Section: Nephrology

Original Research Article

A STUDY ON CLINICO-PATHOLOGICAL FEATURES, COMPLEMENT ABNORMALITIES, MANAGEMENT AND OUTCOMES OF C3 GLOMERULOPATHY IN A TERTIARY CARE CENTRE FROM SOUTH INDIA-RETROSPECTIVE STUDY

Leelavathi V [1], Bhushan C Shetty[2], Sujatha Siddappa[3]

[1]Associate Professor of Nephrology, Institute of Nephrourology, Bangalore, Karnataka, India.
[2]Assistant Professor of Nephrology, Kasturba Medical College Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India.
[3]Professor of Pathology, Institute of Nephrourology, Bangalore, Karnataka, India.

Received : 14/05/2023
Received in revised form : 07/06/2023
Accepted : 17/06/2023

Keywords:
C3 Glomerulopathy, complement abnormalities, treatment, outcome.

Corresponding Author:
Dr. Leelavathi V,
Email: leelavathi.v@gmail.com

DOI: 10.47009/jamp.2023.5.3.416

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5(3); 2113-2118

Abstract

Background:C3 glomerulopathy (C3GP) is a recently described complement-mediated disorder that shows glomerulonephritis on light microscopy (LM), and bright complement 3 (C3) staining in the absence of significant deposition of immunoglobulin (lg) on immune fluorescence microscopy (IF). C3Gp comprises C3 glomerulonephritis (C3GN) and Dense Deposit Disease (DDD). **Materials and Methods:** It is a retrospective cross-sectional study that included patients whose renal biopsy fulfilled the criteria for the diagnosis of C3GP. The demographic details, clinical, biochemical, treatment history, and outcomes of these patients were analysed using appropriate statistical packages. **Results:** Among 1130 renal biopsies done for various indications during the study period, 34 (3.0%) were confirmed to have C3GP. Among these, 97% were C3GN, and 3% were DDD. The mean age was 32 years. The majority of them were male. Hypertension was present in 64.7% of patients. Microscopic haematuria was found in 76.5% of patients. Complement C3 was low in 61.8% of patients. Autoantibodies to factor H were elevated in 50% of patients tested. On LM, mesangial proliferation (47%) was the most common morphological pattern. Patients were treated with steroids, intravenous cyclophosphamide, and plasmapheresis. The mean follow-up was 6.2 months from the date of diagnosis. Complete remission and partial remission were achieved among 12 (38.7%) patients in each group. Five (16.2%) deaths occurred during the study period. **Conclusion:** The incidence of C3GP was 3% during the study period. The most common presentation was acute nephritic syndrome. Most patients responded to steroids and cytotoxic drugs. Plasma therapy was indicated in patients with dialysis-dependent states, cresentic forms on renal biopsy, and elevated CFH autoantibodies.

INTRODUCTION

"C3 glomerulopathy" (C3GP) is a recently described complement mediated disorder that shows glomerulonephritis on light microscopy (LM), bright Complement 3 (C3) staining in the absence of significant deposition of immunoglobulin (lg) on immune fluorescence microscopy (IF). It comprises C3 glomerulonephritis (C3GN), dense deposit disease (DDD), and complement factor H-related protein 5 (CFHR5) nephropathy (familial form).[1] Both C3GN and DDD can be differentiated by electron microscopy (EM) only. EM shows varying degrees of mesangial, subendothelial, intramembranous, and subepithelial deposits in C3GN, while sausage-shaped diffuse intramembranous dense osmiophillic deposits is present along the glomerular basement membranes (GBM) in DDD. Absence or trace Ig staining on IF helps distinguish C3GP from immune-complex mediated glomenrulonephritis.[2]

