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Original Article

ORIGINAL ARTICLE

Poor allograft outcome in Indian patients with post-transplant C3 glomerulopathy

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ABSTRACT

Background. Complement 3 glomerulopathy (C3G) results from dysfunction of the alternative complement pathway (ACP). No data are available on post-transplant C3G in South Asia.

Methods. In this study, renal allograft biopsies of C3G patients performed from 2012 to 2017 were analysed for ACP functional assay (APFA), serum complement levels, complement factor H (CFH), complement factor B (CFB) and autoantibodies to CFH and CFB. Limited genetic screening for *CFH/CFHR5* genes was carried out. All study patients were also followed up.

Results. A total of 21 cases of C3G were included, of which 11 had native C3G disease (that is, recurrent C3G). Of these 11 recurrent cases, 7 presented with allograft dysfunction and 4 with proteinuria and renal dysfunction. Early post-transplant recurrence (<1 month) was noted in six patients, whereas recurrence in five patients occurred within 8–17 months of transplant. Biopsies showed mild focal mesangial expansion with or without endocapillary proliferation and thrombotic microangiopathy. Rejection was also noted in six patients. APFA/C3 levels were low in all cases. Serum CFH levels were low [dense deposit disease (DDD), 44%; C3 glomerulonephritis (C3GN), 25%], whereas CFB levels were normal. Autoantibodies to CFH, CFB and C3 nephritic factor were present in 11, 0 and 44% of DDD cases, respectively, and in 17, 17 and 33% of C3GN cases, respectively. Genetic analysis revealed only non-pathogenic *CFH* gene variants (93%). No novel mutation was found. At follow-up (140 months), stable graft was noted in 28% of cases, progressive renal failure in 19%, graft loss in 34%, and 19% of patients died.

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Conclusion. Post-transplant C3G can present with graft dysfunction and/or proteinuria. Subtle histological findings demand careful interpretation of immunofluorescence results. Autoantibodies to complement pathway regulatory proteins are common, and no novel mutation has been found from limited genetic workup. Clinical outcome is poor.

Keywords: complement, end-stage renal disease, kidney transplantation, membranoproliferative glomerulonephritis, renal biopsy

INTRODUCTION

Complement 3 glomerulopathy (C3G), which includes dense deposit disease (DDD) and C3 glomerulonephritis (C3GN), is associated with abnormalities in the alternative complement pathway (ACP) [1–3]. Data on prevalence, progression, outcome and post-kidney transplant recurrence of C3G are still evolving, and reports from South Asia are scanty. Zand *et al*. published the largest study describing clinical findings, outcomes and detailed pathology of post-transplant C3GN (tx-C3GN) [4]. The authors observed that DDD and C3GN had almost similar rates of recurrence and post-transplant progression. To the best of our knowledge, studies on C3G documenting serological and genetic profiles in post-kidney transplant settings are limited to a few case reports only [5–9]. Most of the studies are from developed nations, and data from low-to-middle-income countries (LMICs) are sparse and likely to be different due to differences in triggering factors and genetic background. Investigating post-transplant C3G (tx-C3G) patients in LMICs would help in planning alternative strategies, such as intensive immunosuppression and plasma exchange, due to non-availability and unaffordability of eculizumab in these countries. Therefore, the aim of our study was to investigate C3G in post-kidney transplant patients in India.

MATERIALS AND METHODS

Study design

In this study, all renal allograft biopsies performed at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, from January 2012 to December 2017 were screened. The inclusion criterion was the presence of C3 as demonstrated by ≥2 magnitude positivity on immunofluorescence (IF), in the absence or presence of trace amounts of other immunoglobulins. Of a total of 1445 renal allograft biopsies, 26 cases of tx-C3G were identified. Electron microscopy (EM) results were not available in five cases. Hence, serological and genetic testing was performed in the remaining 21 cases of tx-C3G (Figure 1). For serological and genetic workup, a total of 6 ml blood was collected (3mL in plain vial and 3mL in EDTA vial). Serum/plasma/DNA samples were isolated and stored at –80°C for subsequent use. Due to limited funding, genetic analysis for CFH and CFHR5 genes were done in all tx-C3G cases.

Informed consent was obtained from all participating patients prior to sample collection. This study was approved by the Institute Ethics Committee (Histopath/13/2088).

Serology

The ACP was assessed by measuring alternative pathway functional assay (APFA) and by measuring complements (C3 and C4), complement factor H (CFH) and complement factor B (CFB) levels. Serum samples were screened for paraproteins and autoantibodies to CFH (ab-CFH), CFB (ab-CFB) and C3 convertase (C3Nef) as described previously by Sethi *et al*. [10] and Nada *et al*. [11]

Genetic workup

All exons of the *CFH* and *CFHR5* genes, as well as their adjoining intronic regions, were screened using polymerase chain reaction, followed by Sanger sequencing. DNA samples of 45 healthy individuals were collected, and all gene variants found in tx-C3G patients were also assessed for control cases [11].

