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Role of C4d and CD68 in kidney biopsy as novel prognostic markers for IgA nephropathy; a single-center study from India

Pritam Khomane[1], Ashwin Somwarpet Prabhakar[2], Shankar Prasad Nagaraju[3*], Indu Ramachandra Rao[3], Kiran Krishne Gowda[4], Mahesha Vankalakunti[4], Ravindra Prabhu Attur[3], Srinivas Vinayak Shenoy[3], Dharshan Rangaswamy[3], Mohan Varadanayakanahalli Bhojaraja[3], Nisha Abdul Khader[3], Divya Datta[3], Shilna Muttickal Swaminathan[3], Vasudeva Guddattu[5]

[1]Department of Nephrology, Jupiter Hospital, Thane, Maharashtra, India
[2]Department of Nephrology, Father Muller Medical College, Mangalore, Karnataka, India
[3]Department of Nephrology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India
[4]Consultant Nephropathologist, Manipal Hospitals Bangalore, Karnataka, India
[5]Department of Apllied Statistics and Data Science, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India

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ABSTRACT

Introduction: IgA nephropathy (IgAN) is a common primary glomerulonephritis worldwide. C4d and CD68 could be useful prognostic markers in this disease.

Objectives: This study was conducted to assess the clinical and prognostic implications of C4d and CD68 staining in patients with IgAN.

Materials and Methods: This is a retrospective single-center observational study. Baseline characteristics and laboratory details were recorded. Renal biopsy was reported according to the MEST-C classification along with further staining for C4d and CD68 by immunohistochemistry. Primary and secondary outcomes were progression to end-stage renal disease (ESRD) and all-cause mortality during follow-up, respectively. The effect of C4d and CD68 along with other risk factors on outcomes was studied.

Results: Sixty patients with primary IgAN were analyzed with a median follow-up of 17 months. Forty were males, with a mean age of 39±16 years, and median estimated glomerular filtration rate (eGFR) of 36.5 mL/min/1.73 m^2 with a median urine protein/creatinine ratio of 1.9 g/g, at the time of kidney biopsy. In our patients, macroscopic hematuria (n=2: 3.3%) was rare, while 15 (25%) of patients had nephrotic-range proteinuria. Most biopsies showed sclerosis 43 (71.7%) followed by interstitial fibrosis and tubular atrophy (IFTA) 32(53.3%). Meanwhile, crescents were seen in 20 (33.3%). About 39 (65%) of patients had glomerular C4d positivity and 10 (16.7%) had tubulointerstitial CD68 positivity while, none having glomerular CD68 positivity. Glomerular C4d and tubulointerstitial CD68 positivity had lower eGFR, higher proteinuria at presentation (P<0.05) and faster progression to ESRD (glomerular C4d-odds ratio [OR]: 5.7 [95% CI: 1.4-22.5]); tubulointerstitial CD68 OR: 5.4 [95% CI: 1.2-23.95]. Other risk factors predicting progression were eGFR at presentation (OR: 0.9 [95% CI: 0.89-0.99], presence of sclerosis OR: 6.5 [95% CI: 1.32-32.06] and IFTA OR: 21.4 [95% CI: 4.3-108]).

Conclusion: In our study, IgAN patients presented in the later stages of chronic kidney disease, with the majority being diagnosed at stage 3 of this disease. Macroscopic hematuria was rare and nephrotic syndrome and crescents were common. Glomerular C4d and tubulointerstitial CD68 were associated with lower eGFR and more rapid progression.

Implication for health policy/practice/research/medical education:
In IgA nephropathy (IgAN) renal function can rapidly worsen to end-stage kidney disease. To develop methods for preventing or delaying disease progression, it is important to identify potential markers that might be helpful for risk stratification of IgAN.

