ORIGINAL ARTICLE

C1Q NEPHROPATHY: A MULTIFACETED DISEASE WITH INFREQUENT DIAGNOSIS

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**Background:** C1q nephropathy (C1qN) is a rare glomerulopathy, with a very low prevalence worldwide varying from 0.2 to 2.5%. Even though more than three decades have passed since this entity was first explained, still, it remains a dilemma for many due to the rarity of this lesion. This study was carried out principally to determine the clinical presentation, morphologic features and distribution of C1qN in our region based on renal biopsies studied by light microscopy (LM), and immunofluorescence (IF) so that this entity is better understood both by nephrologists and pathologists as no such study has ever been conducted in Pakistan to our knowledge. **Methods:** It was a cross-sectional study carried out from 1st January 2012 to 30th December 2016 in Histopathology department, Shifa International Hospital. All cases diagnosed as C1q nephropathy were retrieved from the hospital’s computerized database. Their clinical profiles, morphology and immunohistochemical profiles were studied. **Results:** Over this period a total of 31 cases were diagnosed with C1qN. Mean age of the patients was 32.09±18.66 years. The most common clinical presentation was nephrotic syndrome seen in 22 (71%) patients. The most frequent morphological pattern seen was minimal change disease (MCD) in 13 (41.9%) cases. All cases showed dominant 22 (71%) or codominant 9 (42.9%) mesangial±membranous C1q deposition. No correlation was found (p-value >0.05) between morphological pattern and clinical presentation of the disease or immunofluorescence findings. **Conclusion:** C1qN is a rare entity which is primarily diagnosed on the basis of immunofluorescence findings with a dominant or codominant fluorescent intensity for C1q. It is recommended that C1qN is sought for preferably with immunofluorescence staining of biopsies for immune reactants, especially for C1q. Studies from this part of the world are strongly recommended to predict clinical outcome and treatment options.

**Keywords:** C1q nephropathy; Immunofluorescence; Immune reactants; Histopathological patterns


INTRODUCTION

C1q nephropathy is a rare glomerulopathy, usually in children and young adults. Worldwide it has a very low prevalence; varying from 0.2 to 2.5%. This prevalence is higher in pediatric renal biopsies approaching up to 9.2% in some studies. It is a controversial entity which was first described by Jennette and Hipp in 1985. They evaluated a total of 800 renal biopsies, of which 15 showed predominant C1q mesangial deposition along with C3 and immunoglobulins (Ig). None of the patients had any clinical or serological evidence of systemic lupus erythematosus (SLE). On electron microscopy, these patients had strong electron dense deposits in the mesangium, with few cases showing deposition along capillary walls in addition to the mesangial deposits.

The etiopathogenesis of this rare disease is unclear. C1q is a key member of the complement system. The complement system, in turn, is comprised of about 40 soluble proteins and membrane receptors which take part in host immunity by activation of complement cascades through antibody-dependent and antibody independent pathways. This system plays an important role in immune-mediated disorders. C1 is the first member of the complement system. It is a pentamer that is composed of C1q and two C1r and C1s molecules. The C1q is a 410-kilodalton glycoprotein molecule. It is produced mostly by antigen presenting cells including monocytes and macrophages and plays a key role in the activation of the classical pathway of the complement activation leading to the formation of the membrane attack complex. Receptors specific to C1q are found in the mesangial cells of the kidney. The finding of C1q and immunoglobulin deposition in the glomeruli raise the possibility of an immune complex mechanism involved in the pathogenesis of this disease. However, the precise mechanism by which immune complexes have an affinity to the renal mesangial cells is still uncertain and no specific antigen has been identified.

