Geget (2007) 7 : 13 - 20

Original Article

Ceruloplasmin: A Possible Predictor of Pediatric Renal Vasculitis

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ABSTRACT

Background: Diagnosis of vasculitides remains of paramount importance to ensure appropriate management. Ceruloplasmin is an acute phase protein that has antiprotease and antioxidant properties and inhibits myeloperoxidase.

Objectives: This study has been undertaken to assess the potential role of ceruloplasmin as a marker of pediatric renal vasculitis.

Methods: Ceruloplasmin was studied in 80 subjects between 5 and 15 years of age with different clinical conditions potentially associated with renal vasculitis and 20 controls. Cases included 30 patients with nephritic syndrome, 10 with rapidly progressive glomerulonephritis (RPGN), 20 with systemic lupus and 20 with other vasculitis syndromes.

Results: Ceruloplasmin was significantly elevated in those with nephritis, lupus nephritis and RPGN. Those with positive ANCA (18 cases) had higher ceruloplasmin (0.51 ± 0.3 g/L compared to 0.35 ± 0.2 g/L in negative cases, p = 0.01). Those with pANCA had even higher levels (0.62 ± 0.3 g/L). Those with biopsy evidence of vasculitis had higher ceruloplasmin than those without (0.46 ± 0.3 g/L and 0.28 ± 0.07 g/L respectively, p = 0.01).

Conclusion: Ceruloplasmin is elevated in children with renal vasculitis and may be a useful non-invasive diagnostic test.

INTRODUCTION

Vasculitis is a general term that refers to the inflammation of blood vessels[1]. Because any organ may be involved, enormous panoply of symptoms are expectedly possible. Inflammation of the kidney vasculature may give rise to a range of clinicopathological conditions, ranging anywhere between very mild urinary abnormalities (proteinuria, micro hematuria) all the way up to progressive renal failure[2]. Pediatric diseases associated with renal vasculitis may be renal limited but remain trendy of a systemic presentation. The success of therapy is related to prompt diagnosis, aggressive treatment and careful follow up to be sure that side effects from medications do not develop[3].

ANCAs are autoantibodies directed against intracellular neutrophil antigens, including cytoplasmic (c-ANCA) or perinuclear (p-ANCA). It has been shown that c-ANCAs are directed against a neutrophil and monocyte protease, protease 3 (PR3), whereas p-ANCAs are specific for myeloperoxidase (MPO)[4]. The pathogenic role of ANCAs remains controversial. Anti-myeloperoxidase (MPO) antibodies are

associated with the development of anti-neutrophil cytoplasmic antibody (ANCA) related vasculitis[5]. The imbalance between the protease-antiprotease activity in the neutrophils has been implicated in the pathogenesis of ANCA-related vasculitis[6].

Ceruloplasmin is an acute phase protein that has antiprotease and antioxidant properties and inhibits MPO activity[7,8]. In childhood vasculitis, increase in ceruloplasmin levels during the acute phase could represent an "activation criteria" or else a response to neutrophil mediated tissue injury. Ceruloplasmin takes part in the clearance and inactivation of myeloperoxidase. The impaired inactivation of MPO may have a pathological role in inflammatory diseases characterized by autoantibodies to MPO, such as rapidly progressive glomerulonephritis with P-ANCA (perinuclear anti-neutrophil cytoplasmic antibodies)[9].

Whereas the most important single diagnostic test for vasculitis is often biopsy of the affected organ, its invasiveness could cast a barrier upon its universal feasibility, or else contribute to delay in diagnosis[10].

AIM OF THE WORK

The aim of this study is to assess the potential role of ceruloplasmin as a reliable predictor of pediatric renal vasculitis, which could make it a useful early non-invasive diagnostic test of vasculitis.

SUBJECTS AND METHODS

This study included 100 children aged between five and 15 years, including 80 patients and 20 healthy controls. Patients were recruited from inpatient wards and the General, Nephrology and Rheumatology Clinics of Cairo University Children Hospitals. They included four groups according to the following criteria:

Nephritis group: 30 patients with glomerulonephritis, manifesting with clinical and laboratory criteria of nephritic syndrome with or without nephritic range proteinuria. Nephritis due to systemic lupus erythematosus (SLE) or systemic vasculitis, and cases with rapidly progressive glomerulonephritis (RPGN) were not included in this group.

Rapidlyprogressive glomerulonephritis: 10 patients with RPGN confirmed by biopsy evidence of crescentic glomerulonephritis.