The primary pathogenic process in C3GP is due to uncontrolled alternative complement (AP) pathway activation, deposition, or degradation. AP activation occurs spontaneously at a low level in the circulation due to hydrolysis of the internal thioester bond of the C3 molecule ('C3 tick over'). C3 activation generates fragments C3a (anaphylatoxin) and C3b, the latter binding complement factor B (CF B) to form the AP C3 convertase (C3bBb) of AP, this process is further amplified by a positive feedback mechanism that produces millions of C3b molecules.[3] The binding

ISSN(O): 2687-5365; ISSN(P): 2753-6556

of additional C3b molecules to the AP C3 convertase generates a C5 convertase that activates C5, yielding fragments of C5a anaphylatoxin and C5b. C5b initiates terminal pathway activation, resulting in the formation of the membrane attack complex (MAC, C5b-9) and cell lysis.

The AP is inhibited by several regulatory proteins present both in the circulation and on cell surfaces. Complement factor H (CFH), CF I (CFI), membrane cofactor protein (MCP), and five CF H-related proteins (CFHRI-5) are complement regulators involved in the pathogenesis of C3GP.

Genetic and acquired (mediated through autoantibodies) defects of the complement components and their regulatory proteins leading to uncontrolled activity of the alternative pathway (AP) of complement have been described in patients with C3GF.[4]

The objective of this study was to analyse the clinical features, spectrum of abnormalities in renal biopsy, complement factor abnormalities, treatment, and outcomes in patients with C3GP.

MATERIALS AND METHODS

Present study was conducted at Institute of Nephrourology, Bangalore, Karnataka were studied.

Inclusion Criteria

The patients whose renal biopsy fulfilled the criteria for diagnosis of C3GP (from May 2015 – October 2016), were selected for study.

Exclusion Criteria

Those patients with incomplete biopsy reports (without IF) were excluded from the study.

Method

The light microscopic picture was used to document various morphological patterns like, isolated mesangial proliferation GN. Diffuse exudative GN, membrano proliferative GN (MPGN). Confirmation of C3GP was made when there was bright staining with C3 (3+) on IF and absence or trace staining with immunoglobulin (lg).

Autoantibody to factor H was done by ELISA method. Electron microscopic examination and Genetic defect study done whenever possible. Depending on clinical features, renal biopsy findings, various combinations of treatments advised.

Definitions

Complete remission: Reduction of proteinuria to < 0.3 g/d and creatinine clearance of > 60 ml/min/1.73m2 with serum albumin >3.5gm/dl.

Partial remission: Reduction of proteinuria to <3.5 g/day, or a decrease in proteinuria >50% from baseline stable serum creatinine (with reduction > 25% from baseline). Advanced renal failure: Dialysis dependent renal failure or eGFR<15 ml/min.

Statistical Analysis

The demographic details, clinical, biochemical, treatment history, short term outcomes of patients were analysed using standard Epilnfo and SPSS version 16.0 for frequency and chi square test. informed consent was obtained from all the patients.

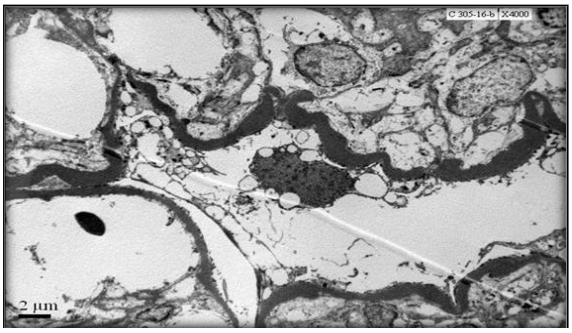


Figure 1: EM showing intramembranous osimophillic electron dense deposit in capillary basement membrane and mesangial deposits in DDD