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*Corresponding author: Shankar Prasad Nagaraju, Email: shankarmmcmed@gmail.com, shankar.prasad@manipal.edu

Original Article

Introduction

IgA nephropathy (IgAN) or Berger's disease, an entity which was initially described in 1968, is a relatively common renal disorder characterized by persistent microscopic hematuria and/or sub-nephrotic proteinuria, with or without hypertension or frequent episodes of upper respiratory tract infections with gross hematuria(1). It has a variable course ranging from isolated urinary abnormalities to end-stage renal disease (ESRD). Various clinical, histological, and serological markers have been used to predict disease outcomes, of which hypertension, elevated serum creatinine, proteinuria greater than 500 g/day, interstitial fibrosis, and tubularatrophy are significant (2-7). Recent evidence highlights the importance of complement activation by alternative and lectin pathways in the pathogenesis and progression of IgAN (8). Complement factors in renal tissue, urine, and serum samples are being evaluated as novel biomarkers to predict the prognosis of IgAN (9-11). IgAN that arises from the activation of an alternative pathway (C4d negative) is considered to have a benign disease course compared with activation by the lectin pathway (C4d positive) (12-15). C4d staining is routinely conducted for diagnosing humoral rejection in a kidney transplant setting. C4d is a part of the classical and lectin pathways, and its staining indicates activation of the lectin pathway in IgAN (12). Roos et al have demonstrated that patients of IgAN who stained positive for the presence of mannose-binding lectin had severe disease and worse outcomes (15). Recent studies have shown that glomerular C4d-positive patients have poor renal survival compared to C4D-negative patients (12,14,16). Whether it can be used as a promising novel tissue biomarker to predict progression in IgAN in different cohorts needs further evidence. CD68 is a monocyte and macrophage lineage marker. The infiltration of macrophages into the tubulointerstitial compartment is being studied and correlated with unfavorable renal outcomes in IgAN (17). Additionally, the identification of glomerular macrophages by immunohistochemistry (IHC) by using CD68 staining can be used as an objective marker for endocapillary hypercellularity (E1) in IgAN (18). There is limited data on the utility of C4d and CD68 in the Indian population as prognostic markers.

Objectives

This study was conducted to assess the clinical and prognostic implications of C4d and CD68 staining in patients with IgAN in an Indian cohort.

Materials and Methods

Study design

This retrospective observational study was conducted from January 2016 to July 2020 at a tertiary care hospital in India.

Inclusion criteria

Patients with biopsy-proven primary IgAN with a minimum follow-up of six months.

Exclusion criteria

Patients with Henoch-Schonlein purpura or secondary IgAN due to systemic disorders like systemic lupus erythematosus, chronic liver disease, seronegative arthritis (particularly ankylosing spondylitis), dermatitis herpetiformis, lymphoma, small cell carcinoma of the lung, and inflammatory bowel disease.

Data collection

Patient demographics and clinical details (age, sex, body mass index [BMI], hypertension, edema, and pallor) along with laboratory parameters (creatinine, estimated glomerular filtration rate [eGFR]) by CKD-EPI (chronic kidney disease epidemiology collaboration) formula (19), 24-hour urine protein, serum albumin, and hemoglobin] were collected at baseline and during follow-up.

The histological diagnosis of IgAN was made by the presence of IgA-dominant or co-dominant immune deposits within glomeruli, as shown by immunofluorescence (20,21). Light microscopic findings were assessed on renal biopsy sections stained with hematoxylin and eosin, periodic acid Schiff, Mason's trichrome, and methenamine silver stains. These findings were categorized as per the Oxford MEST-C classification by two nephropathologists (21-23). The components of the MEST-C score are mesangial hypercellularity (M0/M1), endocapillary hypercellularity (E0/E1), segmental glomerulosclerosis (S0/S1), tubular atrophy/interstitial fibrosis (T0 -25%; T1- 26% to 50%; and T2 >50%) and crescents (C0-no crescents, C1<25% of glomeruli, C2 >25% glomeruli) (21).

C4d and CD68 IHC staining were performed on 3 µm deparaffinized and rehydrated sections of formaldehyde-fixed renal tissue, using rabbit polyclonal anti-human C4d antibody and CD68 antibody. Meanwhile, C4d glomerular staining was recorded as granular staining in the capillary walls, mesangium, and both capillary walls and mesangium; either negative or positive. Likewise, CD68 staining was recorded as glomerular CD68 and tubulointerstitial CD68: either negative or positive. All slides were reviewed by two nephropathologists. The follow-up period was defined as the interval between renal biopsy and the last outpatient or in-patient visit, development of ESRD, or mortality. In patients with crescents with renal dysfunction, nadir creatinine at the end of three months was considered as baseline creatinine to see the progression of the disease on

follow-up. The type of treatment received, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEis/ARBs), and immunosuppression were recorded. All patients received standard treatment as per Kidney Disease Improving Global Outcomes (KDIGO) recommendations by the treating nephrologist (24).

