

# C1q nephropathy among children with nephrotic syndrome: Ten-year experience from a pediatric nephrology unit

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**SUMMARY:** Abu-Shahin N, Al-Khader A, Qattan D, Akl K. C1q nephropathy among children with nephrotic syndrome: Ten-year experience from a pediatric nephrology unit. Turk J Pediatr 2018; 60: 14-21.

C1q nephropathy (C1qN) is a rare glomerulopathy mostly seen in children, and presents with nephrotic syndrome (NS). Diagnosis depends on immunofluorescence or immunohistochemical C1q mesangial deposition, excluding other immune-mediated diseases. We retrospectively investigated C1qN incidence, clinicopathological features, and outcome among pediatric NS in our institution. Clinical data, microscopic slides and corresponding tissue blocks of pediatric renal biopsies were retrieved. According to diagnostic criteria for C1qN, 53 pediatric NS renal biopsies were selected for Anti-C1qA IHC stain microscopic examination. Clinicopathological features and follow up data were recorded. C1qN incidence was 9.4% among pediatric NS biopsies. Mesangial proliferation was the most common histopathological pattern. Steroid dependency with frequent relapses was the most frequent outcome, with a second line immunosuppressant added, yet without impact on progression. Small sample size hinders coherent conclusions; nevertheless, it indicates that C1qN is a rare cause of pediatric NS. C1qN may require second line immunosuppressants more often than non-C1q NS.

**Key words:** glomerulonephritis, nephrotic syndrome, C1q nephropathy.

C1q nephropathy (C1qN) is an idiopathic glomerulopathy typically presenting in children and young adults<sup>1</sup>. Patients classically present with nephrotic syndrome (NS). The key diagnostic features are dominant or co-dominant glomerular mesangial C1q deposition evidenced by immunofluorescence studies (IF), or immunohistochemical stain (IHC) for anti-C1q; provided that other immune complex glomerulonephritides like lupus nephritis are excluded by clinical and serologic tests<sup>2</sup>. Large-scale case studies of C1qN are sparse, with controversy regarding the prognosis and proper patient management.

Examination of C1q deposition by IF or IHCs are currently not performed in our institution, thus, the exact incidence of C1qN and its prognosis is unknown. We aimed to investigate in retrospect using IHC, the prevalence of C1qN in the pediatric renal disease population attending renal pediatric services during the period from 2003 to 2016; and to determine the

impact of C1qN on disease course and patient outcome especially the risk of progression to chronic renal failure at final follow up, providing clinicopathological correlation that can be implemented for therapeutic purposes.

## Material and Methods

In this retrospective analysis, clinical data were gathered from selected pediatric renal patient's medical papers and electronic records following approval from Faculty of Medicine and University Hospital IRB ethics committee/report number (KT/14/2014) according to ethical institutional protocols. A total of 105 renal biopsies were found. Gathered data included age, sex, clinical presentation, serum creatinine at presentation and at final follow up, proteinuria at initial diagnosis and at final follow up, histopathological features, IF results, type of therapy, response to treatment, overall follow up period, and final outcome.

A group of clinical definitions were used to verify the data <sup>2</sup>: NS is a triad of heavy proteinuria, hypoalbuminemia and edema. Steroid therapy is the mainstay of NS therapy according to the International Study of Kidney Disease in Children (ISKDC) regimen. Initial episodes are usually treated with prednisolone dose of 60 mg/m<sup>2</sup> daily for 4 weeks and followed by 40 mg/m<sup>2</sup> on alternate days for a further 4 weeks. Nephrotic proteinuria is defined as  $\geq 50$  mg/kg/day or  $\geq 40$  mg/m<sup>2</sup>/day. Response to primary (steroid) treatment was classified as either: steroid sensitive (SS): complete remission of proteinuria during the treatment and the remission persisted for at least 2 months after stopping steroids; steroid dependent (SD): is the occurrence of a relapse while the child is still on steroid treatment or within 2 weeks after its cessation; and steroid resistant (SR), defined as no remission over a period of at least 4 consecutive weeks of steroid therapy. Final patient outcome was set according to clinical information and laboratory values are as follows: Remission: a  $\geq 0.15$  g/24 h reduction in urine protein concentration. Relapse was defined as reappearance of proteinuria on at least 3 consecutive days. Frequent relapse (FR) was defined as 2 relapses in 6 months or  $\geq 4$  relapses in 12 months.