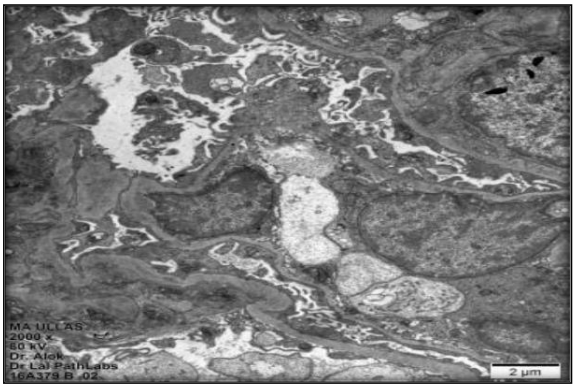


Figure 2: EM showing scattered mesangial&paramesangial& occasional sub endothelial, conventional electron dense deposits are identified in C3GN

RESULTS

The results of 34 diagnosed cases with C3GP are as follows:

Table-1: The mean age of patients was 32 years (range 6 to 72 years). Of these, 11 patients (32.3%) were less than 18 years old. The majority of them were male 25 (73.5%). Pre biopsy diagnoses included acute nephritic syndrome (ANS) in 15 (44.1%), RPGN in 10 (29.4%), nephrotic syndrome (NS) in 5 (14.7%), NDRD in 3 (8.9%), CKD of unexplained aetiology in 1 (2.9%) patient.

Table-2: LM included mesagial proliferative glomerulonephritis (GN) in 16 (47.6%) patients, Mild to moderate IFTA was seen in 30 (88.2%) cases. On IF, majority of the biopsies 29 (85.2%) revealed C3 deposition in both mesangium (M) and capillary wall (CW). Only one (2.9%) of these had tubular deposition of C3. EM was done in 5 (14.7%) patients, 3 revealed sclerosed glomeruli and in remaining 2 patients, 1 was diagnosed as DDD (Fig 1), other one is C3GN (Fig 2).

Table-3: Fifteen (44.1%) patients received combinations of steroids and cyclophosphamide, 5 sessions of plasmapheresis. Eleven (32.5%) patients received prednisone pulse, followed by oral prednisone and IV cyclophosphamide monthly for up

ISSN(O): 2687-5365; ISSN(P): 2753-6556

to 6 months. Five patients (14.7%) were given oral steroids alone. Six (17.6%) patients were dialysis-dependent at the time of presentation (5 RPGN and 1 CKD). Those patients who were dialysis dependent at the end of 3 months discontinued immunosuppressive treatment.

Table-4: A mean follow-up of 6.2 months is available for 31 patients from the date of diagnosis. The majority of 24 (77.4%) of them achieved complete remission or partial remission. One patient (3.2%) (NDRD) has progressed to end stage renal disease at the end of one year. The mean eGFR at the end of the follow up period was 51.5 ml/min/1.73m2.

Table 1: Demographic, clinical and laboratory profile of patients with C3GP

Category	Total (34)	C3GN (33)	DDD (1)
Age(yr)			
< 18	11 (32.3%)	10	1
19 – 30	5 (14.7%)	5	
31 – 40	7 (20.6%)	7	
41 – 50	7 (20.6%)	7	
> 50	4 (11.8%)	4	
Sex			
Female	9 (26.5%)	8	1
Male	25 (73.5%)	24	
Clinical Diagnosis			
ANS	15 (44.1%)	14	1
RPGN	10 (29.4%)	10	
NS	5 (14.7%)	5	
NDRD	3 (8.9%)	3	
CKD (Unexplained aetiology)	1 (2.9%)	1	
Hypertension			
Normotensive	12 (35.3%)	11	1
Hypertensive	22 (64.7%)	22	
Proteinuria (mg/day)			
< 500 mg	8 (23.5%)	8	
500 mg – 3500 mg	10 (29.4%)	10	
> 3500 mg	16 (47.1%)	15	1
Haematuria			
Nil	7 (20.6%)	7	
Microscopic	26 (76.5%)	25	1
Frank	1 (2.9%)	1	
Haemoglobin (g/dl)			
< 10	12 (35.3%)	12	
> 10	22 (64.7%)	21	1
eGFR (MDRD)			
< 15	6 (17.6%)	6	
16 – 30	5 (14.7%)	5	
30 – 60	4 (11.8%)	4	
60 – 90	3 (8.8%)	3	
> 90	16 (47.1)	15	1
S.Albumin (g/dl)			
< 3.5	30 (88.2%)	29	1
> 3.5	4 (11.8%)	4	
Complement C3			
Normal	13 (38.2%)	12	1
Low	21 (61.8%)	21	
Complement C4			
Normal	33 (97.1%)	32	1
Low	1 (2.9%)	1	
CFH Autoantibody			
Normal	8 (23.5%)	8	
High	8 (23.5%)	7	1
Not available	18 (53.0%)	18	