Outcomes

The primary outcome studied was progression to ESRD which was defined as the need for maintenance dialysis or kidney transplantation during follow-up. The secondary outcome studied was all-cause mortality during follow-up.

Statistical analysis

Continuous variables were reported as the mean ± standard deviation (SD) when normally distributed or as the median (interquartile range [IQR]) when the data was skewed. Comparison of continuous variables between two groups was assessed using the unpaired T-test or the Mann–Whitney U test as appropriate. Categorical data were presented as frequencies.

The association between the two groups was assessed by the chi-square test. Factors associated with the progression of kidney disease were assessed using univariate analysis. A *P* value of < 0.05 was considered statistically significant. Analysis of data was conducted using the SPSS software version 22.

Results

Baseline clinical, histopathological characteristics

A total of 60 patients were included (Figure 1). The median follow-up was 17.5 (IQR: 9-28) months. The baseline clinical, laboratory, and histopathological characteristics are shown in Table 1. There was a male preponderance (males – 66.7%; M: F = 2:1). Majority of patients presented between the third and fourth decade of life with an average age of 39 years at the time of diagnosis. The mean BMI observed was 25 kg/m^2. All patients had proteinuria at the time of diagnosis. Hypertension (83.3%), microscopic hematuria (83.3%), and edema (66.7%) were the other

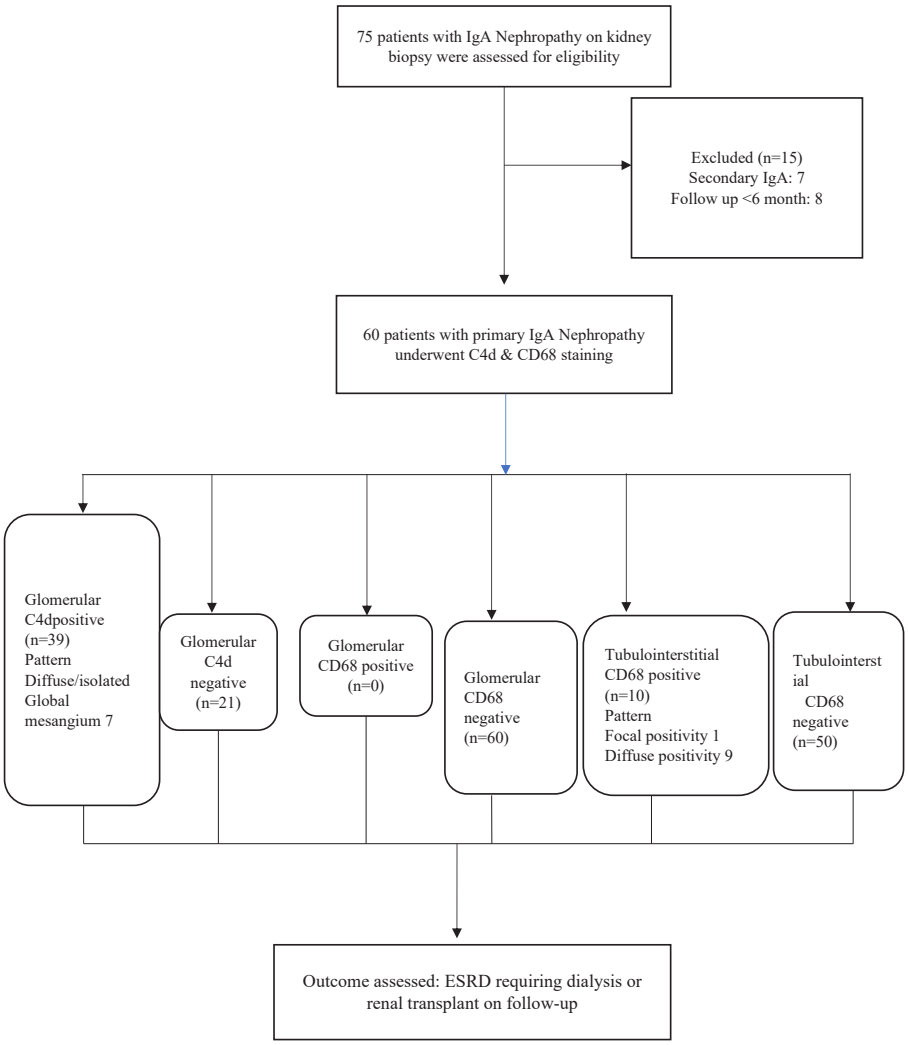

Figure 1. Flow chart of patient selection and study.