Follow-up

Patients were treated as per their treating units' management protocols. All cases were followed up for a period of 5–140 months. End points at follow-up included stable graft, progressive disease/graft loss and death. Other transplant-related events, including rejection, drug toxicity, infections and recurrence of native disease, were also noted.

Statistical analysis

Data were expressed as number, percentage, mean and standard deviation (SD) or median and interquartile range, as appropriate. For comparisons between tx-DDD and tx-C3GN cases, the Statistical Package for Social Sciences, version 21 (SPSS Inc., Chicago, IL, USA) was used. A P-value <0.05 was considered as significant.

RESULTS

Clinical features

The study cohort comprised 21 patients diagnosed with tx-C3G (9 patients with tx-DDD; 12 with tx-C3GN). There was a male predominance, with a male:female ratio of 3:1, and the mean age was 31.33 ± 12.5 years (range 16–60 years). The prevalence of tx-DDD and tx-C3GN was 0.62 and 0.83%, respectively. The native disease was known in 12 patients (DDD: 6 patients; C3GN: 5 patients; type 1 diabetes mellitus: 1 patient), and unknown in 9 cases. Seven (33%) patients had a history of infections before onset of native disease. Three (14%) patients had a family history of renal disease. Live-related and deceased-donor transplantations accounted for 17 (81%) and 4 (19%) patients, respectively (Table 1).

Of 21 tx-C3G cases, 10 (47.6%) patients presented within 1 month of kidney transplant. Of the 11 recurrent cases, 7 patients presented with allograft dysfunction, and 4 with proteinuria and renal dysfunction. There was evidence of rejection and calcineurin inhibitor (CNI) toxicity in seven (33%) and three (14%) patients, respectively. Six (28.5%) patients received induction therapy, and the initial maintenance immunosuppression regimen consisted of tacrolimus, mycophenolate mofetil and oral prednisolone in all cases. Four patients received plasma exchange (PLEX) for the treatment of recurrent disease. Of four treated, immediate reduction in serum creatinine was noted in three patients, and all three patients who showed response to

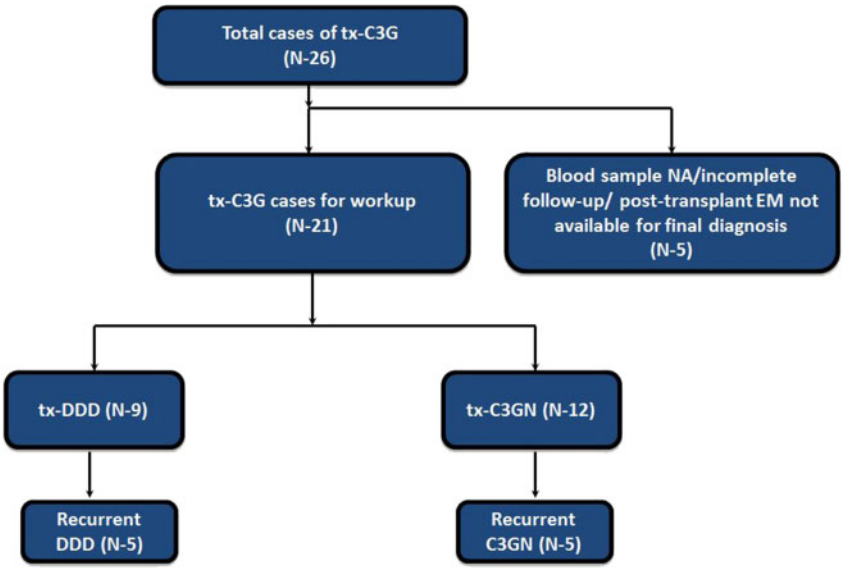

FIGURE. 1: Flow diagram showing an overview of C3GP cases for serological and genetic workup.

plasma exchange (PLEX) had autoantibodies to complement pathway regulators.

DDD

The mean time of progression to end-stage renal disease (ESRD) was <3 months in the majority of cases (78%) after an initial diagnosis of DDD in native biopsies. All nine cases of tx-DDD were diagnosed within 1 year post-transplant, with four (44.4%) patients presenting within 1 month and five (55.6%) patients between 1 and 12 months. Patients had allograft dysfunction either with proteinuria (28%) or without (72%). Patients who presented with DDD within 1 month had relatively higher mean serum creatinine levels (2.9 ± 1.01 mg/dL) and less proteinuria (0.9 ± 0.3 g/day), as compared with those who presented later.