(ANS- Acute nephritic syndrome, RPGN-rapidly progressive glomerular nephritis, NS- nephrotic syndrome, NDRD-nondiabetic renal disease. CKD (chronic kidney disease) eGFR (estimated glomerular filtration rate). MDRD-modified diet in renal disesase.CF H-Complement factor H.).

ISSN(O): 2687-5365; ISSN(P): 2753-6556

Table 2: Histo-pathological pattern of C3 Glomerolopathy

Feature	Total	C3GN	DDD
Light microscopy Pattern of injury/with cresents			
Mesangial proliferation	16 (47.0%) / 9	16 / 9	
Diffuse exudative	11 (32.3%) / 4	11 / 4	
MPGN	7 (20.5%) / 5	6 / 4	1/1
Global sclerosis (% glomeruli)			
Absent	20 (58.8%)	20	
<50%	12 (35.3%)	11	1
>50%	2 (5.9%)	2	
IFTA			
None	2 (5.9%)	1	1
1-25%	23 (67.6%)	23	
26 -50%	7 (20.6%)	7	
>50%	2 (5.9%)	2	
Arteriolar sclerosis			
None	25 (73.5%)	24	1
Mild	7 (20.6%)	7	
Moderate	2 (5.9%)	2	
Immunofluorescence			
C3 mesangium+ capillary wall (CW)	29 (85.2%)	29	
C3 in mesangium (M)	3 (8.8%)	3	
C3 in capillary wall (CW)	2 (5.9%)	1	1
Electron microscopy			
Sub endothelial + subepithelial + mesangial	1(2.9%)	1	
Intra-membranous deposits + mesangial deposits	1 (2.9%)		1

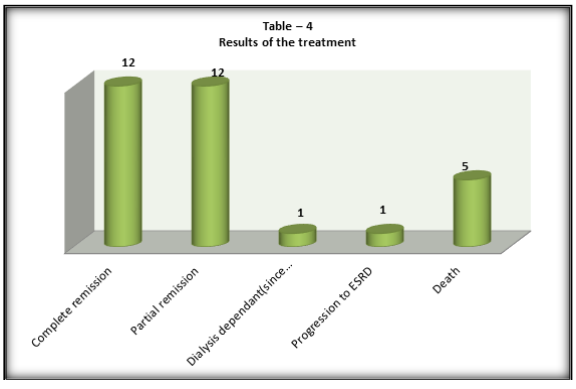
(IFTA-interstial fibrosis, tubular atropy,)

Table 3: The method of treatment

Treatment (n = 31)	No (%)
steroid (S) + cyclophosphamide (CYP) + Plasma Pheresis (PP)	15 (44.0%)
S+cyclophosphamide (CYP)	11 (32.5%)
steroid (S) alone	5 (14.7%)
Treatment not received	3 (8.8%)

Table 4: Results of the treatment

Outcome (n = 31)	No (%)
Complete remission	12 (38.7%)
Partial remission	12 (38.7%)
Dialysis dependant (since beginning)	1 (3.2%)
Progression to ESRD	1 (3.2%)
Death	5 (16.2%)



DISCUSSION

The present study provides a clinical description of 34 patients diagnosed to have C3GP as defined by IF microscopy and their clinical and histopathologic features, complement abnormalities, and short-term outcomes.