Table 1. Baseline characteristics and comparison of glomerular C4d and tubulointerstitial CD68 positive patients with C4d and CD68 negative patients with IgA nephropathy

Parameters	Overall (N=60)	C4D positive (n=39)	C4D negative (n=21)	*P* value	CD 68 positive (n=10)	CD 68 negative (n=50)	*P* value
Demographic							
Age[a] (years)	39±16	36±13	44±16	0.037	40±18	39±14	0.87
Males, No. (%)	40 (66.7)	24 (61.51)	16 (76.2)	0.25	2 (20)	18 (36)	0.32
BMI (kg/m^2)[a]	25±4.9	24.8±5	25.3±4	0.705	27.04±4	24.6±4	0.11
Duration of follow-up (months)[b]	17.5 (9-28)	21 (12-30)	12 (6-20)	0.13	18 (11-22)	15 (10-19)	0.23
Clinical and biochemical characteristics							
Systolic BP,[a] (mm Hg)	149±22	150± 23	149±21	0.876	173± 23	145±19	0.001
Diastolic BP,[a] (mm Hg)	88±15.5	87±17	89±10	0.75	91±29	87±11	0.60
MAP,[a] (mm Hg)	108±15.7	108±17	109±12	0.89	118±24	108±12	0.134
Hypertension, No. (%)	50 (83.3)	32 (82.1)	18 (85.7)	0.717	10 (100)	40 (80)	0.121
Pedal edema, No. (%)	40 (66.7)	25 (64.1)	15 (71.4)	0.56	7 (70)	33 (60)	0.806
Haemoglobin,[a] g/dL	11.65±2.2	11.15±2	12.54±2.4	0.02	10.47±2.09	11.89±2.2	0.117
Microscopic hematuria, No. (%)	50 (83.3)	33 (84.6)	17 (81)	0.717	8 (80)	42 (84)	0.757
Macroscopic hematuria, No. (%)	2 (3.3)	2 (5.1)	0	0.219	0 (0)	2 (4)	0.52
Nephrotic syndrome, No. (%)	15 (25)	12 (30.8)	2 (9.5)	0.06	3 (30)	11 (22)	0.65
eGFR (ml/min/1.73m^2)[b]	36.5 (19.3-56)	27.85 (14.2-55.5)	51 (30.7-65.2)	0.009	19.16 (10-25.9)	42.4 (24-61.7)	0.002
Serum creatinine (mg/dL)[b]	1.95 (1.2-3.2)	2.6 (1.4-3.9)	1.6 (1.2-2.2)	0.02	3.5 (2.6-6.3)	2 (2.9-4.2)	0.002
24-hour urine protein (g/day)[b]	1.9 (1.2-3.1)	2 (1.5-3.9)	1.5 (0.9-2)	0.02	2 (1.5-3.9)	1.5 (0.9-2)	0.02
Serum albumin,[a]	3.6±0.72	3.4±0.72	3.8±0.7	0.039	3.5±0.4	3.6±0.8	0.56
Histological characteristics							
(M) Mesangial hypercellularity, No (%)	35 (58.3)	25 (64)	10 (47.6)	0.21	5 (50)	30 (60)	0.55
(E) Endocapillary hypercellularity, No (%)	24 (40)	18 (46)	6 (28)	0.18	4 (40)	20 (40)	1.0
(S) Sclerosis, No. (%)	43 (71.7)	30 (76.9)	13 (61.9)	0.21	6 (60)	37 (74)	0.37
IFTA No. (%)	32 (53.3)	26 (66.7)	6 (28.6)		7 (70)	25 (50)	0.24
T1 (25-50%)	17 (28.3)	13 (33)	4 (19)		2 (20)	15 (30)	0.5
T2 (>50%)	15 (25)	13 (33)	2 (9.5)	0.005	5 (50)	10 (20)	0.04
(C) Crescents, No. (%) C1 (<25% glomeruli) C2 (>25% glomeruli)	20 (33.3) 18 (30) 2 (3.3)	17 (43.6) 16 (41) 1 (2.6)	3 (6.7) 2 (9.5) 1 (4.8)	0.02	5 (50) 4 (40) 1 (10)	15 (30) 14 (28) 1 (2)	0.221 0.45 0.19
Treatment and outcome							
ESRD	22 (36.7)	19 (48.7)	3 (14.3)	0.008	3 (30)	26 (52)	0.204
Oral steroid, No. (%)	29 (48.3)	18 (46.2)	11 (52.4)	0.64	3 (30)	30 (60)	0.08
RAS inhibitors, No. (%)	33 (55)	18 (46.2)	15 (71.4)	0.55	1 (10)	10 (20)	0.4
Steroid + added immunosuppression, No. (%) (cyclophosphamide, mycophenolate mofetil)	11 (18.3)	8 (20.5)	3 (14.3)	0.13	7 (70)	15 (30)	0.017