C3GN

The mean time of progression to ESRD was 12.5 ± 11 months (range 4–24 months) after an initial diagnosis of C3GN in native biopsies. Six (50%) patients presented with ESRD within 1 month post-transplant, while the remaining six presented within 1–27 months. Patients had allograft dysfunction either with proteinuria (25%) or without (75%), as observed in patients with DDD. Patients who presented with C3GN within 1 month had a mean serum creatinine level of 2.87 ± 1.23 mg/dL and proteinuria of 2.5 ± 0.92 g/day. Patients who presented later had a mean serum creatinine level of 2.3 ± 0.96 mg/dL and proteinuria of 2.53 ± 2.19 g/day.

Pathology

In patients presenting early with tx-DDD, subtle histopathological findings were observed, including mild focal and segmental mesangial expansion with or without endocapillary proliferation. Cases of tx-DDD diagnosed after 1 year post-transplant showed an membranoproliferative glomerulonephritis (MPGN) pattern (Table 2; Figure 2). Patients with C3GN in native biopsies ($n = 3$) showed a mesangioproliferative pattern with crescents in 16.6% of cases. Thrombotic microangiopathy (TMA) was present in three cases of tx-DDD, but was not found in cases of tx-C3GN. Patients presenting with tx-C3GN also displayed similar histopathological findings to those who presented with tx-DDD after 1 year (Table 3; Figure 2). In the majority of cases presenting early (<1 month) with tx-C3G, IF showed focal and segmental staining for C3, with granular deposits mostly in the mesangium; in contrast, in cases that presented late, IF revealed global and diffuse C3 staining, similar to that seen in native MPGN cases. In all graft biopsies analysed, EM showed deposits limited in distribution and amount in early-presenting cases, compared with cases that presented late post-transplant. Late-presenting cases displayed intramembranous/mesangial osmiophilic deposits in DDD, and subendothelial/mesangial deposits in C3GN.

Serological profile

Low complement activity with APFA was obtained in all cases of tx-C3G. Low C3 levels were found in all cases of tx-C3GN and 89% of tx-DDD cases, whereas C4 levels were normal in all tx-C3G cases. CFH levels were low in 44% of tx-DDD cases and 25% of tx-C3GN cases. CFB levels were normal in the majority of tx-C3G cases, except in one case of tx-DDD and two cases of tx-C3GN that had low CFB levels. ab-CFH was present in one (11%) case of tx-DDD and two (17%) cases of tx-C3GN, whereas ab-CFB was present in only two (17%) cases of tx-C3GN. C3Nef was present in 44% of tx-DDD cases ($n = 4$) and 33% of tx-C3GN cases ($n = 4$) (Table 4). ab-CFB, ab-CFH and C3Nef were present in 62% of tx-C3G cases. Serum samples tested negative for paraproteins and chronic viral markers (hepatitis B, hepatitis C and human immunodeficiency virus I and II) in all tx-C3G cases. There was no statistically significant difference when we compared all the serological findings between tx-DDD and tx-C3GN cases ($P > 0.05$).

Genetic workup for *CFH* and *CFHR5* genes

A total of five *CFH* gene variants were detected in this post-transplant series. These variants were in exon 2 (rs800292, p.Val62Iso), exon 3 (IVS2-18TT insertion), exon 7 (rs1061147,

Table 1. Clinical details of pre-/post-transplant workup for DDD and C3GN patients