For the 105 renal biopsies, de-identified, hematoxylin & eosin-stained microscopic glass slides and the corresponding paraffin-fixed tissue blocks were retrieved from the archives of pathology department. The slides were reviewed by a nephropathologist.

According to a set of diagnostic criteria for C1qN as determined by Jennette et al.<sup>1</sup>, the diagnosis depends on C1q deposition detected by IHC or IF, after exclusion of immune complex GN like lupus nephritis. A wide spectrum of LM findings were allowed, including: Minimal change disease (MCD), focal and segmental sclerosis pattern (FSGS), mesangial proliferative pattern (MP), and crescentic GN (CGN). Also, as a disease typically presenting with NS, we aimed to investigate incidence among NS cases. Keeping that in mind, selection of cases to perform anti-C1q antibody (anti-C1q) by IHC was done, and included:

- a) A clinical presentation of nephrotic proteinuria (N.Pr)/NS;
- b) Cases with documented negative or

inconclusive previous IF reports that lacked testing for C1q.

c) Exclusion of cases with diagnostic features of other immune complex glomerulonephritides (like lupus nephritis (LN), IgA nephropathy, membranous nephropathy, etc) by clinical and serologic tests.

Three samples were initially excluded for being inadequate for evaluation. Categorization of cases (n=102) was then performed as previously mentioned into Group 1 (criteria-positive; n=59) and Group 2 (criteria-negative; n=43). In Group 1, six tissue blocks were excluded because they were either not found or considered destitute to perform IHC. Accordingly, a total of 53 biopsies (Group 1) were selected to perform the anti-C1q IHC staining as described below.

IHC stains were performed manually on the corresponding tissue blocks, using Anti-C1qA antibody [clone: EPR2980Y] Rabbit IgG monoclonal concentrated antibody (Gennova/Spain). For the formalin-fixed, paraffin-embedded tissue, a concentration of 1  $\mu$ g/ml was used. Heat-mediated antigen retrieval was done before commencing with IHC protocol. Positive and negative tissue controls were run with each set of slides. The results were interpreted by a nephropathologist using a LM, including the presence or absence of glomerular stain, stain intensity, and location within the glomeruli.

Clinicopathological correlation was performed using the LM findings, C1q deposition, and the clinical parameters of the corresponding cases, with center of attention on the mode of therapy, response to treatment, disease progression and outcome. Descriptive statistics of mean was used for continuous variables, and numbers (percentages) for categorical variables.

## Results

### *Diagnostic categories of renal biopsies among pediatric cases*

The pediatric renal biopsies included 102 native kidney biopsies and 3 kidney transplant biopsies. The patients were 51 females and 54 males (ratio of 0.95:1). The mean age was 7.4 years (ages ranged from 3 months to 18 years). Clinical follow up (FU) was available for 96 cases. The follow up periods

**Table I.** Clinical Data of Group 1 (C1q+ versus C1q-).

Criteria	Group1 (criteria+)	
	C1q+	C1q-
Number of patients	5	48
Patients with follow-up	4	43
% of Group 1	9.4%	90.6%
% of all samples	4.9%	47.1%
Initial creatinine (mean±SD)	0.4± 0.23 mg/dl	0.47 ±0.75 mg/dl
Initial creatinine (mean±SD)	0.28± 0.15mg/dl	1.8±2.5 mg/dl
Initial urinary protein (mean±SD)	1.89± 1.26g/day	1.47 ±2.3g/day
Initial urinary protein (mean±SD)	2.6 ± 2.3 g/day	1.92±2.46 g/day
Mean follow-up	42 month +/- 15.49	36.69months +/- 39.79
Treatment		
Number of patients	5	43
Steroid sensitive	0 (0%)	21 (48.8%)
Steroid dependent	0 (0%)	7 (16.3%)
Steroid dependent +2 <sup>nd</sup> drug	3 (60%)	6 (14%)
Steroid resistant	1 (20%)	4 (9.3%)
No treatment	1 (20%)	5 (11.6%)
Outcome		
Alive at FFU	4	37
Remission	1 (20%)	21 (48.8%)
Frequent relapse	3 (60%)	6 (14%)
Persistent proteinuria	1 (20%)	7 (16.2%)
ESRD	0(0%)	3 (7%)
Deaths*	1(20%)*	6 (14%)