BP: Blood pressure; eGFR: estimated glomerular filtration rate; BMI: Body mass index; IFTA: Interstitial fibrosis tubular atrophy; ESRD: End-stage renal disease; MAP: Mean arterial pressure; RAS: Renin-angiotensin system.
[a]Mean±SD; [b] Median (IQR).

common findings. Macroscopic hematuria was seen only in 3.3% of subjects. In addition, 25 % of patients had nephrotic-range proteinuria. At presentation, majority of patients were at CKD stage 3 or later with median eGFR of 36.5 mL/min/1.73 m^2. The median serum creatinine and urine protein-urine creatinine ratio (UPCR) was 1.95 mg/dL and 1.9 g/g, respectively. The median number of glomeruli per biopsy examined was 12. Based on Oxford classification, MEST-C scoring was evaluated on light microscopy. The commonest histological finding noted in our study was sclerosis (71.7 %) followed by interstitial fibrosis and tubular atrophy (IFTA) (53.3%). Moreover, crescents were present in 33.3% of patients. Overall glomerular C4d staining was positive in 65% of patients. None were positive for glomerular CD68 staining. Tubulointerstitial CD68 positivity was seen in 16.7% (Table 1).

Glomerular C4d positive

Glomerular C4d was seen in 39/60 (65%) (Figures 2a and b). The most common pattern observed was diffuse and global mesangial and capillary wall positivity (22/39). The comparison of baseline characteristics between C4d-positive and C4d-negative patients is shown in Table 1. C4d-positive patients were younger ($P=0.03$), had lower hemoglobin at presentation ($P=0.02$), significantly lower eGFR (27.85 mL/min/1.73 m^2, $P=0.009$), lower serum albumin ($P=0.03$) and more proteinuria [UPCR 2 g/g (1.5-3.9) ($P=0.02$)] at the time of diagnosis. Meanwhile, C4d-positive patients also had a significantly higher IFTA ($P<0.005$) and crescents ($P<0.02$) on biopsy. The proportion of patients treated in the follow-up with ACEi/ARBs, prednisone, or immunosuppressive agents was similar in both groups.

CD68 staining

CD68 staining was conducted to find macrophages in both glomerular and tubulointerstitial compartments. Tubulointerstitial CD68 staining (Figures 2d, 2e) was seen in 10/60 (16.6%). The majority had diffuse staining patterns (90%). Tubulointerstitial CD 68 positive patients had lower eGFR (median 19.16 mL/min/1.73 m^2) ($P=0.002$) and higher proteinuria (UPCR 2 g/g) ($P=0.02$) at presentation. They had high systolic blood pressure ($P=0.001$) and pallor ($P=0.017$) compared to patients who were CD68-negative at the time of diagnosis. The proportion of patients treated with ACEi/ARBs, prednisone, or immunosuppressive agents on follow-up was similar between groups (Table 1).

Glomerular CD68 staining

In this study, surprisingly, no patients had glomerular staining for CD68 (Figure 2c). Glomerular CD68 staining is considered as an endocapillary proliferation marker. Out of 24 patients with endocapillary hypercellularity none had glomerular CD68 positive.

Outcomes and renal survival

The median follow-up period was 17 months. Of 60 patients, 22 (36.7%) progressed and reached ESRD. None of the patients required kidney transplantation or peritoneal dialysis. There was no mortality. Among 39 patients with C4d positivity, 19 (48.7%) progressed to ESRD ($P=0.008$), and out of 10 patients with CD68 positivity, 7 (70%) reached ESRD ($P=0.017$).

