31 ± 2.15	0.33

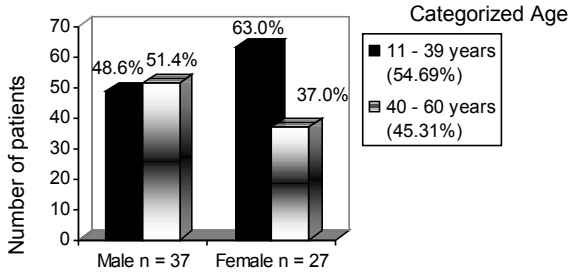

Fig. 1: *Age groups between two genders groups (n = 64).*

In this study, biopsy examination confirmed the diagnosis and included diffuse proliferative glomerulonephritis, rapid progressive glomerulonephritis, focal segmental glomerulosclerosis and mesengial proliferative glomerulonephritis. Levels of p-ANCA and c-ANCA in different groups are listed in Table 2. These groups were compared for the levels of c-ANCA and p-ANCA but their *p*-values were statistically non-significant (Table 2). Among 64 patients, 57 (89.06%) patients had acute glomerulonephritis while 7 (10.94%) had chronic glomerulonephritis. The ratio between acute glomerulonephritis and chronic glomerulonephritis was 8.1:1. Spectrum of different patients of glomerulonephritis is shown in Fig. 3. This study included patients of confirmed glomerulonephritis and patients of clinically diagnosed glomerulonephritis. The first group contained 31 (48%) patients and among these 9 (29%) patients had confirmed disease on biopsy whereas 12 (71%) patients were confirmed after renal function tests. Clinically diagnosed glomerulonephritis group contained 33 (52%) patients (Fig. 2). Mean value of p-

ANCA and c-ANCA in confirmed patients and in clinically diagnosed patients is shown in Table 3. Mean values of p-ANCA and c-ANCA in male and female is shown in Tab le 4. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of p-ANCA and c-AN-CA are shown in Table 5.

DISCUSSION

The present study was carried out to determine the levels of p-ANCA and c-ANCA in the blood of patients with glomerulonephritis. A total of sixty-four patients were included in the study. Male to female ratio was 1.8: 1, similar observation regarding male to female ratio was found by Obana *et al* as 1.9: 1.[18]

The comparison of p-ANCA between different age groups (group A and group B) was statistically significant (p = 0.03) (Table 2) whereas comparison of c-ANCA between group A and group B was not statistically significant (p = 0.33) (Table 2). These findings suggested that levels of p-ANCA were low in young patients compared to old patients while there was no difference in the levels of c-ANCA between two age groups. Jalali *et al* observed male patients with relatively high levels of c-ANCA compared to female patients. [9] On the other hand in the present study, seropositivity of ANCA was non-significant between two genders.

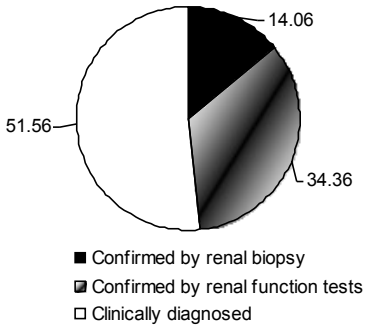

Fig. 2: *Pattern of diagnosis in all patients.*

Table 3: *Levels of p-ANCA and c-ANCA on the basis of diagnosis.*

	Mean ± SD	*p*-

Table 2: *Levels of p-ANCA and c-ANCA with duration of disease and on biopsy diagnosis.*

	Mean ± SD		*p*-value
	p-ANCA	c-ANCA	
< 12 months (n = 55)	2.14 ± 6.71	1.08 ± 1.57	0.25
12 – 60 months (n = 5)	0.31 ± 0.09	0.99 ± 0.42	0.01
>60 months (n = 4)	0.45 ± 0.19	1.44 ± 0.53	0.01
Diffuse proliferative glomerulonephritis (n = 3)	0.63 ± 0.20	0.86 ± 0.17	0.20
Rapidly progressive glomerulonephritis (n = 1)	0.60 ±0.0	0.31 ±0.0	---
Focal segmental glomerulosclerosis (n = 5)	1.23 ± 0.99	0.51 ± 0.16	0.14
Mesengial proliferative glomerulonephritis (n = 4)	0.63 ± 0.12	0.94 ± 0.47	0.24

