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ORIGINAL ARTICLE

Immune Complex-positive Glomerulonephritis in Antineutrophil Cytoplasmic Antibody-positive Patients

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ABSTRACT

Antineutrophil cytoplasmic antibody (ANCA)-positive glomerulonephritis (GN) has traditionally been considered pauci-immune (PI); however, various reports have demonstrated the presence of immune reactants in a subset of these cases. The study was done to evaluate the prevalence of immune deposits in ANCA-positive GN by direct immunofluorescence (DIF). Renal biopsies of 35 patients with ANCA-positive GN were retrospectively analyzed for light microscopic and DIF findings to look for the presence of immune reactants and to correlate these with the corresponding histologic features. Twenty-seven cases (77.2%) showed PI-GN while 8 (22.8%) cases showed ≥2+ positivity for one or more immune reactants in the mesangium and/or capillary loops and were categorized as ANCA-positive immune complex GN. Of these, seven were positive for perinuclear (P-ANCA) and one for cytoplasmic (C-ANCA). Immunoglobulin M (IgM) was the most frequent immunoglobulin. On light microscopy, these cases exhibited significantly increased mesangial cellularity (p = 0.033) and more number of normal glomeruli as compared with PI cases (p = 0.015).

Keywords: Antineutrophil cytoplasmic antibody, Direct immunofluorescence, Immune complex glomerulonephritis, Pauci-immune glomerulonephritis, Renal biopsy.

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INTRODUCTION

Pauci-immune glomerulonephritis is associated with circulating ANCA predominantly directed against either myeloperoxidase (MPO) or proteinase 3 (PR3). It is characterized by mild or absent Igs and/or staining for complement components on DIF and few or no electron-dense deposits on electron microscopy (EM).[1,2] Antineutrophil cytoplasmic antibody-mediated GN is typically referred to as "pauci-immune"; however, some immune complex (IC) deposition may be found within the glomeruli on DIF or on EM. The composition as well as clinical and pathological significance of IC deposits in ANCA-associated GN is not well known, although they are believed to promote development of more severe GN. These ANCA-associated GN cases with immune deposits are often referred to as ANCA-positive IC-GN.[3]

This study was done to determine the frequency of IC deposits in 35 renal biopsies from ANCA-positive patients and to study their histopathological features.

MATERIALS AND METHODS

A detailed retrospective analysis of renal biopsies of 35 patients of ANCA-positive GN, referred to Immunopathology Department, during 8-year period was done and the cases were reclassified based on DIF findings. Subsequently, histopathological features were compared between these two groups, i.e., PI-GN and IC-GN. Antinuclear antibodies (ANAs) by indirect immunofluorescence (IIF) using Hep2 slides were negative in all cases. Patients with anti-GBM disease, systemic lupus erythematosus, postinfectious GN, IgA nephropathy, and Henoch–Schonlein purpura (all IC-mediated renal diseases) were excluded based on clinical picture, ANA, and renal biopsy findings (both light microscopy and DIF).

Direct Immunofluorescence and Light Microscopy of Renal Biopsies

Direct immunofluorescence for IgG, IgA, IgM, and C3 was carried out on frozen sections of renal biopsies using fluorescein isothiocyanate (FITC)-labeled monospecific immunoreactants. All the DIF patterns were recorded systematically. The intensity (0 to 4+) and location of positive immunoreactants was recorded. Taking into account the definition of pauci-immunity on DIF, given by Falk and Jennette et al,[4] <2+ glomerular immunostaining was interpreted as PI.

Two histopathologists, blinded to clinical history, reviewed the light microscopy of all these renal biopsies. The histopathological features were then compared between these two groups. Mesangial hypercellularity

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Table 1: Demographic, histological, and ANCA profile of patients (n = 35)

Parameter	*ANCA-positive IC-GN (8 cases)*	*ANCA-positive PI-GN (27 cases)*
Mean age in years (range)	29 (11–58)	54.5 (11–83)
Gender (M:F)	1:1	13:14
Histopathological diagnosis		
Crescentic GN	6	23
Diffuse global sclerosis	–	3
Membranoproliferative GN	2	1
ANCA status		
P-ANCA positive	7	14
C-ANCA positive	1	13

was reported when more than three nuclei were seen in mesangium.[5]

Antineutrophil Cytoplasmic Antibody Assay

Antineutrophil cytoplasmic antibody was tested using an IIF assay with "in house" ethanol-fixed blood group O+ve neutrophil preparations in all cases. The staining patterns were recorded as C-ANCA, P-ANCA, or negative.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Statistical Package for the Social Sciences version 17 (Chicago, IL). The differences between means of both groups were compared using Fisher's exact test. A p value of less than 0.05 was considered statistically significant.