Systemic lupus erythematosus: 20 patients with the clinical and laboratory diagnostic criteria of SLE, including ten patients with evidence of lupus nephritis.

Vasculitis group: 20 patients with vasculitis from other causes, including Henoch Schonlein Vasculitis and Kawasaki disease.

Informed parental consent was obtained and patients were subjected to:

- History taking and clinical examination.
- Initial laboratory tests including CBC, ESR, C3, C4, ASOT, urea, creatinine, serum proteins, cholesterol, urine analysis and estimation of protein excretion by urinary protein/creatinine ratio. ANA and anti DNA were done for cases of SLE.
- Ultrasonographic assessment of renal size, echogenicity and any abnormalities.
- Ultrasound guided renal biopsy for all cases with lupus nephritis and RPGN, and according to standard indications in other cases.
- Testing for ANCA and measurement of

serum ceruloplasmin for cases and controls:

- Serum samples were aseptically controlled, immediately centrifuged and frozen at -20°C.
- ANCA testing was done by indirect immunofluorescence using multiple well substrate slides containing ethanol fixed human neutrophils and anti-human IgG fluorescent conjugate.
- Ceruloplasmin was measured by an immunohistochemical reaction in which immune complexes formed between ceruloplasmin and specific antiserum scatter a beam of light passed through the sample. Concentration was quantified by nephelometry analyzing increase in turbidity as measured by increasing right angle scatter of laser light.

Data analysis:

Frequency and percentage were used to describe nominal data and mean, range and standard deviation for numerical data. Means were compared using t tests and nominal data using the Chi Square test. Pearson's correlations were used to test for association of numerical values. P values less than 0.05 were considered significant.

RESULTS

Patients were 28 males (35%) and 52 females, with a mean age (± SD) of 9.13 ± 2.6 years, not significantly different from controls ($p > 0.05$). Table 1 summarizes the clinical and laboratory features of the study groups. Eleven patients (18%) were positive for cytoplasmic ANCA (c-ANCA), seven (9%) for perinuclear ANCA (p-ANCA) and 62 (77%) were ANCA negative.

Table 2 and Figure 1 show the ceruloplasmin levels in studied groups of patients and controls. Table 3 illustrates the relation between ceruloplasmin levels and ANCA status. 72% of ANCA +ve cases had ceruloplasmin levels of > 0.3 g/L, compared to 65% of ANCA –ve cases (OR 1.4, $p > 0.05$). Considering a higher cut off level of 0.4 g/L, 61% of positive cases had elevated ceruloplasmin levels compared to 18% of -ve cases (OR 7.1, $p < 0.001$). Considering p-ANCA, 84% of positive cases had ceruloplasmin levels above 0.3 g/L (OR 2.8, $p > 0.05$) and 71% above 0.4 g/L (OR 11.2, $p = 0.002$).

Thirty two patients underwent renal biopsy. Table 4 shows that those with biopsy evidence of renal vasculitis had significantly higher ceruloplasmin levels. Twenty three patients with evidence of vasculitis (88%) had ceruloplasmin level > 0.3 g/L compared to 33% of those without (OR 15.3, $p = 0.003$). Half of those with vasculitis and none of those without had ceruloplasmin > 0.4 g/L ($p = 0.02$). Other than a negative correlation with complement C4 ($r = -0.31$, $p = 0.004$), ceruloplasmin levels had no significant relation to age, gender, sonographic or other laboratory findings.

Table 1: General features of the study groups.