	Pre-transplant workup					Post-transplant workup							
Patient ID	Age/sex	Native disease	Time to progress to ESRD	Other findings	Donor	Induction and immunosuppression	Post-tx diagnosis	Post-tx presentation	Serum creatinine at presentation (mg/dL)	Proteinuria at presentation (g/day)	Serum creatinine at last follow-up (mg/dL)	Graft status	Post-transplant follow-up
1	27/M	DDD	3 months	History of TB	Deceased	ATG and TAC/MMF/steroids	DDD	<1 week	1.5	0.85	1.3	Graft loss	19 months
2	16/M	DDD	2 months	NA	Mother	None and TAC/MMF/steroids	DDD	<1 month	3.8	0.53	4.2	Graft loss	35 months
3	20/F	DDD	12 months	History of UTI	Mother	ATG and TAC/MMF/steroids	DDD	<1 week	2.0	3.4	1.3	Expired with stable graft function	18 months
4	19/F	DDD	3 months	History of UTI	Grandmother	ATG and TAC/MMF/steroids	DDD	2 weeks	2.9	1.2	6.5	Graft loss	40 months
5	26/M	Unknown	<1 month	Family history of renal disease	Wife	None and TAC/MMF/steroids	DDD	5 months	1.8	2.5	3.6	Progressive disease	87 months
6	23/M	Unknown	2 months	NA	Deceased	ATG and TAC/MMF/steroids	DDD	5 months	1.6	1.2	1.2	Expired with functioning graft	40 months
7	28/F	DDD	14 months	History of UTI	Mother	None and TAC/MMF/steroids	DDD	8 months	2.5	2.3	3.8	Graft loss	58 months
8	60/M	Unknown	<1 month	Alternative medicine	Son	None and TAC/MMF/steroids	DDD	8 months	2.0	NA	5.2	Graft loss	73 months
9	30/M	DDD	<1 month	Family history of renal disease	Mother	None and TAC/MMF/steroids	DDD	11 months	1.15	0.97	4.2	Progressive disease	32 months
10	25/M	Unknown	6 months	History of TB	Mother	None and TAC/MMF/steroids	C3GN	<1 week	5.0	0.9	3.1	Progressive disease	64 months
11	27/M	C3GN	<1 months	NA	Mother	None and TAC/MMF/steroids	C3GN	<1 week	2.4	NA	3.8	Expired	5 months
12	36/M	T1DM	30 months	NA	Wife	Basiliximab and TAC/MMF/steroids	C3GN	<1 week	2.6	2.5	1.5	Stable graft function	81 months
13	45/F	Unknown	6 months	NA	Deceased	Basiliximab and TAC/MMF/steroids	C3GN	<1 week	4.8	NA	1.3	Stable graft function	34 months
14	32/M	C3GN	12 months	NA	Mother	None and TAC/MMF/steroids	C3GN	1 week	1.45	2.5	2.9	Graft loss	28 months
15	48/M	Unknown	2 months	History of TB	Wife	Basiliximab and TAC/MMF/steroids	C3GN	<2 weeks	3.1	NA	1.2	Stable graft function	47 months
16	23/M	C3GN	8 months	NA	Mother	None and TAC/MMF/steroids	C3GN	12 months	2.2	3.4	5.6	Expired (graft loss)	24 months
17	21/M	C3GN	12 months	Family history of renal disease	Deceased	ATG and TAC/MMF/steroids	C3GN	12 months	1.9	NA	1.5	Stable graft function	48 months
18	23/M	C3GN	24 months	History of TB	Mother	None and TAC/MMF/steroids	C3GN	17 months	2.8	0.8	1.4	Stable graft function	42 months
19	40/M	Unknown	8 months	Alternative medicine	Sister	ATG and TAC/MMF/steroids	C3GN	24 months	1.9	NA	1.2	Stable graft function	51 months
20	35/M	Unknown	36 months	NA	Mother	None and TAC/MMF/steroids	C3GN	27 months	1.2	NA	3.3	Progressive disease	80 months
21	47/F	Unknown	6 months	NA	Mother	None and TAC/MMF/steroids	C3GN	15 months	3.7	3.9	4.2	Graft loss	140 months

ATG, anti-thymocyte globulin; F, female; M, male; MMF, mycophenolate mofetil; NA, not available; TAC, tacrolimus; TB, tuberculosis; UTI, urinary tract infection.

Table 2. Detailed histopathological findings in DDD

Patient ID	Pre-transplant morphology	Post-transplant morphology	IF findings	EM findings	Histological lesions (as per Banff classification)
1	MPGN	Mild mesangial proliferation	C3 (3+)	Intramembranous, mesangial	Non-specific changes
2	Mesangioproliferative pattern	Mild mesangial proliferation	C3 (3+), IgM (1+), kappa (1+)	Intramembranous	Non-specific changes
3	MPGN	Mild mesangial proliferation	C3 (2+), kappa (1+)	Intramembranous	Non-specific changes—first Bx Suggestive of CNI toxicity—second Bx
4	MPGN	Mild mesangial proliferation	C3 (3+), kappa (1+), lambda 1+	Intramembranous, mesangial	Chronic active TCMR (grade 1B)—first Bx Suggestive of ABMR (C4d negative)—second Bx IFTA grade 3—third Bx
5	NA	Mild mesangial proliferation	C3 (3+)	Intramembranous	IFTA grade 3, suggestive of CNI toxicity—first Bx Chronic active TCMR (grade 1B)—second Bx
6	NA	Mild focal and segmental mesangial expansion	C2 (2+)	Intramembranous	Active TCMR (grade 1A)
7	MPGN	Focal and segmental endocapillary and mesangioproliferative pattern	C3 (2+)	Intramembranous	Non-specific changes
8	NA	Mild mesangial proliferation	C3 (3+)	Intramembranous	IFTA grade 1B
9	Mesangiocapillary pattern	Mild mesangial proliferation	C3 (2+), IgM (1+)	Intramembranous	ATI, ANS

ABMR, antibody mediated rejection; ANS, arterionephrosclerosis; ATI, acute tubular injury; Bx, biopsy; IFTA, interstitial fibrosis and tubular atrophy; MPGN, membranoproliferative glomerulonephritis; NA, not available; TCMR, T cell mediated rejection.

