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Original Article

Early Prediction of Unfortunate Outcome of Acute Post-Streptococcal Glomerulonephritis (APSGN) in Children

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Abstract

Objectives: The aim of this work was to study the prevalence and significance of antineutrophil cytoplasmic autoantibodies in sera of patients with acute post-streptococcol glomerulonephritis.

Methods: We investigated 41 children with acute post-streptococcal glomerulonephritis (APSGN) and 13 children with acute streptococcal infection (without nephritis) in addition to 15 healthy age and sex matched controls for the presence of antineutrophil cytoplasmic antibodies (ANCA) to elaborate their pathophysiologic role in APSGN.

Results: ANCA were detected only in sera of 5 children (12%) of APSGN patients, three of them (60%) showed atypical cytoplasmic pattern "C-ANCA" and two (40%) showed perinuclear pattern 'P-ANCA". On the other hand ANCA were not detected in children with acute streptococcal infection or the controls.

We found that hypertension, blood urea, serum creatinine, proteinuria and haematuria were significantly higher in ANCA positive than in ANCA negative APSGN patients.

On follow-up after one month, the ANCA positive APSGN patients showed the same titre of these autoantibodies and did not show a significant improvement of renal function tests in contrast to the ANCA negative APSGN children.

Conclusions: The positive correlation found in our study between the level of ANCA titre and the disturbed renal function tests pointed to the possible role of these auto-antibodies in the pathogenesis of APSGN and the disease progression. The absence of ANCA in the acute streptococcal infection suggested that their presence is not just an epiphenomenon of the heterogenous humoral immune response due to the streptococcal infection.

We concluded that ANCA testing in children with APSGN could predict those with an unfortunate outcome and orientate the clinicians to focus their efforts in prevailing chronic renal damage.

INTRODUCTION

Acute post-streptococcal glomerulonephritis (APSGN) is a post-infectious "benign" glomerular disease, mediated by immune complexes that occasionally may follow a rapidly progressive course with the presence of extensive crescents in the renal biopsy[1]. APSGN is probably mediated by inflammatory effector cells, such as neutrophils, that are attracted to glomeruli by products of complement activation before oxidants and proteases are released that directly induce capillary wall injury[2]. The identification of markers that could predict an unfortunate progression and orient the clinicians to focus their efforts in prevention of chronic renal damage is the only way to support any change in our traditional conservative therapeutic attitude[3].

Antineutrophil cytoplasmic autoantibodies (ANCA) are a class of autoantibodies directed against certain components of

neutrophil cytoplasm[4]. They have been recognized as a useful tool in the diagnosis of vasculitis such as microscopic polyangiitis and necrotizing crescentic glomerulonephritis with a high sensitivity and specificity[5-7], and increasing evidence about their potential pathogenic role has been reported[8,9] APSGN, on the other hand, is an immune complex glomerular disease characterized by neutrophil infiltration (acute diffuse exudative glomerulonephritis), mesangial matrix increase and occasional formation of epithelial crescents, with intramembranous and subepithelial electron dense deposits[10] in which presence of ANCA has been little studied.

The aim of this work was to study the prevalence and significance of antineutrophil cytoplasmic autoantibodies in sera of patients with acute post-streptococcol glomerulonephritis.

SUBJECTS AND METHODS

This study included 69 children as two groups of patients and a healthy control group. It was carried out in Benha University Hospital during the period from August 1997 to March 1999.

APSGN group: The sera used in this study were obtained from 41 well-documented APSGN patients at the time of admission to hospital and during convalescence (after one month of onset). Their ages ranged from 4 to 11 years (mean 7.4 ± 2.6) and they were 28 males and 13 females. All the patients had a history of a preceeding streptococcal infection, (9 with preceeding pharyngitis and 32 with preceeding skin infection), with elevated levels of antistreptolysin O, and reduced levels of complement 3 at the moment of APSGN diagnosis. No patient showed evidence of other non-streptococcal infection at the moment of the study.

Acute streptococcal infection group: Sera obtained from 13 patients (8 males 5 females) with acute streptococcal infection, 8 with pharyngitis and 5 with skin infection, and without evidence of glomerular involvement, were included in the study. Their ages ranged from 5 to 11 years (mean 7.54 ± 1.78).

Control group: Sera from 15 healthy children (8 males and 7 females) were included. Their ages ranged from 4 to 11 years (mean 7.63 ± 2.01).

All patients and controls were subjected to thorough history taking, clinical examination and laboratory investigations including:

- Erythrocyte sedimentation rate (ESR) by Westergren's method[11] and the mean of the ESR in the first and second hours was calculated.
- Blood urea and serum creatinine[12].
- Serum complement 3 by radial immunodiffusion technique[13].
- Complete urine analysis.
- Protein in 24-hr urine collection[14].
- Indirect immunofluorescence technique on ethanol fixed neutrophil for detection of ANCA[15]. The kit was supplied by IMMCO Diagnostic.