		Control	All cases*	p value	Case subgroups				
					Nephritis	RPGN	SLE	LN	Vasculitis
No. of cases		20	80		30	10	10	10	20
Hypertension	*no,%*	0	23 (29%)	< 0.01	9 (30%)	6 (60%)	0	4 (40%)	4 (20%)
Urine:									
RBCs	*no,%*	0	57 (71%)	< 0.001	30 (100%)	10 (100%)	0	6 (60%)	11 (55%)
WBCs	*no,%*	0	53 (66%)	< 0.001	30 (100%)	8 (80%)	1 (10%)	6 (60%)	8 (40%)
Casts	*no,%*	0	44 (55%)	< 0.001	16 (53%)	7 (70%)	0	10 (100)	11 (55)
Protein/creatinine ratio	*Mean, SD*	0.26 ± 0.1	0.97 ± 0.05	< 0.001	0.83 ± 0.5	1.66 ± 1.3	0.29 ± 0.1	1.59 ± 0.4	0.88 ± 0.6
High creatinine	*no,%*	0	23 (29%)	< 0.01	8 (27%)	10 (100%)	0	4 (40%)	1 (5%)
Hypo albuminemia	*no,%*	0	59 (74%)	< 0.001	19 (63%)	7 (70%)	9 (90%)	9 (90%)	15 (75%)
Leucocytosis	*no,%*	0	19 (24%)	0.02	7 (23%)	5 (50%)	0	0	7 (35%)
Thrombocytopenia	*no,%*	0	4 (5%)	> 0.05	0	1 (10%)	0	2 (20%)	1 (5%)
ESR	*Mean, SD*	5.45 ± 1.5	66.3 ± 28	< 0.001	64.3 ± 26.8	57 ± 24.6	69.1 ± 22	68.5 ± 29	71.7 ± 34
High ASOT	*no,%*	6 (30%)	46 (58%)	0.03	27 (90%)	8 (80%)	3 (30%)	3 (30%)	5 (25%)
High CRP	*no,%*	0	80 (100%)	< 0.001	30 (100%)	10 (100%)	10 (100%)	10 (100%)	20 (100%)
C3	*Mean, SD*	107 ± 14	63.8 ± 27	< 0.001	49 ± 21.6	66.3 ± 31.2	76.4 ± 21.3	69.9 ± 26.4	75.5 ± 26.8
C4	*Mean, SD*	21.1 ± 4.2	21 ± 8.7	> 0.05	23.2 ± 5.8	24.1 ± 1	15.5 ± 7.1	11.2 ± 8.4	23.9 ± 5.5
c-ANCA	*no,%*	0	11 (14%)	> 0.05	1 (3%)	0	5 (50%)	3 (30%)	2 (10%)
p-ANCA	*no,%*	0	7 (9%)	> 0.05	0 (0%)	0	4 (40%)	3 (30%)	0

RPGN = rapidly progressive glomerulonephritis, SLE = systemic lupus erythematosus (without nephritis), LN = lupus with nephritis

• all groups of cases, taken together

Table 2: Ceruloplasmin levels (g/L) in different study groups.

	Mean ± SD	Range	p value*
Control	0.24 ± 0.1	0.08 - 0.28	
Neph	0.36 ± 0.1	0.12 - 0.62	< 0.001
RPGN	0.36 ± 0.2	0.27 - 0.49	< 0.001
SLE	0.45 ± 0.3	0.12 - 0.95	> 0.05
LN	0.64 ± 0.4	0.34 - 1.72	0.004
Vasc	0.27 ± 0.1	0.08 - 0.62	> 0.05

Neph = nephritis
SLE = systemic lupus erythrematosus
Vasc = vasculitis
RPGN = rapidly progressive glomerulonephritis
LN = lupus nephritis
* Compared to normal controls

Table 3: Ceruloplasmin levels in relation to ANCA status in the study group.

ANCA	No. of cases	Ceruloplasmin (g/L)		p value*
		Mean ± SD	Range	
Negative	62	0.35 ± 0.2	0.08 - 1.72	
All positive	18	0.51 ± 0.3	0.12 - 0.95	0.01
p-ANCA positive	7	0.62 ± 0.3	0.12 - 0.94	0.03
c-ANCA positive	11	0.45 ± 0.2	0.16 - 0.95	> 0.05

* Compared to ceruloplasmin in ANCA negative cases.

Table 4: Ceruloplasmin levels in relation to biopsy evidence of renal vasculitis.

	No. of cases	Ceruloplasmin (g/L)		p value
		Mean ± SD	Range	
Vasculitis	26	0.46 ± 0.30	0.07 - 1.72	0.01
No Vasculitis	6	0.28 ± 0.07	0.16 - 0.36	