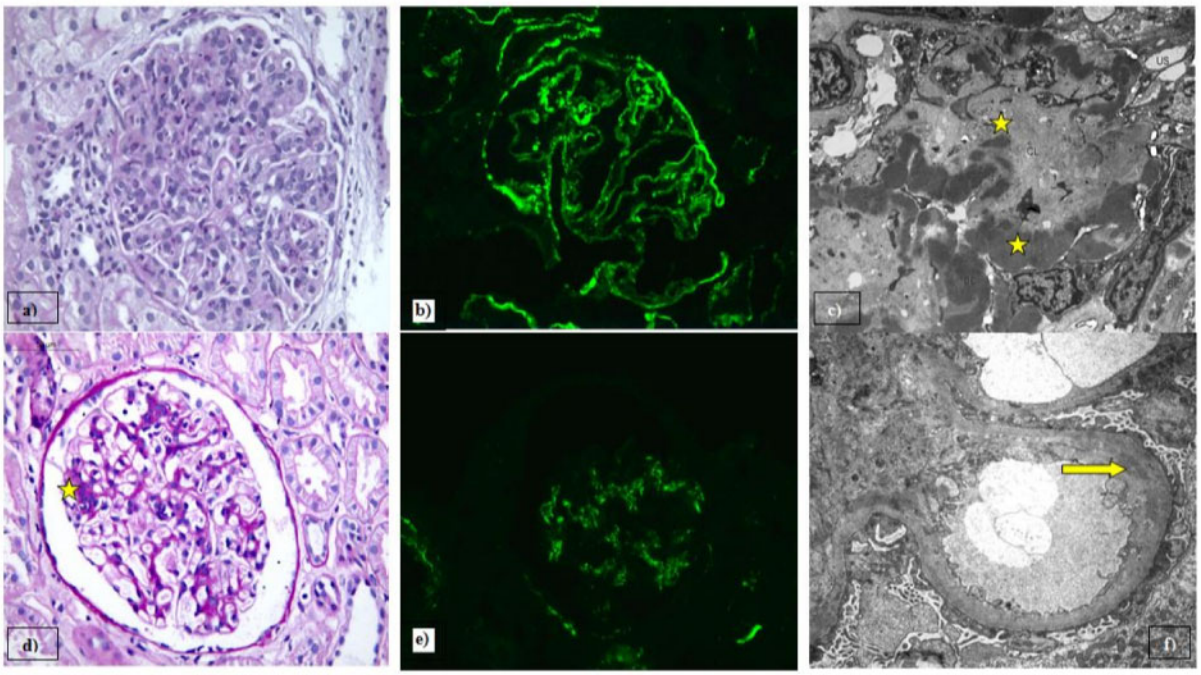

FIGURE. 2: A case of tx-DDD showing (**a**) a membranoproliferative pattern and interstitial foam cells; (**b**) IF showing coarse granular C3 in subendothelial (green color), mesangial (green color) and Bowman's capsule; and (**c**) EM showing dense osmiophilic intramembranous deposits (asterisks) with occasional mesangial deposits. A case of tx-C3GN showing (**d**) segmental mesangial proliferation (asterisk); (**e**) IF showing coarse granular C3 deposits in the mesangium and membranes and along Bowman's capsule; and (**f**) EM showing subendothelial immune complex-type deposits (arrow). (a) and (d) x20: Periodic Acid -Schiff (PAS) staining; (b) x20 and (e) x10: IF staining for C3 using fluorescein isocyanate (FITC) antibody; (c) x1500 and (f) x1200: Uranyl Acetate staining for EM, original magnification.