RESULTS

On reviewing patient records, histopathology, and DIF findings, we found that out of 35 cases with ANCA-positive GN, eight cases showed ≥2+ positivity for at least one immunoreactant. These eight cases were labeled as ANCA-positive IC-GN and their histopathological features were compared, with the remaining 27 cases of ANCA-positive PI-GN. Clinically, all 35 patients had rapidly progressive renal failure. Patients with ANCA-positive IC-GN were younger (mean age = 29 years) than those with PI-GN (mean age = 54.5 years; Table 1).

Histopathological Findings in Renal Biopsies

The histopathological features in two categories are summarized in Table 2. On comparison, cases with IC-GN showed significantly more normal glomeruli as compared with the PI-GN ($p < 0.05$). Though the percentage of crescents was slightly more in PI-GN group (96.3 *vs* 87.5%), the difference was statistically insignificant ($p = 0.41$). The mesangial cellularity was significantly higher in cases with immune deposits (mean, 50%) as compared with PI-GN (mean, 11.1%; $p < 0.05$). However, there was no significant difference between interstitial and vascular features among the two groups (Table 3).

Table 2: Histopathological features of ANCA-positive GN

Histological feature	*ANCA-positive PI-GN (27)*	*ANCA-positive IC-GN (8)*	*p-value*
Normal glomeruli	4 (14.8%)	5 (62.5%)	0.015
Crescents	26 (96.3)	7 (87.5)	0.41
Glomerulosclerosis			
Segmental	7 (25.9%)	2 (25%)	1.00
Global	13 (48.1%)	4 (50%)	1.00
Mesangial cellularity	3 (11.1%)	4 (50%)	0.033
Mesangial matrix	1 (3.7%)	1 (12.5%)	0.41
Disruption of BM	–	1 (12.5%)	0.229
Tubular casts	19 (70.4%)	6 (75%)	1.00
Interstitial fibrosis	11 (40.7%)	2 (25%)	0.68
Interstitial inflammation	25 (92.6%)	8 (100%)	1.00
Arteriolar hyalinosis	7 (25.9%)	2 (25%)	1.00
Medial hypertrophy	7 (25.9%)	2 (25%)	1.00
Intimal proliferation	5 (18.5%)	1 (12.5%)	1.00

Table 3: Details of immunoreactant positivity at various locations in glomerulus in renal biopsies of patients with ANCA-positive IC-GN (eight cases)

Location	*Immunoreactant*	*No. of cases*
Mesangium (eight cases)	IgM + others	6
	IgG + others	5
	IgA + others	2
	C3	8
	C3 only	2
	C3 + others	6
Capillary loops (three cases)	IgM + others	3
	C3 + others	3
	IgG + others	1

Direct Immunofluorescence findings on Renal Biopsies

ANCA-positive IC-GN (n = 8)

A ≥2+ staining for immunoreactants was found in mesangium in all these eight cases, while capillary loops showed immune deposits in three cases (38%). Positivity for C3 was seen in all cases; IgM positivity was seen in six patients, IgG in five cases, and IgA in two cases. Brightest intensity (4+) was noted for C3 followed by IgM. In six out of eight (75%) cases, mesangial deposits showed combination of different immunoreactants while two cases showed only C3 deposits (Table 3).

ANCA-positive PI-GN (n = 27)

These cases showed 0 to <2+ glomerular immunostaining.

Antineutrophil Cytoplasmic Antibody Serology

Of eight cases with ANCA-positive IC-GN, seven (88%) showed P-ANCA positivity and one (12%) showed

Table 4: Brief review of literature of cases with ANCA-positive IC-GN

Author	*No. of cases*	*ANCA serology*	*DIF findings on renal biopsy*	*Histology diagnosis on renal biopsy*
Komatsuda et al[6]	1	P-ANCA/MPO	IgG (mesangial and capillary loops)	IC-NCGN
Allmaras et al[7]	3	P-ANCA/MPO	IgA, C3c, C3 (mesangial and capillary wall)	IC-NCGN
Ramirez et al[8]	1	P-ANCA/MPO	IgA (mesangial)	NCGN
Rollino et al[9]	1	C-ANCA/PR3	IgA, C3 (mesangial and capillary loops)	IC-NCGN
Haas et al[17]	6	2 C-ANCA, 1 P-ANCA, 1 C-ANCA and P-ANCA (both), 4 PR3, 1 MPO, 1 PR3 and MPO (both)	IgA, IgM, C3 (mesangial)	IC-NCGN
Aasarød et al[10]	2	2 C-ANCA/PR3	IgA, IgM, IgG, C3 (mesangial)	IC-CGN
Soylu et al[11]	1	P-ANCA and C-ANCA	–	IC-CGN
Neumann et al[3]	8/45	5 C-ANCA, 1 P-ANCA, 2 C-ANCA and P-ANCA (both)	5 IgA, 2 IgM, 1 C1q	IC-CGN
Ayada et al[12]	1	MPO	IgG and C3 (mesangial and capillary loops)	IC-focal necrotizing CGN
Morizane et al[13]	1	MPO	IgG, IgM, C3	CGN with type 3 MPGN
Kawashima et al[14]	20	MPO	IgG, C3 (focal segmental)	IC-GN
Bălgrădean et al[15]	1	P-ANCA	IgG, C3, κ, λ	IC-CGN
Present study (2015)	8	7 P-ANCA, 1 C-ANCA	8 C3, 6 IgM, 5 IgG, 2 Ig A	6 IC-CGN, 2 MPGN