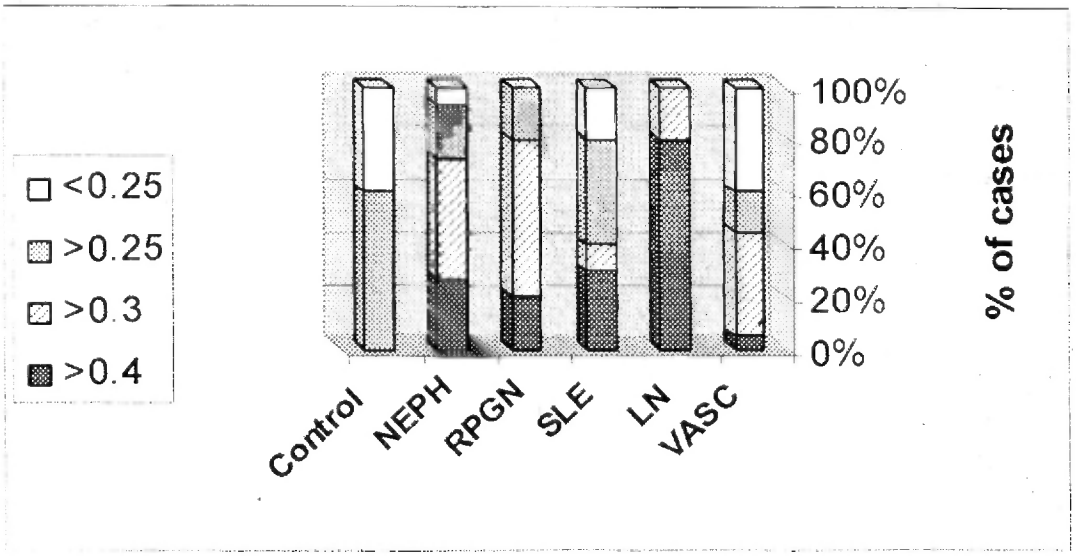

Fig. 1: Ceruloplasmin levels in different study groups.

Ceruloplasmin is expressed in g/L
NEPH = nephritis
SLE = systemic lupus erythrematosus
VASC = vasculitis
RPGN = rapidly progressive glomerulonephritis
LN = lupus nephritis

DISCUSSION

Vasculitis remains one of the conditions whose diagnosis is frequently difficult. Immune complexes play a key role in the pathophysiology of many vasculitis syndromes, thus complement levels can be helpful in establishing a diagnosis of vasculitis. Nevertheless, complement levels are generally normal in antineutrophil cytoplasmic antibody (ANCA) associated small vessel vasculitides, Henoch Schonlein purpura and polyarteritis nodosa[11].

ANCA testing has revolutionized the diagnosis and treatment of various immune mediated vasculitides. Positive cytoplasmic (c-ANCA) or perinuclear (p-ANCA) strongly suggests a diagnosis of small vessel vasculitis[4].

Although tissue biopsy is generally considered the gold standard for diagnosis of vasculitis[12], non-invasive tests that could provide early and reasonably accurate diagnosis would be of benefit in the management of these cases. The present study has been designed to assess the potential role of ceruloplasmin as a reliable predictor of pediatric renal vasculitis.

In this study, ceruloplasmin has been found to be elevated in children with nephritis, lupus nephritis, and RPGN. The fact that patients with systemic lupus erythematosus without evidence of nephritis had significantly lower ceruloplasmin levels than those with lupus nephritis may support the suggestion that renal affection is linked to an increase in the ceruloplasmin level compared to the same vasculitis disorder (in this case SLE) without nephropathy. To the same effect is the elevation of ceruloplasmin found in study groups representing different disorders in which renal involvement is the major common factor.

The relation between ceruloplasmin and ANCA was impressive. Significantly

higher concentrations of ceruloplasmin were found in ANCA positive cases and especially in p-ANCA positive cases. Those with c-ANCA had ceruloplasmin levels not significantly exceeding controls. At a ceruloplasmin cut-off level of 0.3 g/L (higher than all controls), ceruloplasmin was elevated in 72% of ANCA +ve cases and 65% of ANCA -ve cases. However, using a higher cut-off of 0.4 g/L, the difference has been more marked and significant (OR 7.1, $p < 0.001$).

Similar results were published by Esra et al.[13] describing significantly higher levels of ceruloplasmin in studied cases with p-ANCA associated vasculitis with renal involvement and positively correlated with MPO which decline on remission. Moreover, the same study showed increased ceruloplasmin levels during the acute phase suggesting that this might be attributed to the inhibitory effect of ceruloplasmin on MPO[14].

These findings support that increased ceruloplasmin levels together with ANCA positivity may be predictive for renal involvement. When biopsied cases have been studied to determine the relation between ceruloplasmin and biopsy findings, ceruloplasmin levels were significantly higher in those with vasculitis. These results propose ceruloplasmin, possibly in combination with p-ANCA, as a potential non-invasive and accurate test for renal vasculitis.

In conclusion, ceruloplasmin is elevated in children with renal vasculitis and especially in those with p-ANCA. Although more work is needed to question the long – believed credo describing biopsy as being the sole irrefutable evidence for final diagnosis, early use of ceruloplasmin in vasculopathy-nephritidis would be helpful. The value of serial measurements in monitoring disease activity and response to therapy needs to be addressed.

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