The C3GP comprised 3.0% of the total renal biopsy done during the study period. Studies.[6] found that 0.7% of their renal biopsies over a period of five years (2007-2012) were C3 GP, and studies over a period of 20 years (1992–2012) found that 1.34% of their renal biopsies were C3GP. A previous study over a period of three years (2010-2013) found that 1.16% of the renal biopsies were C3GP. It was described that, 12 patients with C3GN in 2012. These are retrospective studies done after the publication of the new consensus report in 2013 about the revised classification of membranoproliferative patterns (MPGN). The higher incidence of C3GP in the present study is due to increased recognition, it being a prospective one.[7]

Among the 34 patients diagnosed with C3GP, 97.0% had C3GN and 3% had DDD. The group with C3GN may comprise many cases of DDD that require EM for diagnosis.

In this study, most of them were males, 73.5% and according to previous studies.[8]51% were males, and according to recent studies, the sex distribution was equal.

ISSN(O): 2687-5365; ISSN(P): 2753-6556

Among diabetics biopsied with unexplained acute worsening of renal function or worsening of proteinuria, C3GP was present in 3 cases. One of them had monoclonal gammopathy of undetermined significance. It was observed in 10 patients in the retrospective study of 41 patients with C3GN, with a mean age of 54.5 years. Monoclonal Ig may also cause kidney injury indirectly through dysregulation of the alternative pathway (AP) of complement, which could result in C3GN.[9]

The prevalence of HTN was 47.37%. In this study, the mean 24 hour urinary protein was 3.0 gm/24 hours (range 0.08 gms to 8.6gms). The prevalence of the nephrotic range of proteinuria was found to be 47.1% in C3GP. It was also found that the nephrotic range of proteinuria was present in 40.7% of patients, which was comparable to our study.[10] found a nephrotic range of proteinuria in 15.7% of patients with C3GN.(11) Microscopic haematuria was present in 76.5% of the study subjects in this study.

Complement C3 and C4 levels were low in 21(61.8%) and 1(2.9%) patients. Autoantibodies to factor H done by ELISA method were elevated in 50% of patients tested (8 out of 16). Genetic studies to detect defect in complement components were done in 2 (5.8%) patients which did not find any pathogenic variants.

LM in this study showed mesangial proliferation at 47% was the most common form in C3GN, which is different from other studies done. Where MPGN was the most common finding, in this study cresents were found in mesangial proliferation (9 patients), diffuse exudative (4 patients), and MPGN pattern (5 patients). Many authors did not find cresentic forms in their studies.[11]

C3GP is a recently diagnosed entity for which there are no disease-specific effective therapies. Supportive treatment consists of ACEI or ARBs to control protenuria. In patients with pathogenic antibodies against complement regulatory proteins (CFH, CFI, and CFB), steroids, cytotoxic drugs, and rituximab are useful. Therapies, such as corticosteroids, cyclophosphamide, and calcineurin inhibitors, have been used in small numbers of patients with varied results in both DDD and C3GN. In patients with regulatory factor deficiency or with circulating C3 nephritic factor, plasmapheresis is the treatment of choice. Complement deficiency can be corrected by plasma infusion. The frequency and duration of plasma therapy are unclear.[12]

All patients were informed about the treatment aspects. It consists of steroids, cyclophosphamide (intravenous, monthly dose), and plasma exchanges (5 sessions) in view of the lack of genetic and serological testing for various complement abnormalities. Due to the recognised efficacy of plasmapheresis with fresh frozen plasma as a replacement fluid in patients with autoantibody and complement factor deficiency, it was recommended as a part of treatment. Plasma therapy is indicated in patients with progressive disease, a dialysis-dependent state, and documented CFH autoantibodies, cresentic form on renal biopsy. 44.1% of patients received a combination of steroids, cyclophosphamide, and plasma exchange. Others denied plasmapheresis given pulse steroids and IV monthly cyclophosphamide in 32.5% of patients. Those patients who refused hospital admission were treated with oral steroids in 14.7% of cases on an outpatient basis, as mentioned in Table 3. It was suggested that, to use steroids, cytotoxic drugs (cyclophosphamide, mycophenolatemofitil). None of these retrospective studies mentioned the use of plasmapheresis.(2,12)