Table 3. Detailed histopathological findings in C3GN

Patient ID	Pre-transplant morphology	Post-transplant morphology	IF findings	EM findings	Histological lesions (as per Banff classification)
10	NA	Mild focal and segmental mesangial expansion	C3 (2+), IgM (1+)	Subendothelial, mesangial	Active TCMR (grade 1A), suggestive of active ABMR
11	MPGN	Mild mesangial proliferation	C3 (2+)	Subendothelial, mesangial	Non-specific changes
12	MPGN with refractile membranes	Mild mesangial proliferation	C3 (3+)	Subendothelial, mesangial	Non-specific changes
13	NA	Mild mesangial proliferation	C3 (3+), IgM (1+)	Subendothelial, mesangial	Non-specific changes, suggestive of CNI toxicity
14	Mesangial expansive pattern with collapse	Mild mesangial proliferation	C3 (3+), kappa (2+)	Subendothelial, mesangial	Non-specific changes—first Bx Non-specific changes, suggestive of CNI toxicity—second Bx
15	NA	Mild focal and segmental mesangial expansion	C3 (3+)	Mesangial	Acute TCMR (grade 1B)—first Bx Suspicious of ABMR (C4d equivocal positivity)- second Bx
16	MPGN	Mild mesangial proliferation	C3 (2+)	Subendothelial	Acute TCMR (grade 1A), suggestive of ABMR
17	MPGN	Mild focal and segmental mesangial expansion	C3 (3+)	Subendothelial, mesangial	Non-specific changes
18	MPGN	Mild mesangial proliferation	C3 (1+)	Subendothelial	Non-specific changes
19	NA	Mild mesangial proliferation	C3 (3+)	Subendothelial, mesangial	ANS
20	NA	Mild focal and segmental mesangial expansion with focal basement membrane thickening	C3 (3+)	Mesangial	Non-specific changes
21	NA	Mild mesangial proliferation	C3 (3+), IgM (1+)	Subendothelial, mesangial	Non-specific changes

ABMR, antibody mediated rejection; ANS, arterionephrosclerosis; Bx- biopsy; CNI, calcineurin inhibitor; MPGN, membranoproliferative glomerulonephritis; NA, not available; TCMR, T cell mediated rejection.

p.Ala243Ala), exon 9 (rs1061170, p.His402Tyr) and exon 10 (rs2274700, p.Ala473Ala) (Table 4). Mutational screening of all exons in the *CFHR5* gene did not show any variants. At least one *CFH* gene variant was present in 93% of cases. Two or more gene variants in combination were noted in a single case (Table 4). There was no statistically significant difference between the tx-DDD and tx-C3GN groups ($P > 0.05$).

Outcome

Mean follow-up period for tx-C3G patients was 50 months (5–140 months). We expressed patient outcome in terms of stable graft function, progressive renal failure, graft loss and death, and not according to treatment of recurrent disease. Of the 21 tx-C3G patients, stable graft function was observed in 33% ($n = 7$), progressive renal failure in 43% ($n = 9$), graft loss in 5% ($n = 1$) and death in 19% ($n = 4$) of patients. Patients in the tx-DDD group were followed up for 19–87 months. Two patients (22%) had progressive renal failure, and five (56%) progressed to graft loss. Two patients (22%) died with functioning graft at 16 and 40 months post-transplant, respectively, due to an unrelated cause (Table 1). In the tx-C3GN group, patients were followed up for 5–140 months. Stable graft function was observed in six patients (50%), and progressive renal failure in two (17%). Two patients (17%) died, one as a result of graft failure within a year (due to non-compliance and financial constraints) and the other at 24 months post-transplant with unknown reasons (Table 1). No correlation was found when we compared serological profiles with final patient outcomes.

DISCUSSION

In the present study, we evaluated the ACP (using serological and limited genetic analyses) in tx-C3G patients and compared these findings with clinical outcomes. Demographic profiles of tx-DDD and tx-C3GN patients were consistent with those from previous studies [1, 2, 4, 12]. In the present study, patients who presented early post-transplant (within 1 month) had increased serum creatinine levels only, whereas those who presented late post-transplant (>1 month) had proteinuria. Native biopsies from recurrent cases also showed MPGN/mesangiocapillary patterns, as noted in other case series [4, 13, 14]. Time of progression to ESRD was shorter for both tx-DDD and tx-C3GN patients (<1 year) in our study cohort, compared with Western patient populations (within 10 years for both DDD and C3GN) [4, 8, 15–17]. This rapid progression to ESRD in our study could be due to late presentation and unavailability/unaffordability of disease-specific therapy (eculizumab). Histological findings at the time of post-transplant diagnosis were subtle and included focal mesangial proliferation in both tx-DDD and tx-C3GN patients, in agreement with other histological reports in the literature [3, 4, 7, 8, 14–16, 18, 19].