To date, only a limited number of large-scale studies have been carried out for a better understanding of this rare glomerulopathy. Variable clinical presentations have been reported in the literature with most of the studies emphasizing on either isolated proteinuria or nephrotic syndrome as the dominant clinical picture. These patients also frequently show resistance to treatment by steroids. On routine light microscopy, different morphological patterns have been
described. A study by Satoshi et al of 61 renal biopsies showed that predominant morphological pattern was that of minimal change disease (MCD) in 46 patients (75%), focal or diffuse mesangial proliferative glomerulonephritis (PGN) in 7 (12%) and focal segmental glomerulosclerosis (FSGS) in 8 (13%) patients.9

On immunofluorescence all cases of C1q nephropathy show C1q deposition in mesangium in either dominant or co-dominant pattern. There is associated deposition of IgG and IgM in many cases since they serve as ligands for immune complex formation.6 In a study by Visjak of 72 cases of C1q nephropathy, a full house pattern was seen in 22 (30.6%) cases, there was associated IgG, IgM and IgA deposition seen in 66.7%, 80.6% and 47.2% of the cases. C3 and C4 deposits were also seen in many patients.10

Clinical prognosis and outcomes depend not only on clinical presentations of the patients but also on the morphological patterns seen on routine light microscopy. Few studies have hinted that C1q nephropathy may be clinically more aggressive as compared to other glomerulopathies, with a greater proportion of patients presenting with the steroid-resistant nephrotic syndrome.5 Other studies suggest that patients with isolated proteinuria and nephritic syndrome along with those having minimal change disease-like the pattern on routine light microscopy tend to have a more favorable outcome.11 Whatever the case may be, this spectrum of glomerulopathy poses a diagnostic challenge for nephrologists. Due to a variable clinical presentation, a clinical diagnosis is seldom warranted. Timely intervention and early management may be started if a close liaison is established with the nephropathologists.

Even though more than three decades have passed since this entity was first explained, still, it remains a dilemma for many due to the rarity of this entity. This study is undertaken to take into account the clinical presentations and histological patterns seen in our population as to date no such study has been published regarding C1q nephropathy from this region.

MATERIALS AND METHODS

It was a cross-sectional study. All renal biopsies received in the histopathology department of the tertiary care hospital from 1st January 2012 to 30th December 2016 were retrieved from the hospital’s computerized database. All cases diagnosed as C1q nephropathy during this time period were reviewed for the purpose of study. Their clinical profiles, histological and immunofluorescence patterns were studied. Patients of all ages and both genders were included. Cases showing clinical and/or serological evidence of systemic lupus nephritis (SLE) were excluded. Cases with negative lupus serology but showing full house positivity were also excluded if demonstrating hypocomplementemia so as to avoid confusion with “seronegative lupus”. Also excluded were cases showing features of Type 1 membranoproliferative Glomerulonephritis.

All the biopsy samples were subjected to 2–3 μ- thick paraffin sections for light microscopy (LM) and were stained with hematoxylin and eosin (H&E), Periodic acid Schiff (PAS), Gomori’s silver stain (GMS) and Trichrome stain. For direct immunofluorescence (IF) studies, anti-human IgA, IgG, IgM, C1q, and C3 anti-sera were used on the 3-μ-thick frozen sections. IF findings were graded as 0, ±, +1 through +3. Here, 0 stands for negative staining, ± for trace, +1 to +3 represent progressively increasing levels of positivity. Tubules and vessels near the glomerulus were considered as control.

Clinical presentations were documented using laboratory parameters and clinical information from patient’ record files. Following defining, criteria were used.

C1q nephropathy: It is defined by the presence of mesangial immune deposits that stain dominantly or co-dominantly for C1q accompanied by negative antinuclear antibodies (ANA) in patient’s serum and absence of clinical evidence for SLE.5 Cases with Type 1MPGN are considered as exclusion criteria.6

Nephrotic syndrome: Nephrotic-range proteinuria (urinary protein excretion >3.0 g/dl) hypoalbuminemia, hyperlipidemia, and edema.10

Nephritic syndrome: It is a collection of signs associated with renal disorder and includes hematuria, oliguria, mild proteinuria, and renal failure.12