In this method, the sera were incubated on optimized preparations of human neutrophils to allow binding of antibodies to the substrate. Any antibodies not bounded were removed by rinsing. Bound antibodies of

IgG class were detected by incubation of the substrate with fluorescence labeled, anti-human IgG conjugate. Reactions were observed under a fluorescent microscope equipped with appropriate filters.

At least three different patterns of fluorescence can be distinguished:

- Cytoplasmic pattern with accentuation of the fluorescence intensity in the area within the nuclear lobes (cytoplasmic or C-ANCA).
- Perinuclear pattern (P-ANCA).
- More diffuse finely speckled cytoplasmic staining pattern (atypical or a-ANCA). The titre is determined by testing serial dilutions.
- *These previous investigations were repeated for all APSGN patients after one month of prescribed treatment.*
- Renal biopsies were obtained from patients with APSGN whose renal functions remained abnormal on follow-up (after one month of onset of disease).

RESULTS

See Tables 1 - 5.

Table (1): Clinical, laboratory data in ANCA positive APSGN patients

No.	Age (years)	Sex	Blood Pressure	ESR	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)	Comp-lement 3 (mg/dl)	Protein-uria (mg/24h)	Haema-turia (Cell/HPF)	ANCA		Crescent in Biopsy
										Type	Titre	
1	4	F	160/120	40-80	80	1.7	50	440	50	P	1/20	+
2	11	M	180/130	68-105	90	1.8	48	320	45	a	1/20	+
3	7.5	F	160/110	60-98	55	1.8	40	650	65	P	1/40	+
4	10	M	185/140	52-78	110	2.1	25	800	100	a	1/80	+
5	6	M	160/130	80-103	82	2	20	920	80	a	1/40	+

HPF = High power field

Table (2): The different parameters in ANCA positive and ANCA negative APSGN compared to control

	ANCA + ve (n = 5)	ANCA – ve (n = 36)	Control (n = 15)
Age	7.7 ± 2.86	7.1 ± 2.31	7 ± 1.6
Diastolic B.P.	126 ± 10.20	104.68 ± 10.38	58.66 ± 3.39
ESR	76.40 ± 20.69	71.77 ± 23.27	6.13 ± 2.39
Blood Urea (mg/dl)	83.4 ± 17.73	41.13 ± 16.79	15.70 ± 5.65
Serum Creatinine (mg/dl)	1.88 ± 0.15	1.08 ± 0.32	0.72 ± 0.03
Complement 3 (mg/dl)	36.6 ± 12.09	78 ± 20.76	145 ± 11.9
Proteinuria (mg/24hr)	622 ± 221.59	410 ± 131.63	72.8 ± 32.38
Haematuria (cell/HPF)	68 ± 20.15	17.15 ± 5.68	1.63 ± 0.77
Sex (M/F)	3/2	25/11	8/7
Site of Strep. infection (Sk/Ph)	4/1	28/8	-

M = Male
F = Female

Sk = Skin
Ph = pharynx

Table (3): Statistical significance of different parameters between APSGN and control groups

	Age	Diastolic Blood Pressure	ESR	Blood Urea	Serum Creatinine	Comple-ment 3	Protein-uria	Haema-turia	Sex	Site
I Versus II	0.599	0.0001**	0.68	0.0001**	0.0001**	0.0001**	0.018*	0.0001**	0.51	0.7
I Versus III	0.496	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.60	-
II Versus III	0.879	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.43	

I = ANCA positive APSGN patients
II = ANCA negative APSGN patients
III = Control group

$p < 0.05$ = Significant
$p < 0.01$ = Highly significant

Table (4): Mean and standard deviation of the studied parameters at diagnosis and follow-up

	ANCA + ve (n = 5)			ANANA – ve (n = 36)		
	Initial	**Follow**	**P**	**Initial**	**Follow**	**P**
Diastolic B.P.	126.00 ± 10.20	92.00 ± 2.45	0.0001**	104.68 ± 10.38	60.00 ± 3.16	0.0001**
ESR	76.40 ± 20.69	42.50 ± 10.09	0.011*	71.77 ± 23.27	14.09 ± 9.99	0.001**
Blood Urea (mg/dl)	83.40 ± 17.73	59.20 ± 11.36	0.033*	41.13 ± 16.79	26.21 ± 8.53	0.011*
Serum Creatinine (mg/dl)	1.88 ± 0.15	1.75 ± 0.11	0.157	1.08 ± 0.32	0.82 ± 0.25	0.001**
Complement 3 (mg/dl)	36.60 ± 12.09	56.00 ± 8.60	0.019*	78.00 ± 20.76	124.74 ± 13.52	0.001**
Proteinuria (mg/24h)	622.00 ± 221.59	528.00 ± 190.41	0.475	410.00 ± 131.63	226.32 ± 80.28	0.0001**
Haematuria (cell/HPF)	68.00 ± 20.15	64.00 ± 22.23	0.773	17.15 ± 5.68	11.35 ± 4.28	0.0001**