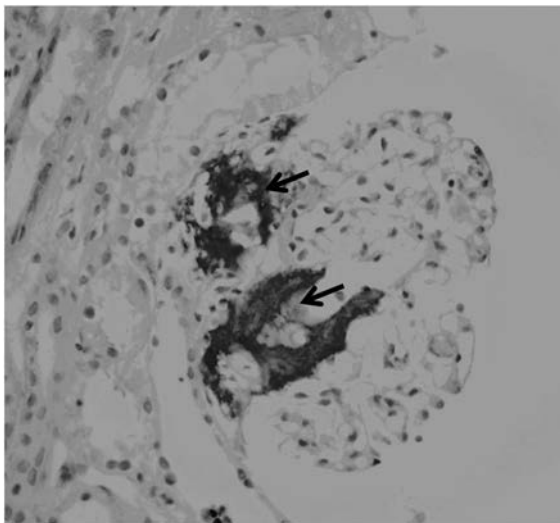
SD: standard deviation; FUP: follow up period; FFU: final follow up; ESRD: end stage renal disease; \* refers to death due to ESRD

(FUP) ranged from 1 to 156 months (mean= 38.56mo). According to clinical presentation and initial laboratory work up, the most common presentation was nephrotic-range proteinuria (N.Pr) in 59 patients (57.9 %), 39 others had non-nephrotic proteinuria, and 18 had no proteinuria. Ten patients initially presented with renal failure (9.5% of all). Initial serum creatinine (Cr) values ranged from 0.09 to 5.78 mg/dl (0.66± 0.96). Cr readings at final follow up (FFU) ranged from 0.04 to 13.50 mg/dl (1.56± 2.55). Initial urine tests showed no hematuria in 83 cases, microscopic hematuria in 6, and macroscopic hematuria in 13 patients. 3 patients presented initially with hypertension (HTN). Two patients had renal anatomical defects (posterior urethral valves) in addition to nephrotic range proteinuria.

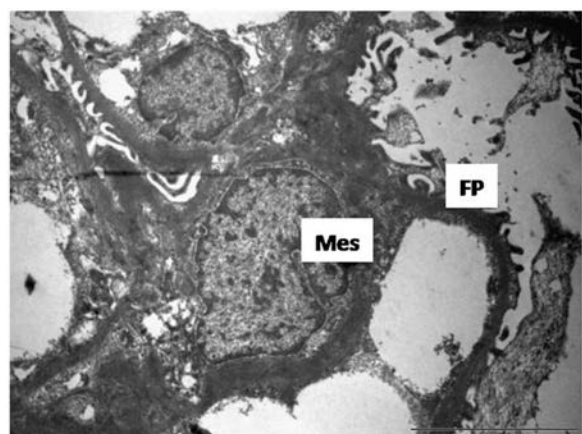
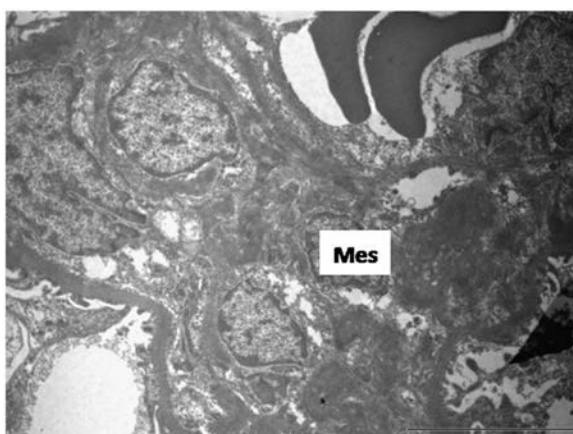
Among the 59 cases selected for the current study, 6 were excluded since the tissue blocks were not found or considered meager for staining (n=59-6= 53). Out of the 53 cases, 40 cases had a histopathological diagnosis of MP (75.5 %), 8 had FSGS (15.1 %), 7 had MCD (13.2 %), with one had NS post renal allograft, 2 cases of congenital nephrotic syndrome, 2 cases with crescentic GN. FU of biopsied NS cases showed the most common outcome to be remission (41%). Persistent proteinuria was observed in 18%, and 16% eventually progressed to ESRD. Clinical data of cases with and without C1q detection are summarized in Table I.