	Confirmed cases (n=31)	Clinically diagnosed cases (n=33)	value
p-ANCA	3.07 ± 8.78	0.78 ± 1.3	0.14
c-ANCA	1.17 ± 1.99	1.10 ± 0.68	0.65

Table 4: *p-ANCA and c-ANCA between genders.*

	Mean ± SD		*p*-value
	Male (n = 37)	Female (n = 27)	
p-ANCA	1.66 ± 6.41	2.2 ± 6.11	0.17
c-ANCA	1.09 ± 1.83	1.08 ± 0.72	0.38

Table 5: Sensitivity and specificity of p-ANCA and c-ANCA:

Parameter	p-ANCA	c-ANCA
Sensitivity	95.24%	100%
Specificity	100%	25%
Positive predictive value	100%	95.24%
Negative predictive value	25%	100%
Diagnostic Accuracy	95.31%	95.31%

Our study included 4 (6.3%) cases of membranous glomerulonephritis, 5 (7.8%) of focal segmental glomerulosclerosis, 1 (1.6%) patient of rapidly progressive glomerulonephritis and 2 (2.1%)

cases of idiopathic membranous glomerulonephritis. A similar study was performed in Pakistan by Ashraf *et al* where both focal segmental glomerulosclerosis and proliferative glomerulonephritis were reported as 7%.[19] In another study, Obana *et al* observed idiopathic membranous glomerulonephritis as 2.4%. Both the observations closely related to the present study.[18]

In the present study, it was observed that mean levels of ANCA (p-ANCA and c-ANCA) is confirmed glomerulonephritis were almost equal to clinically diagnosed glomerulonephritis. In confirmed glomerulonephritis, mean value of p-ANCA was 3.07 ± 8.78 and c-ANCA was 1.18 ± 2.00. The comparison between p-ANCA and c-ANCA in confirmed glomerulonephritis was statistically non-significant (p = 0.24). These findings could be due to correct clinical diagnosis of glomerulonephritis.

It could be assumed from the present study that ANCA, weather c-ANCA or p-ANCA, could not be a useful marker for the diagnosis of glomerulonephritis but on the contrary Oda *et al* reported ANCA as a useful marker and they suggested that it would be possible to substantiate diagnosis of idiopathic crescentic glomerulonephritis by performing this test.[20] The difference of opinion could be due to disparity in methodology used in these two studies.

Enzyme linked immunosorbant assay (ELISA) technique was used in this study and reliability of this method has been documented by various studies as reported by Aslam *et al* who showed high sensitivity (82%), high specificity (97%), increased positive predictive value (92%) and increased negative predictive value (93%) of this technique. [21] Similarly, Harria *et al* reported ELISA as a superior technique to immunofluorescent assay (IFA) and he documented 97% specificity and 73% positive predictive value of ELISA technique while specificity of 88% and positive predictive value of 50% for IFA technique.[17] It is believed that ELISA is more specific method for the detection of these antibodies than immunoflorescent technique but sensitivity of florescent technique is more than ELISA technique. Therefore, we can assume that results obtained in this study could be due to comparatively less sensitivity technique of ELISA. Similar results were also obtained in another study where two patients of glomerulonephritis were screened by ELISA technique and none of the patients were found positive for either p-ANCA or c-ANCA.[17]

It is **concluded** that the comparison in the levels of myeloperoxidase antineutrophil cytoplasmic antibodies (MPO-ANCA) in different age groups was significant but it was not significant between genders. The comparison in levels of protenase 3 antineutrophil cytoplasmic antibodies (PR3-ANCA) was not significant between different genders and age groups. Level of p-ANCA and c-ANCA was found high in both clinically diagnosed and laboratory confirmed patients.

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