Isolated proteinuria: Isolated proteinuria is defined as non-nephrotic range proteinuria without abnormalities in the urinary sediment, including hematuria, or a reduction in glomerular filtration rate (GFR), as well as the absence of hypertension or diabetes.13

Isolated hematuria: More than 5 red blood cells per high power field on microscopic examination of the urinary sediment.3

Statistical analysis: Data was analyzed using SPSS version 20.0. Mean and the standard deviation was calculated for quantitative variables like the patient’s age and Mean Immunofluorescent scores. Frequency and percentages were calculated for qualitative variables like gender, clinical presentation, morphological pattern and IF findings. For comparison, two groups were formed on the basis of morphological patterns. Minimal change disease (MCD) and Focal segmental glomerulosclerosis (FSGS) were considered as one group whereas Proliferative glomerulonephritis (PGN) was considered as the second group. For comparison of categorical variables, chi-square test was applied. Whereas, comparison of Mean was done through independent sample t-test. A p-value of <0.05 was considered to be statistically significant.
RESULTS

In our study period from 1st Jan 2012 to 30th December 2016, we received a total of 1700 medical renal biopsies. Of these 31 renal biopsies were included in our study which fulfilled the criteria for C1q nephropathy. The age range of the patients varied from 3.00 to 75.0 years with a mean age of 32.0±18.66 years. As regards gender distribution; there were 21 (67.7%) males and 10 (32.3%) females.

The most common clinical presentation was that of nephrotic syndrome seen in 22 (71%) patients, followed by isolated proteinuria in 4 (12.9%), nephrititic syndrome in 2 (6.5%), acute renal failure in 2 (6.5%) and isolated hematuria in 1 (3.2%) case.

Regarding histological features, the dominant morphological pattern seen on H&E was that of minimal change disease (MCD) seen in 13 (41.9%) cases, followed by proliferative glomerulonephritis (PGN) in 11 (35.5%) cases, Focal segmental glomerulosclerosis (FSGS) in 9 (2.9%) cases and combined FSGS + Mesangial proliferation (MP) in 2 (6.5%) cases. Two of the cases didn’t show any of these morphological patterns. 01 of them showed global sclerosis of all glomeruli whereas other case exhibited marked interstitial inflammation. Figure-1 & Table-1

On immunofluorescence 22 (71%) biopsies showed dominant C1q deposition whereas 9 (29%) cases showed co-dominant C1q deposition. This deposition was seen in mesangium only in 22/31 biopsies (71%) whereas 9/31(29%) biopsies showed deposition in both mesangium along with glomerular basement membranes. Full house immune complex deposition IgG, IgA, IgM, C3, and C1q was seen in 5/31 (16.1%) cases. Other immune reactants also showed variable percentage positivity along with C1q. Figure-2 & Table-2

Mean immunofluorescence scores (MFS) for C1q and other immune reactants was calculated for all C1q cases as well as separately for MCD + FSGS and PGN. The results showed that C1q had a much greater MFS of 2.70±0.46 when compared with other immune reactants. A comparative analysis was made between two morphological groups including MCD and FSGS on one hand since they both are podocytopenias and PGN which is recognised to be immune- complex-mediated on the other.10 The correlation between these two groups was analyzed in relation to differences in age, clinical presentations and immunofluorescence findings. However, the results were not found to be statistically significant (>0.05) and no correlation was found between morphological patterns and clinical presentations or immunofluorescence findings. Table-3.