**Results of IHC with anti-C1q: Group 1a (C1q+) vs. group 1b (C1q-)**

Out of the 53 anti-C1q -tested tissue blocks, 5 blocks (9.4% of stained cases) displayed C1q deposition. The deposition was moderate intensity and mesangial in location in all cases. Light microscopic findings are displayed in Figure 1. One paraffin-fixed tissue block for one C1q-positive sample was de-parafinized for electron microscopic (EM) examination by transmission electron microscope, following retrieval procedures, and the glomeruli showed mesangial immune deposits, as shown in Figure 2. Due to the small number of samples that were C1q +, statistical analysis was not justified; however, the clinicopathological features of those cases are detailed below.



**Fig. 1.** Light microscopic findings in C1qN. IHC stain with anti-C1q showing a glomerulus with mesangial C1q deposition (arrows) as well as in segmental sclerosis. (original magnification 100X).



**Fig. 2.** Ultrastructural features of C1qN. Electron microscopic examination reveals mesangial (Mes) expansion by electron dense deposits, and adjacent podocyte foot process (FP) effacement. (Transmission Electron Microscope, 8,000X).

### **Clinicopathological features of C1q+ cases**

Our case series included 5 patients (n=5). The patient ages ranged from 2 to 12 years (mean = 7.4 years). They were 4 males and 1 female, clinically presented with NS. Urinary protein values at FFU were nil in one patient and 3+ (heavy) in the other three. Initial Cr values ranged from 0.13 to 0.7 mg/dl. Cr values at FFU were similar, ranging from 0.13 to 0.45.

The FUP ranged from 24 to 60 mo. (mean= 44 mo.). One (patient 1) was lost to follow up (LFU). Patient 2 had a relapse 4 year following initial biopsy, yet is doing well now, with Cr of 0.45 and no proteinuria. The remaining cases (patients 3, 4 and 5) were NS-SD; with FR; they had an immunosuppressant drug added as a second line of therapy (cyclophosphamide and cyclosporine). Except for patient 1 who was LFU and unfortunately found to be dead due to ESRD, none of the cases with FU showed progression to ESRD. Examination of the microscopic slides revealed MP pattern in all cases. Review of the old IF reports that did not include testing for C1q deposition, was negative for immunoglobulins and complements in 2 cases, one case showed mesangial IgM 1+, and trace IgA, while the IF was not performed in 1 case. In this study, all five cases showed mesangial location of C1q deposits, with weak to moderate intensity.

### **Patient 1**

A 12-year-old boy, presenting with edema and N.Pr, was initiated on prednisolone therapy but did not complete his therapy course and was discharged from hospital by family against



medical advice. Unfortunately, he was LFU and upon medical archive retrieval was found to be dead due to renal failure.

#### **Patient 2**

The patient is a 6-year-old boy, who presented to pediatric clinics with NPr. He was treated with steroids and his clinical course was significant for SD. Second line drug (cyclosporine) was added after 2 yrs. He had his last relapse on June 2008 after 4 years of initial diagnosis. At final FU, he was found well with no proteinuria.