NCGN: Necrotizing crescentic glomerulonephritis; CGN: Crescentic glomerulonephritis; MPGN: Membranoproliferative glomerulonephritis

C-ANCA positivity; however, those with PI-GN showed an almost equal incidence of P-ANCA (n = 14) and C-ANCA (n = 13) positivity.

DISCUSSION

Antineutrophil cytoplasmic antibody-mediated GN was traditionally considered to be PI; however, we found IC deposition in 23% patients with ANCA-positive GN. Neumann et al[3] reported presence of immune deposits in 20% of patients with ANCA-positive GN. Various other reports describing immune deposits in renal biopsies of patients with ANCA-positive GN have been summarized in Table 4.

Neumann et al[3] observed C-ANCA positivity in 63% (5/8) of IC-GN cases, the remaining being P-ANCA positive. Other studies have also found a more frequent association of C-ANCA with IC-GN.[6-15] However, in our series, P-ANCA (88%) was more frequently associated with IC-GN.

A review of literature reveals IgA as the most common Ig detectable in cases with IC-GN (Table 4). However, we found IgM as the most common Ig detected in 75% of our cases. Although this might possibly suggest a nonspecific deposition of larger IgM molecules, however, on subsequent correlation of these findings with the histopathological features, we could identify significant changes in the form of mesangial hypercellularity in these cases, thereby negating the possibility of nonspecific deposition.

In this study, we also attempted to search for characteristic histopathological features to distinguish ANCA-positive IC-GN from PI-GN. We observed that there was significantly increased mesangial cellularity (Table 3) in ANCA-positive IC-GN (4/8; 50%), as compared with ANCA-positive PI-GN (3/27; 11% cases), with presence of neutrophils in two of four cases and mononuclear cells in remaining two cases. Haas and Eustace[16] also observed significantly increased glomerular cellularity in IC-GN as compared with the PI-GN. However, Haas et al[17] in another study did not observe significant increase in glomerular cellularity in cases of ANCA-positive GN compared with IgA nephropathy.

Based on these findings, we postulate that neutrophils and monocytes predominate in the kidney during early stage of ANCA-associated GN, when it is a predominantly IC-GN and this causes an increase in mesangial cellularity as observed in our series. Also, significantly more number of normal glomeruli was seen in ANCA-positive IC-GN, reiterating the fact that these cases represent early forms of the same disease that evolves to PI-GN. The neutrophils and monocytes that engulf ICs might represent pathognomonic feature of ANCA-positive IC-GN. Thus, our observation supports the view that the presence of ICs is a time-dependent phenomenon. Immune complexes might be present in early stages of disease and are later phagocytosed by neutrophils and monocytes, and eventually undergo apoptosis as the disease evolves to a classical PI-GN.[3] Eight cases in our series, which have shown immunoreactant deposition on renal DIF, might be the ones picked up at an early stage, as hypothesized.

Some authors have reported a higher degree of proteinuria and high serum creatinine values in ANCA-positive IC-GN than PI-GN.[3] We could not look at this aspect due to non-availability of clinical details at the time of data

analysis and lack of follow-up in these patients. The biggest limitation of this study is the lack of clinical data, and thus it falls short of comparing the clinical picture, extrarenal manifestations, treatment response, and outcome of the cases. Future prospective studies can be carried out to elucidate the clinical presentation and to correlate the disease severity with histopathology and DIF.

Larger studies are required to determine the impact of IC deposition in ANCA-positive GN and its influence on renal function and prognosis. Further research is desirable to look into whether these patients with IC deposition represent a distinct sub-group among ANCA-positive GN with a disparate clinical course from PI patients, or an early form of ANCA-positive GN which becomes PI as the disease progresses.

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