Risk factors for progression

A univariate logistic regression analysis was done to evaluate the relationship between the clinicopathological features and primary outcome (ESRD). Lower eGFR at presentation (odds ratio: 0.9 [0.89-0.99]), presence of segmental sclerosis (OR: 6.5 [1.32-32.06]), IFTA (OR: 21.4 [4.3-108]), positive C4d staining (OR: 5.7 [1.4-22.5]), CD68 staining (odds ratio-5.4 [1.2-23.95]), and positive staining for both C4d and CD68 (OR: 8.4 [1.56-45.02]) were found to be the significant risk factors predicting disease progression ($P<0.05$) as shown in Table 2. Given the small sample size and fewer events with wide confidence intervals on univariate analysis, we did not do multivariate logistic regression/analysis in our cohort.

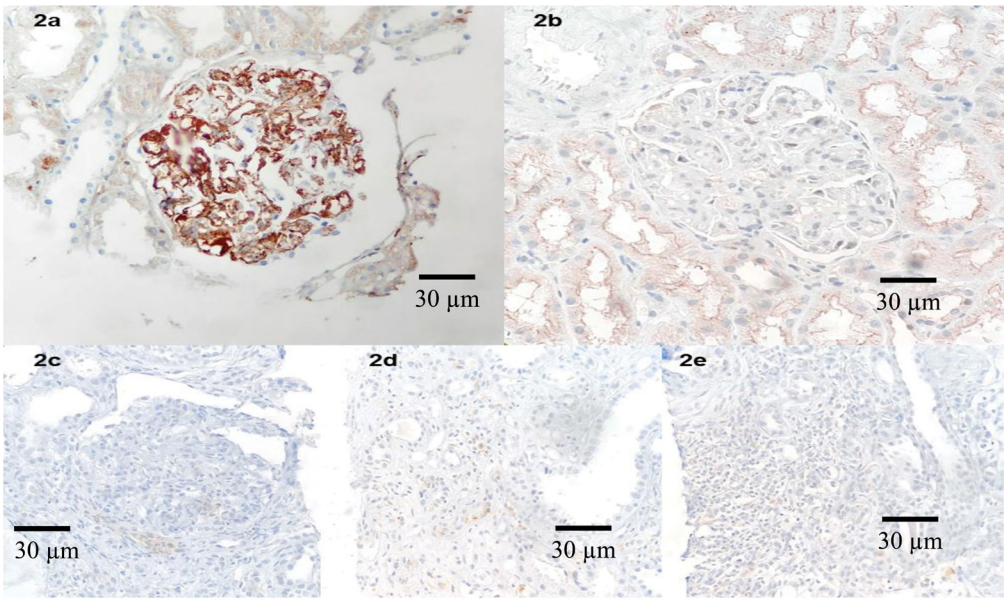

Figure 2. Immunohistochemistry pictures. (a) Glomerular tufts with granular deposits of C4d globally, (b) Glomerular tufts with negative C4d, (c) Glomerular tufts with negative CD68 staining, (d) Interstitial mononuclear cells reveal cytoplasmic positivity with CD68, (e) Interstitial mononuclear cells with negative CD68 staining.

Table 2. Risk factors predicting the progression in IgA nephropathy patients

Variables	Odds ratio	95% CI	*P* value
Age (y)	0.9	0.1-1.7	0.34
MAP at presentation	1.02	0-07-1.95	0.143
BMI >25 kg/m^2	1.23	0.4-3.5	0.6
M (mesangial hypercellularity)	0.58	0.2-1.6	0.32
E (endocapillary hypercellularity)	1.06	0.3-2.7	0.91
S (sclerosis)	6.5	1.32-32.06	0.02
T (interstitial fibrosis and tubular atrophy)	21.4	4.3-108	<0.001
Crescents	1.2	0.4-3.7	0.705
eGFR at presentation	0.9	0.89-0.99	<0.001
24-hour urine protein (g/d)	1.2	0.9-1.67	0.08
C4d staining	5.7	1.4-22.5	0.013
CD68 staining	5.4	1.2-23.95	0.02
C4d and CD68 positivity	8.4	1.56-45.02	0.01

MAP: Mean arterial pressure; BMI: Body mass index; eGFR: estimated glomerular filtration rate.