In the present study, complete remission and partial remission were achieved among 12 (35.29%) patients in each group, and one patient (2.9%) progressed to ESRD.

Death occurred in 16.1% of patients in this study group, either due to sepsis or cardiovascular causes.

Older age, severe renal impairment, dialysis-dependent state, and cresentic form of renal biopsy were predictors of poor prognosis in patients with C3GP.

CONCLUSION

The present study includes a cohort of patients with C3GP and is reported from south India. The incidence of C3GP was 3% during the study period. The commonest presentation was ANS or RPGN. Mesangial proliferation with cresents was the commonest morphological pattern on renal biopsy. Renal function was normal in nearly half of the patients. Dialysis-dependent states were seen in a significant minority of the patients. Most patients responded to steroids and cytotoxic drugs. Plasma therapy is indicated in patients with dialysis-dependent states, cresentic forms on renal biopsy, and elevated CFH autoantibodies. Older age, severe renal impairment, dialysis-dependent state, and cresentic form of renal biopsy were predictors of poor prognosis in patients with C3GP. A larger study to evaluat findings.

Limitation of study

Due to lack of EM, genetic test, serology tests for complement nephritic factors, we have limited results and findings.

- There is no conflict of interest.
- Self-Funded.

REFERENCES

1. Pickering MC, D'Agati VD, Nester CM - C3 glomerulopathy: consensus report. Kidney Int. 2013 Dec;84(6):1079–89. PMID: 24172683;
2. Sethi S, Fervenza FC, Zhang Y - C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. Kidney Int. 2012 Aug;82(4):465-73. PMID: 22673887;
3. Barbour TD, Pickering MC, Cook HT - Recent insights into C3 glomerulopathyNephrol Dial Transplant. 2013 Jul;28(7):1685-93. PMID: 23479095;
4. Smith RJ, Alexander J - Dense Deposit Disease Focus Group New approaches to the treatment of dense deposit disease. J Am SocNephrol. 2007 Sep;18(9):2447–56. PMID: 17675665;

ISSN(O): 2687-5365; ISSN(P): 2753-6556

5. Gale DP, de Jorge EG - Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis Lancet. 2010 Sep 4;376(9743):794–80 PMID: 20800271.
6. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and a proposal for a new classification. SeminNephrol. 2011 Jul;31(4):341–8. PMID: 21839367.
7. Bomback AS, Appel GB. Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. Nat Rev Nephrol 2012; 8:634–42.
8. Fakhouri F, Frémeaux-Bacchi V - C3 glomerulopathy: A new classification. Nat Rev Nephrol 2010; 6:494–9.
9. Hou J, Markowitz GS - Towards a working definition of C3 glomerulopathy by immunofluorescence. Kidney Int 2014; 85:450–6.
10. Barbour TD, Pickering MC - Dense deposit disease and C3 glomerulopathy. SeminNephrol. 2013 Nov; 33(6): 493-507. PMID: 24161036;
11. Viswanathan GK, Nada R - Clinico-pathologic Spectrum of C3 Glomerulopathy: An Indian Experience DiagnPathol. 2015 Mar 17;10:6. PMID: 25889427.
12. Medjeral-Thomas NR, O'Shaughnessy MM - C3 glomerulopathy: clinicopathologic features and predictors of outcome. Clin J Am SocNephrol. 2014 Jan;9(1):46-53. PMID: 241789743.

ISSN(O): 2687-5365; ISSN(P): 2753-6556