There are few studies/case reports documenting the serology, ab-CFH/CFB or C3Nef and gene variants for C3G/atypical haemolytic uraemic syndrome (aHUS) in post-transplant

Table 4. Serological and genetic profiles of post-transplant DDD and C3GN

Patient ID[a]	C3 values (g/L)	C4 values (g/L)	APFA values (%)	CFH levels (mg/mL)	CFB levels (mg/mL)	ab-CFH	ab-CFB	C3Nef	CFH gene variants
1	0.31	0.26	12.81	161.24	264.57	0	0	0	ND
2	0.29	0.19	0.50	401.50	165.61	ND	0	0	rs1061147 c.921A>C, rs1061170 c.1204C>T
3	1.69	0.34	9.15	368.47	183.90	0	0	Positive	ND
4	0.67	0.33	1.35	106.13	185.69	0	0	Positive	rs800292 c.184G>A, rs1061147 c.921A>C, rs1061170 c.1204C>T, rs2274700 c.1419G>A
5	0.66	0.35	8.31	35.82	208.63	ND	0	Positive	rs800292 c.184G>A, IVS2-18TT, rs1061170 c.1204C>T, rs2274700 c.1419G>A
6	0.43	0.23	3.17	295.79	96.76	0	0	0	ND
7	0.23	0.33	10.61	136.23	217.96	Positive	0	0	ND
8	0.14	0.23	0.84	370.92	154.13	0	0	0	ND
9	0.36	0.35	10.50	444.63	38.68	ND	0	Positive	rs800292 c.184G>A, rs1061147 c.921A>C, rs1061170 c.1204C>T
10	0.33	0.19	9.15	409.32	127.13	0	0	0	rs1061147 c.921A>C, rs1061170 c.1204C>T
11	0.45	0.31	1.70	141.49	156.1	Positive	0	0	rs1061147 c.921A>C, rs1061170 c.1204C>T
12	0.23	0.25	8.65	223.71	30.76	0	Positive	0	IVS2-18TT, rs1061170 c.1204C>T
13	0.77	0.30	16.38	369.22	138.13	ND	0	0	rs1061147 c.921A>C, rs1061170 c.1204C>T
14	0.73	0.20	8.99	375.28	102.79	0	0	0	ND
15	0.69	0.19	9.49	393.44	125.19	3	0	Positive	rs1061170 c.1204C>T
16	0.23	0.22	9.32	359.89	112.45	0	0	Positive	rs1061147 c.921A>C, rs2274700 c.1419G>A
17	0.56	0.32	10.16	164.69	126.74	Positive	0	0	IVS2-18TT, rs1061170 c.1204C>T
18	0.23	0.30	9.83	396.71	114.57	0	0	Positive	ND
19	0.74	0.26	9.15	373.76	211.71	ND	0	Positive	ND
20	0.31	0.33	16.21	366.70	64.75	0	Positive	0	rs800292 c.184G>A, rs1061147 c.921A>C, rs1061170 c.1204C>T, rs2274700 c.1419G>A
21	0.34	0.32	8.99	354.35	99.90	3	0	0	rs800292 c.184G>A, IVS2-18TT, rs1061170 c.1204C>T

Normal values: C3, 0.89–1.87 g/L; C4, 0.165–0.38 g/L; APFA, 28–51%; CFH, 225–760 mg/mL; CFB, 85–227 mg/mL.
[a]Patients 1–9: DDD; patients 10–21: C3GN.
ND, not done.

Table 5. Comparison of tx-C3G findings

	Present study ($n = 21$)		Regunathan-Shenk *et al.* [30] ($n = 19$)		Zand *et al.* [4] ($n = 21$)
	Median follow-up 50 months		Median follow-up 76 months		Median follow-up 73.9 months
	DDD	C3GN	DDD	C3GN	C3GN
Recurrence rate (%)	67	42	85	83	67
Time to recurrence (months)	8 (<2 weeks in 45% of patients)	16 (<2 weeks in 50% of patients)	15	14	28
Graft loss (%)	56	38	85	25	–
Time to graft loss (months)	10	44	42		77
Abnormal ACP (%)	100	100	80		–
Low CFH (%)	44	25	–		–
Low CFB (%)	11	17	–		–
Low CFI (%)	–	–	10		–
C3Nef (%)	44	33	50		–
C5Nef (%)	–	–	10		–
ab-CFH (%)	11	17	10		–
ab-CFB (%)	C3GN (17%)	–	–		–
All gene variants (%)	–		30		–
CFH/CFHR5 (%)	93 (CFH variants)		–		–

ab-CFB, autoantibodies to CFB; ab-CFH, autoantibodies to CFH; CFI, autoantibodies to CFI.

settings [5, 20–25]. There are reports documenting low C3 levels, as well as low levels of other complement regulatory proteins, in C3G and low serological factors as probable causes of complement dysregulation [26–29], although all data are from native biopsies. No study has evaluated the serological profile post-transplant. Regunathan-Shenk *et al.* [30] recently published a series of 21 cases with autoantibody profiles similar to those in our present study, except for ab-CFB, which, to our knowledge, has not been reported in other post-transplant studies. The authors [30] described a high recurrence rate and a relatively longer post-transplant period in their study patients with DDD, compared to those in our study. A comparative summary of our study results and those from Regunathan-Shenk *et al.* [30] and Zand *et al.* [4] is given in Table 5.