Discussion

IgAN has varied presentation and complex trajectories (1,25). Our cohort consisted of predominantly males and the majority were young belonging to 3rd and 4th decades of life which is similar to other Indian and Western cohorts (22,26,27). Compared to the classical description in the Western population, the Indian cohort of IgAN presents late (28).

Our cohort also presented late, the majority being in CKD stage 3 or 4 (median eGFR 36.5 mL/min/1.73 m^2). The nephrotic presentation is common in our population (25%) and macroscopic/frank hematuria (3.3%) is rare (28). The clinical presentation in our cohort is similar to other studies on Indian subjects done by Chacko et al, Mittal et al and Alexander et al (27-29).

The distribution of histopathological patterns seen in our study population is different compared to the patterns seen in other studies. The most common histopathological pattern seen in our study group was segmental glomerulosclerosis (71.7%) followed by IFTA (53.3%). Besides 33.3% of our patients had crescents at presentation.

The recent GRACE-IgAN cohort study by Alexander et al from India had sclerosis (80%) as the most common histopathological finding followed by IFTA (77.9%), consistent with our study. However, crescents in their study had low prevalence (8.6%) (28). A study conducted in Western India by Mittal et al reported mesangial hypercellularity as a common finding while in our population it accounted for only 40% of the cases. The sclerosis was the second most common lesion after mesangial hypercellularity in the European, Chinese, and Japanese cohorts (27,28,30,31). Differences in the clinical and histopathological manifestations in various studies including our cohort can be due to differences in race, geographic variations in kidney disease screening patterns, and timing of diagnosis and kidney biopsy.

C4d positivity in patients with IgAN is a novel histological marker that signifies lectin pathway activation and is associated with a more rapid disease progression (32-35). In the present study, we found evidence of C4d staining in the glomeruli in two-thirds of our cohort, which is higher than previously reported (14,36). Similar to previous studies, C4d-positivity was associated with higher proteinuria, lower eGFR, and greater chronicity on kidney biopsy. Our population had a significantly lower eGFR of 27.85 (14.2-55.5) mL/min/1.73 m^2 as compared to the studies by Espinosa et al and Yang et al (14,36). Lower eGFR at presentation in our cohort is probably due to delayed presentation at the time of diagnosis. The most common histopathological lesion found in C4d-positive patients in our study was sclerosis followed by IFTA while in the Spanish and Chinese cohorts, the most common lesion was IFTA and mesangial hypercellularity, respectively(14,36). Similar to previous studies, C4d-positivity was associated with poor renal survival on follow-up even in our cohort (OR: 5.7 [1.4-22.5]). In glomerulonephritis, macrophages mediate immune responses and tissue damage, including antigen presentation, cell signaling, and mesangial matrix remodeling. Prior studies showed that CD68 is a marker for the infiltration of monocytes and macrophages (37). Macrophages' presence in the tubulointerstitium signifies progressive disease with poor outcome. The number of tubulointerstitial macrophages was also correlated with the magnitude of urinary protein excretion and lower eGFR at presentation and severe IFTA (17). In our study, we performed both glomerular and tubulointerstitial

staining for CD68. In our cohort, tubulointerstitial CD68 positivity was seen in only 16.6%. They had significantly lower eGFR (19.16 mL/min/1.73 m^2), higher proteinuria, and significant IFTA (T2) at presentation. We observed a significant correlation between tubulointerstitial CD68 positivity and the poor outcome ($P=0.01$). Glomerular CD68 is also emerging as a potential marker for endocapillary proliferation in the glomeruli (17,37,38). However, glomerular CD68 staining was negative in all of our patients. We presume it is likely due to advanced disease in our patients which may be the probable reason for the paucity of macrophages in glomeruli. Our finding further confirms the utility of novel biomarkers of glomerular C4d ($P=0.008$) and tubulointerstitial CD68 positivity ($P=0.017$) on IHC in predicting progression and poor renal survival. The data in the Asian population like our population is still sparse (33,37). Our study strengthens the existing evidence regarding the importance of the use of C4d and CD68 in clinical practice.