Figure 1: Morphological patterns as seen on H & E. (A) Showing minimal change disease (MCD) like pattern. (B) Showing focal segmental glomerulosclerosis (FSGS). (C & D) Showing focal and diffuse proliferative glomerulonephritis. (PGN) (Original magnifications A & B [100X], C & D [200X])

Figure 2: Immuno-fluorescence staining intensity shown by different immune reactants: (A) Immunofluorescence staining for C1q shows, high-intensity (3+) positivity in the mesangial areas, with comma-like pattern. (B) C3 showing negative Immuno-fluorescence staining. (C) IgA showing 1+ immuno-fluorescence staining. (D) IgG showing 2+ immunofluorescence staining. (E) IgM showing 3+ immunofluorescence staining.
Table-1: Morphological patterns in relation to clinical presentation and immuno-fluorescence findings of 31 cases with C1q nephropathy

<table>
<thead>
<tr>
<th>Clinical Presentations</th>
<th>Morphological Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Data</td>
</tr>
<tr>
<td>No of cases</td>
<td>31</td>
</tr>
<tr>
<td>Age range (In Yrs)</td>
<td>3-75</td>
</tr>
<tr>
<td>Male:Female</td>
<td>2:1</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>22 (71%)</td>
</tr>
<tr>
<td>Isolated Proteinuria</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Nephritic syndrome</td>
<td>2 (6.5%)</td>
</tr>
<tr>
<td>Isolated haematuria</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td>ARF</td>
<td>2 (6.5%)</td>
</tr>
<tr>
<td>Distribution of C1q deposits</td>
<td></td>
</tr>
<tr>
<td>Mesangium only</td>
<td>22 (71%)</td>
</tr>
<tr>
<td>Mesangium + GBM</td>
<td>9 (29%)</td>
</tr>
<tr>
<td>Full House Positivity</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td>MFS* for C1q</td>
<td>2.70±0.46</td>
</tr>
</tbody>
</table>

*MFS= Mean Immuno-fluorescence score, †For statistical analysis FSGS (Focal segmental glomerulosclerosis) and MCD (Minimal change disease) were considered as one group where as PGN (Proliferative Glomerulonephritis) was considered as a separate group.

Table-2: Showing percentage positivity and Mean fluorescence score of various immune reactants seen in C1q nephropathy

<table>
<thead>
<tr>
<th>C1q</th>
<th>No of positive cases (%)</th>
<th>Mean Immuno-fluorescence Score (MFS) when positive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>100%</td>
<td>2.70±0.46</td>
</tr>
<tr>
<td>IgG</td>
<td>23 (74.2%)</td>
<td>1.71±0.92</td>
</tr>
<tr>
<td>IgA</td>
<td>10 (32.3%)</td>
<td>1.55±0.76</td>
</tr>
<tr>
<td>IgM</td>
<td>23 (74.2%)</td>
<td>1.26±0.70</td>
</tr>
<tr>
<td>C3</td>
<td>24 (77.41%)</td>
<td>1.77±0.84</td>
</tr>
</tbody>
</table>

*MFS= Mean Immuno-fluorescence score, †For statistical analysis FSGS (Focal segmental glomerulosclerosis) and MCD (Minimal change disease) were considered as one group where as PGN (Proliferative Glomerulonephritis) was considered as a separate group.

Table-3: Showing comparative analysis of Mean Immuno-fluorescence scores of individual immune reactants in FSGS+MCD and PGN

<table>
<thead>
<tr>
<th>Immune Reactants</th>
<th>Mean ± SD of Immuno-fluorescence score when positive (Group1)</th>
<th>Mean ± SD of Immuno-fluorescence score when positive (Group2)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Group1) MCD + FSGS</td>
<td>(Group2) PGN</td>
<td></td>
</tr>
<tr>
<td>C1q</td>
<td>2.72±0.46 (n=18)</td>
<td>2.63±0.50 (n=11)</td>
<td>0.64</td>
</tr>
<tr>
<td>IgG</td>
<td>1.92±0.10 (n=13)</td>
<td>1.27±0.56 (n=9)</td>
<td>0.10</td>
</tr>
<tr>
<td>IgA</td>
<td>2.00±0.63 (n=6)</td>
<td>0.83±0.28 (n=3)</td>
<td>0.02</td>
</tr>
<tr>
<td>IgM</td>
<td>1.20±0.72 (n=15)</td>
<td>1.37±0.69 (n=8)</td>
<td>0.58</td>
</tr>
<tr>
<td>C3</td>
<td>1.75±0.75 (n=14)</td>
<td>1.75±1.00 (n=8)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*MCD= Minimal change disease, FSGS= Focal segmental glomerulosclerosis, PGN=Proliferative glomerulonephritis.