#### **Patient 3**

A 5-year-old male presented with NS/SD, his serologic work up showed normal serum C3. His disease course was significant for FR. Cyclosporine as a second line drug was added after 3 yrs on Nov, 2008. He was last seen on Dec, 2009, when he was doing well 1 year after last relapse.

#### **Patient 4**

A 2-year-old female with NS/SD, started on steroids initially, then cyclophosphamide was added 6 months later. She was still symptomatic at her final follow up, when she started to develop resistance to both steroids and cyclophosphamide. She also complained of precocious puberty 2 years since her initial diagnosis.

#### **Patient 5**

A 12-year-old male, presenting with 3+ urine dipstick protein, was evaluated and treated with steroids. An outside electron microscopic report for his kidney biopsy was suspicious of Fabry's disease, which is a lysosomal storage disease that frequently leads to proteinuria and progression to renal failure by adulthood<sup>3</sup>. During the FUP of 48 months his clinical course was significant for many admissions, NS- SD, and had FR. Cyclosporine was added 24 months of initial diagnosis as a steroid sparing agent.

### **Discussion**

C1 is a complement component of the classical complement pathway. C1q plays a role in several immunological processes, like triggering of bacterial phagocytosis, neutralization of retroviruses and safeguarding of immune tolerance via the removal of apoptotic cells<sup>4</sup>. C1q receptors are expressed on different cell types, including leukocytes, fibroblasts,

platelets, endothelial cells and renal mesangial cells<sup>4</sup>. Furthermore, auto-antibodies against C1q have been found in a number of autoimmune diseases, specifically LN<sup>5</sup>, were they have a high negative predictive value in the detection of active disease.<sup>5</sup> Knowledge of pathogenesis of immune-mediated diseases will help in development of targeted therapy to block complement activation.<sup>6</sup>

C1qN is an uncommon entity, where C1q-containing immune deposits are detected in the mesangium. It is considered an immune complex-mediated glomerulonephritis. The term was initially proposed by Jennette and Hipp<sup>1</sup> in 1985. Pathophysiology of C1qN had been an issue of controversy for the past 30 years, mostly regarding whether C1qN is a single disease, or a mixture of several diseases. Many researchers think of C1qN as subgroup of primary FSGS<sup>7</sup>. However, many others proposed it as a separate disorder as evidenced by different clinical, histopathological features, therapeutic responses and prognoses.<sup>8</sup> There are other uncertainties about C1qN that require further investigations. First, detection of C1q deposition in other types of GN, like LN. However, LN can be distinguished from C1qN<sup>4</sup>, on clinical and serologic backgrounds, and by the fact that C1q deposition is accompanied by a full-house pattern of immunoglobulin deposition.<sup>5</sup> Therefore, C1qN should be only diagnosed after exclusion of various types of immune complex-mediated GN.<sup>8</sup> Second, is the clinical and microscopic heterogeneity, as detailed below:

From a histopathological perspective, this disorder refers to C1q deposition escorted by glomerular injury with a broad range of potential findings, including MCD-like disease, FSGS, and MP pattern. The diagnostic criteria of C1qN were set in 1985 by Jennette et al.<sup>1</sup>, and those include: (1) dominant or co-dominant mesangial IF staining for C1q; (2) mesangial electron-dense deposits by EM; and (3) and absence of clinical and serologic (antinuclear antibodies) evidence of LN. It has been disputed that the LM heterogeneity in C1qN is akin to that of IgA nephropathy, and thus the diagnosis should be determined by C1q staining<sup>1,9</sup> either by IHCs or IF. However, several points should be excluded first: the presence of another immune-complex disease