Several studies have demonstrated various clinical or histological factors like male gender, serum creatinine/eGFR, proteinuria, hypertension, mesangial hypercellularity, glomerular sclerosis, and IFTA as the independent predictors of ESRD (39). In our study, in addition to positive glomerular C4d (OR: 5.7 [1.4-22.5]) and tubulointerstitial CD68 staining (OR: 5.4 [1.2-23.9]), we found lower eGFR at presentation (OR: 0.9 [0.89-0.9]), segmental sclerosis, IFTA (OR: 21.4 [4.3-108]) are significant risk factors, which predicting disease progression ($P<0.05$). However, because of the small sample size and the low number of events, multivariate analysis could not be conducted.

Strengths of the study

Although this is a single-center study, the demographic and clinical profile of our cohort is consistent with other studies from the Indian subcontinent and thus, appears to be representative of the IgAN population in this ethnic group. The findings of our study further reinforce the fact that IgAN has varied presentation and outcomes. For the first time, we are looking into both novel biomarkers, C4d and CD68, as prognostic factors in IgAN. Since all the biopsies have been reported by the same two nephropathologists independently, this has ensured consistency in the reporting of histomorphology and immunostaining. We have kept ESRD requiring renal replacement therapy as the primary outcome which is a hard clinical endpoint. However, considering that this was a small retrospective study with a short duration of follow-up, our findings should be interpreted with caution. The applicability of our findings to other ethnic groups also needs further validation. Electron microscopy and CD68+ macrophage counting was not performed. Given the small sample size, prognostic significance of CD68 and C4d positivity independent of all the other parameters could not be performed.

Conclusion

IgAN in our cohort predominantly occurs in males in their 3rd and 4th decade of life while most of them were in CKD stage 3 at the time of diagnosis. Additionally, we found crescents and nephrotic presentation are common; however, macroscopic hematuria is infrequent. The most common histopathological finding was sclerosis followed by IFTA rather than endocapillary proliferation. We also found C4d positivity is high, whereas CD68 positivity is infrequent. In addition to lower eGFR at presentation and IFTA, the presence of glomerular C4d and tubulointerstitial CD68 in kidney biopsy predicted poor renal survival. We conclude that, C4d and CD68 staining appear as promising novel biomarkers for predicting renal survival in IgAN. We postulate that, staining for these novel objective markers should be incorporated into routine renal biopsy evaluation of IgAN.

Limitations of the study

The study was a retrospective single centered with a small cohort.

Authors' contribution

Conceptualization: Pritam Khomane, Ashwin Somwarpet Prabhakar, Shankar Prasad Nagaraju, Indu Ramachandra Rao, Kiran Krishne Gowda, Mahesha Vankalakunti, Ravindra Prabhu Attur, Srinivas Vinayak Shenoy, Dharshan Rangaswamy, Mohan Varadanayakanahalli Bhojaraja.

Data curation: All authors.

Formal analysis: Pritam Khomane, Indu Ramachandra Rao, Kiran Krishne Gowda, Mahesha Vankalakunti, Ravindra Prabhu Attur, Srinivas Vinayak Shenoy, Dharshan Rangaswamy, Mohan Varadanayakanahalli Bhojaraja, Nisha Abdul Khader, Divya Datta, Shilna Muttickal Swaminathan, Vasudeva Guddattu.

Investigation: All authors.

Methodology: All authors.

Project administration: Pritam Khomane, Ashwin Somwarpet Prabhakar,Srinivas Vinayak Shenoy, Shankar Prasad Nagaraju.

Resources: All authors.

Software: Indu Ramachandra Rao and Srinivas Vinayak Shenoy and Divya Datta.

Supervision: Shankar Prasad Nagaraju, and Mohan Varadanayakanahalli Bhojaraja and Ravindra Prabhu Attur.

Validation: Shankar Prasad Nagaraju, Mohan Varadanayakanahalli Bhojaraja, Ravindra Prabhu Attur,

Indu Ramachandra Rao and Dharshan Rangaswamy. **Visualization**: Pritam Khomane, Ashwin Somwarpet Prabhakar, Shankar Prasad Nagaraju, Indu Ramachandra Rao, Kiran Krishne Gowda, Mahesha Vankalakunti, Ravindra Prabhu Attur.
Writing–original draft: All authors.
Writing–review & editing: All authors.

Conflicts of interest

The authors declared no competing interests.

Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki, as revised in 2008. This study was approved by approved Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee (#IEC-584). Written consent was taken from all the patients. The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

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