DISCUSSION

C1q nephropathy is a rare glomerulopathy, with only a few studies previously reported from South Asia.14,18 Our study is the first study being reported in Pakistan. The diagnosis of C1q nephropathy is primarily histopathological. This entity is missed and under-recognized even in many tertiary care centers due to mostly to unavailability of immunohistochemistry and immuno-fluorescence-based techniques to detect immune deposits or due to a lack of awareness resulting in underutilization of anti C1q antibodies.

In our study, the mean age of the patients was 32.09±18.66years, with a wide age range varying from 3 to 75 years. Previous studies have emphasized that this disease is mostly seen in children and young adults with a mean age varying between 17–20 years in studies done worldwide.7,9 A retrospective study by Wong et al in children presenting with the steroid-resistant nephrotic syndrome, found that C1q nephropathy presents at a younger median age of 2.7 years.15 However, studies done in centers located in South Asia report a higher mean age.14 Our study establishes the fact that this disease has a much wider age variation than was previously thought and diagnosis of this entity must be considered in all ages.10,14

C1q nephropathy may have varied clinical presentations, ranging from isolated hematuria and non-nephrotic range proteinuria to frank nephritic and
nephrotic syndromes. Presence of renal insufficiency at the time of diagnosis may occasionally be encountered. The cases discussed herein also displayed heterogeneous clinical features, the most common of which was a nephrotic syndrome, followed by isolated proteinuria which is similar to findings documented in previous literature. Hitasho et al reported asymptomatic hematuria and/or proteinuria in 36 (59%) of his patients which were the most prevalent clinical finding. Gunasekara reported among pediatric patients, that out of 35 patients who fulfilled the criteria for C1q nephropathy, 31 (88%) had NS whereas remaining 4 (12%) had nonnephritic range proteinuria with or without hematuria. These remarkable diversifications in clinical presentation makes the disease difficult to suspect clinically and the diagnosis remains heavily dependent on histopathological features.

The current study revealed MCD to be the most frequent morphological pattern observed in 13 (38.1%) biopsies, this was closely followed by PGN in 11 (35.5%) biopsies and FSGS with or without mesangial proliferation in 5 (16.1%) cases. In the original study by Jennette and Hipp, the authors evaluated 800 renal biopsies of which 15 were identified as having C1q nephropathy. In this study, 13 cases in which light microscopic findings were available; MCD was seen in 2 cases, mesangial proliferation in 3, focal proliferative GN was seen in 5 and diffuse proliferative GN in 5 cases. Vizjak et al, with the largest number of cases (72) depicted that the most common histological pattern was that of MCD followed by FSGS (11) and proliferative GN (20). Another study carried out in Saudi Arabia, over a period of 11 years from 2001 to 2011, identified 11 patients with C1q nephropathy. Of these, 9 biopsies showed a variable degree of mesangial hypercellularity, whereas the remaining 2 cases showed FSGS. The results obtained from our study and literature review establish that this multifaceted entity may present with any of the aforementioned histopathological patterns.