**Table II.** Summary of Pediatric C1qN Case Series.

Series	Current study	Mokhtar et al. 17	Tullus et al. 16	Roberti et al. 15	Wong et al. 14	Fukuma et al. 13	Kernisk et al. 12	Lau et al. 11	Iskandar et al. 10
Year	2016	2015	2014	2009	2009	2006	2005	2005	1991
Sample size, N	53	750	50	264	55	2221	131	319	91
Biopsy	NS	Native	Native with C1q deposition	Native & Tx	Refractory NS	Native	Native	native	NS, nephritis
Number of C1qN	5	11	35	14	9	30	12	20	15
Incidence, %	9.4	1.5	*	6.6	16.3	1.4	9	6.3	16
Gender, M:F	4:1	5:6	24:11	7:7	5:4	18:12	4:8	11:9	5:10
Mean age, years	7.4	3.7	4.5	10.7	2.3	10.5	10.2	10.4	8.8
Follow-up, mo.	42	56.4	49	34	60	58	48	36	30
Presentation	NS	NS	NS	NS	NS	NS	NS	NS	Renal insuff.
Light microscopy	MP	MH	MCD	MP	MCD	MCD	MCD; FSGS	FSGS	MCD
FR, %	75	73	20	30	44	67	0	0	22
SD, %	75	18	20	0	33	0	50	33	22
SR, %	25	82	47	40	22	8	38	33	44
ESRD, %	20	9	6	0	0	8	50	22	11

NS nephrotic: syndrome; Tx: transplant; FUP: follow up period; insuff: insufficiency; FR: frequent relapse; SD: steroid relapse; SR: steroid resistant; ESRD: end stage renal disease.

associated with co-deposition of C1q, like LN, IgA, or IgM nephropathy; and second, the false positive results of C1q trapping in sclerosed segments or globally sclerosed glomeruli, as previously noted.<sup>7</sup>

Due to the recent awareness of C1qN and its rarity, the number of comprehensive large-scale studies on C1qN had been modest. Besides some case reports and small adult case series, there had been eight exclusively pediatric case series of C1qN.<sup>9-17</sup> Major clinicopathological parameters of those pediatric case series are summarized in Table II.

Generally, the incidence of C1qN shows a broad range among different pediatric and adult studies, ranging from as low as 0.2% to as high as 16.5% in pediatric cases with persistent NS.<sup>6,12</sup> Our study had an incidence of 9.4% among pediatric NS cases. The wide spectrum of incidence among the different studies is due to the study population, as it is much higher in NS patients compared to random studies on general biopsy populations. Another recent study from Middle East, focusing on SR-NS patients, had showed that the incidence of C1qN was about 8%.<sup>18</sup> The mean age among those studies ranges from as young as 2.3 to 10.7 yrs. The gender distribution had moved back and forth in favor of male gender in 4 previous studies, while it had a female predominance in 3 others, and showed equal incidence in one other study.

The optimal management of C1qN is not clearly defined yet. Corticosteroids have been used as a first line with diverse results, with some studies showing poor response to oral corticosteroid therapy<sup>10</sup>, while others shows superior results.<sup>14</sup>

There had been substantial disagreement over the outcome of patients with C1qN.

As it had been previously described, the outcomes of patients with C1qN can be predicted by an array of clinical and histopathological variables.<sup>6</sup> Precautious evaluation of the previous pediatric case series makes it clear that the dominant LM pattern had a higher impact in predicting disease progression and outcome at FFU, even regardless of C1q deposition.<sup>8</sup> Tulluset al.<sup>16</sup> had confirmed that cases presenting with a LM picture of MCD had better general outcome compared to those with FSGS, or MP. They

also show better response to steroids, although not statistically significant.<sup>16</sup> Nevertheless, patients with MCD/C1q deposition seemed to need second-line drugs as cyclosporine despite having comparable renal outcome at FFU.<sup>14</sup>

Regarding overall disease progression to ESRD, some studies failed to show any statistical significance distinguishing cases with C1q from non-C1q patterns, and both patient groups exhibited an analogous outline.<sup>16</sup>

In conclusion, our current study had comparable results to previous pediatric case series in clinical and histologic parameters. MP was the most common LM pattern. All cases presented clinically as NS. SD-NS was the most frequent outcome, however, with no statistical impact on progression to renal failure. The small number in our study hinders accurate statistical assurance, however, our findings also point towards a superior role of LM pattern over C1q deposits regarding disease progression. It may, also signify need of second line drugs in C1qN. Larger studies are needed to establish solid management guidelines of C1qN.

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