Initial = at diagnosis
Follow = at follow-up

* = Significant
** = Highly significant

Table (5): Correlation between the degree of ANCA positivity and other parameters

	ANCA	
	r	**p**
Age	0.28	> 0.05
Sex (M/F)	0.33	> 0.05
Blood Pressure	0.42	> 0.05
ESR	-0.11	> 0.05
Blood Urea	0.35	> 0.05
Serum Creatinine	0.88*	< 0.05
Complement 3	-0.80	> 0.05
Proteinuria	0.80	> 0.05
Haematuria	0.97*	< 0.05

* r 0.8745 = significant

DISCUSSION

In our study ANCA were detected only in 5 (12%) of sera of children with APSGN, where 60% of them showed atypical cytoplasmic pattern (a-ANCA) and 40% showed perinuclear pattern (P-ANCA). However, no patient with streptococcal infection (without glomerular involvement) showed positive ANCA. Previous studies found ANCA in 9-20% of APSGN[3,16,17], whereas a-ANCA was present in 50-77.6%.

We found a significant association between the presence of ANCA and hypertension, blood urea, serum creatinine, proteinuria and haematuria as these parameters were significantly higher in ANCA positive patients than in ANCA negative patients. This coincides with the previous studies[16,17] that found an association of some of these parameters with the intensity of glomerular disease. So, the presence of positive ANCA testing, particularly if its grade of intestity is high (high ANCA titre), in children with APSGN is an indicator of severe renal impairment.

Our ANCA positive APSGN patients did not show a significant decrease in serum creatinine level one month after diagnosis, while ANCA negative APSGN patients showed a significant improvement of their renal function. Furthermore the renal biopsies of ANCA positive patients showed crescent formation, and it is tempting to speculate that the presence of these autoantibodies could play a pathogenic role in this disease. Detection of ANCA in a patient with immune complex type crescentic glomerulonephritis has been described[18]. ANCA were also found in many cases of idiopathic crescentic glomerulonephritis[19], and the immune complex disease was found in 15-30% of crescentic glomerulonephritis[20].

In this study, ANCA positive APSGN patients still showed positive ANCA testing at follow-up and the titre remained the same in all patients. In a previous study[16] the grade of intensity of ANCA positivity remained the same in the majority of cases and increased in the minority, and another study[3] reported persistence of ANCA positivity in 30% of patients for up to six months after the acute nephritic syndrome.

The positive correlation found in our study between the level of ANCA titre and the impairment of renal function tests points to the possible role played by these autoantibodies in the pathogenesis of APSGN. It was suggested that these autoantibodies are simply an epiphenomenon of chronic inflammation[21]. However, in-vitro studies suggested that they are initimately involved in the pathogenesis[22,23]. In our study, absence of ANCA in the acute streptococcal infection group (without nephritis) might suggest that their presence is not just an epiphenomenon of the heterogeneous humoral immune response due to the streptococcal infection.

The presence of ANCA in the plasma leads to the existence of potential for vascular inflammation. The production of interleukin-8 by ANCA-stimulated neutrophils within the intravascular compartment may frustrate neutrophil transmigration, encourage intravascular stasis and contribute to damage of glomerular endothelial cells[24]. Recent histological demonstration

of anti-glomerular basement membrane nephritis in a patient with ANCA-associated pauci-immune glomerulonephrits had blamed ANCA to be responsible for initial vasculitis insult to the kidney[25].

Our study showed that ANCA had a significant relation to the presentation and course of APSGN, and we may predetect patients with unfavorable course by testing for these autoantibodies in conjunction with other indices of disease activity.

Recent studies showed that therapeutic removal of nephritogenic factors (circulating immune complexes as well as ANCA) from the circulation by plasma exchange in rapidly progressive glomerulonephritis (RPG) was associated with improved renal functions[26,27]. Also, prophylactic haemodialysis was tried in glomerulonephritis before clinical evidence of uraemia[28]. To summarize, this study showed that ANCA have a significant relation to the severity and outcome of APSGN. Renal function tests were significantly more disturbed in ANCA positive patients compared with ANCA negative patients and there was a significant correlation between ANCA positivity and these tests. The ANCA positive patients shared positive ANCA testing at follow-up with the same titre and showed crescents in renal biopsies. Thus, the presence of ANCA could play a possible role in the disease pathogenesis and its estimation is important to determine the severity and outcome of APSGN.

We recommend early ANCA testing in children with APSGN to predict those with an unfortunate outcome and orientate the clinicians to focus their efforts in preventing chronic renal damage through the recently proposed therapeutic interference.

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