On immunofluorescence, all of our biopsies showed intense granular deposition of C1q which was mostly dominant (71%) in the mesangium. Nine out of thirty-one cases (29%) cases showed deposition along capillary walls in addition to mesangium. Other studies have also shown almost similar findings. Markowitz et al described a cohort of 19 patients with C1q nephropathy, his results showed mesangial C1q deposition in 17/19 (89.5%) cases whereas 2/19 (10.5%) cases showed C1q deposition in mesangium along with capillary walls. Most of the cases (57.9%) showed dominant C1q deposition, the remaining showed co-dominant immune complex deposition. In our study full house immune complex positivity was seen in 5/31 (16%) cases, it is particularly important in such type of cases to rule out even the remote possibility of SLE through clinical history, signs & symptoms and serological tests before labeling it as a case of C1q nephropathy. C3, IgG and IgM deposition was also seen in 70-80% of cases in addition to C1q in varying combinations. Deposition of IgA in addition to C1q was seen in the lowest proportion of cases accounting for 10/31 (32.3%) cases. However, a particularly important finding noted in our study was that the mean fluorescence intensity of immune complex deposition on IF was significantly higher for C1q (MFS=2.7) in contrast to other immune reactants (MFS=1.26–1.77). Other studies have also shown that various other immunoglobulins can be positive in C1q nephropathy, as they serve as a ligand for C1q binding. The original study carried out by Vizjak showed the frequency of positivity for IgG, IgA, IgM, C3, and C4 to be 66%, 34%, 80%, 83%, and 35% respectively However this staining is either equal to or less than that of C1q. A study by Jamila et al carried out in Saudi Arabia, reported C1q nephropathy in two sisters of Pakistani origin both had full house positivity on immunofluorescence. In a study among local Saudi population, 3/11 biopsies showed full house immune complex deposition on IF whereas others showed C1q deposition in a dominant or codominant pattern in addition to other immunoglobulins.

In order to evaluate the correlation between morphological patterns in relation to clinical and histopathological parameters. We divided our data into two groups, one of which included podocytopenies (MCD + FSGS) whereas other included immune complex-mediated disease, i.e., PGN. Our results showed that except for mean immunofluorescence score of IgA, there was no correlation between these two groups and clinicopathological features. A literature search revealed only one study to date, which has compared the two morphological groups in terms of clinical and histological data. Vizjak et al in his study found a strong association of these two groups to most of the clinical, morphological and immunofluorescence parameters (p-value<0.05). This seems to be in striking contrast to our study which shows no such correlation between the two groups. As, C1q nephropathy, continues to be a lesser-known disease and to the best of our knowledge this was the only study apart from our own which has compared these two groups in terms of clinicopathological data, therefore, we believe that more large-scale studies need to be carried out in order to authenticate the findings. Moreover, this difference could have arisen as a result of the difference in population characteristics. Our cases were all of the South Asian descent whereas the correlation study presented by Vizjak was conducted in North America. In addition,
the difference might have been due to a limited number of cases in our study as although our hospital is a renal referral center but owing to the rarity of the disease the no of cases is limited. Likewise, on a global level, only a few large-scale studies have been published regarding this entity.19,20 We believe that a multiple center study must be undertaken to learn more about this disease which will open doors for research and treatment options.

The prognosis of C1q nephropathy depends not only on the clinical presentation but also on the morphological presentations. Studies have shown that patients with FSGS have worse clinical outcome than those with MCD.13 Other studies have also shown that children with diffuse and extensive C1q had difficult nephrosis and follow a more complicated course compared to those with mild to moderate C1q deposition or just patchy staining.8 The main limitation of our study was that we were unable to do follow-up with our patients, as many of them were outside referrals who continued with their treatment in their respective areas.

CONCLUSION
C1q nephropathy, is a rare but distinct clinicopathological entity with varying presentations and histological patterns. It is primarily diagnosed on the basis of immunofluorescence findings with a dominant or codominant fluorescent intensity for C1q. It is important to exclude SLE on the basis of clinical features and antinuclear antibodies test particularly if a full house of immune reactants is detected. It is a rare disease mostly detected in younger (mean age=33yrs) age group. It is recommended that C1q nephropathy is sought for preferably with immunofluorescence staining of biopsies for immune reactants, especially for C1q. Studies from this part of the world are strongly recommended to predict clinical outcome and treatment options.

AUTHORS’ CONTRIBUTION
NT, HN, TA: Study design, data collection, data analysis, write-up, proof reading. MU: Data analysis, data collection, write-up, proof reading. KSA: Design of manuscript, data collection, critical review.

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