In our study, 88.9% of tx-DDD patients and 100% of tx-C3GN patients had low C3 levels. In a series of 32 DDD patients, Nasr *et al.* reported low C3 levels in 100% of paediatric and 41% of adult patients [29]. Sethi *et al.* described low C3 levels in 75% of C3GN patients [2], and Servais *et al.* reported low C3 levels in 45% of DDD patients and 37% of C3GN patients [1]. C4 levels were normal in all tx-C3G cases, as observed by Servais *et al.* and Sethi *et al.* [1, 2]. APFA showed very low complement activity in all cases of DDD and C3GN, similar to previous studies in Western patient populations [2, 3, 10].

In our study, a higher proportion of tx-DDD patients (44.4%) had low CFH levels compared with tx-C3GN patients (25%). However, both results were higher than those reported by Servais *et al.* [1] (18.2% of tx-DDD patients and 3.8% of tx-C3GN patients with low CFH levels). Moreover, the percentages of tx-DDD (11%) and tx-C3GN (17%) patients with low CFB levels were comparable in our study, but lower than the percentages reported by Servais *et al.* [1] (27% of tx-DDD and 26% of tx-C3GN with low CFB levels).

The majority of studies evaluating ab-CFH were conducted in patients with aHUS and MPGN [24, 31–33]. Only a few studies have reported on autoantibodies in C3G. ab-CFH were present in 17% of tx-DDD patients and 25% of tx-C3GN patients. Interestingly, cases with ab-CFH had low CFH levels. In two separate studies, Sethi *et al.* reported ab-CFH in 8–20% of DDD and C3GN cases [2, 10]. Zhang *et al.* also described ab-CFH in 3% of cases of DDD [3].

In the present study, we found ab-CFB in 17% of tx-C3GN cases, whereas these autoantibodies were previously documented by others in 8–9% of DDD cases only [3, 34]. C3Nef levels were slightly lower than most previous reports in DDD patients (41–86%) and comparable to levels found in C3GN patients (45%) [1–3, 10]. In our study, we did not find paraproteinaemia in tx-C3G cases, whereas Zand *et al.* reported paraproteinaemia in 31% of patients, which also resulted in early recurrences [35]. Moreover, Sethi *et al.* reported paraproteinaemia in 71.4% of cases [36]. This difference in findings could be explained by the younger age of our study patients, compared with relatively older patients (mean age 54.5 years) in the studies by Zand *et al.* and Sethi *et al.*

CFH gene variants were detected in 10–17% of DDD cases [26, 27]. Recent report showed CFH gene variants in 24–37% of C3G patients [37, 38]. The single-nucleotide polymorphisms (SNPs) rs800292, rs1061147, rs1061170 and rs2274700 are synonymous variants that have been described as significant in aHUS, age-related macular degeneration (AMD) and DDD [39–42]. The IVS2-18insTT insertion is a splice site variant that changes the natural acceptor site and was previously reported in DDD [40] and AMD [42]. In the present study, this insertion was more common in C3GN compared with DDD. The variant rs1061170 is the most common SNP (Tyr402His) described in DDD [39–41]. The significance of these variants is still unclear but they have been found to be associated with adverse outcomes in tx-C3G patients in our study. Graft loss reported by Barbour and Gill and Zand *et al.* was found in 43–50% of patients within 18–21 months [4, 15]. In our study, graft loss was found in one case of tx-DDD at 16 months, while 38% of tx-C3G cases had graft loss, which is slightly lower than in Western patient populations. In addition, 19% of tx-C3G patients in present series died. Three patients who had plasma exchange showed a good initial response and had autoantibodies against C3Nef and ab-CFH [43] (published data, doi: 10.4103/ijot.ijot_78_18). Only limited data are available on use of plasma exchange in the treatment of tx-C3G [22, 24, 32, 44].

In conclusion, our study demonstrated a shorter time to recurrence and graft loss in our patients, which could be

explained by differences in management policies in different countries. Our study also showed poor overall outcome of patients with tx-C3G. A limitation of our study is the limited genetic workup assessing for only two genes in the ACP. Other approaches, including whole-genome sequencing/targeted sequencing or multiplex ligation-dependent probe amplification (MLPA) testing, would be more appropriate for performing genetic screening in these patients.

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CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

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