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Retrospective Study
Clinicopathological features and medium-term outcomes of histologic variants of primary focal segmental glomerulosclerosis in adults: A retrospective, observational study

Prognostic significance of FSGS variants

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Abstract
BACKGROUND
The Columbia classification identified five histological variants of focal segmental glomerulosclerosis (FSGS). The prognostic significance of these has remained controversial.

AIM
This study aimed to evaluate the relative frequency, clinicopathologic characteristics, and medium-term outcomes of FSGS variants at a single center in Pakistan.

METHODS
Methods: This retrospective study was conducted at the Department of Nephrology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan on all consecutive adults (≥16 years) with biopsy-proven primary FSGS from January 1995 to December 2017. Studied subjects were treated with steroids as a first-line therapy. The response
rates, doubling of serum creatinine, and kidney failure with replacement therapy were compared between histological variants using ANOVA or Kruskal Wallis, and Chi-square tests as appropriate. Data was analyzed in SPSS version 22.0. P-value ≤0.05 was considered significant.

RESULTS

Results: A total of 401 patients were diagnosed with primary FSGS during the study period. Among these, 352 (87.7%) had a designated histological variant. The not otherwise specified (NOS) variant was the commonest, being found in 185 (53.9%) patients, followed by the tip variant in 100 (29.1%) patients. Collapsing, cellular, and perihilar variants were seen in 58 (16.9%), 6 (1.5%), and 3 (0.7%) patients, respectively. Cellular and perihilar variants were excluded from further analysis because of the small numbers.

The mean follow-up period was 36.5±29.2 months. Regarding response rates of variants, patients with tip lesions achieved remission more frequently (59.5%) than NOS (41.8%) and collapsing (24.52%) variants (p<0.001). The hazard ratio of complete response among patients with the collapsing variant was 0.163 (95% confidence interval [CI]: 0.039-0.67) as compared to patients with NOS. The tip variant showed a hazard ratio of 2.5 (95%CI: 1.61-3.89) for complete remission compared to NOS. Overall, progressive kidney failure was observed more frequently in the collapsing variant, 43.4% (p<0.001).

Among these, 24.53% of patients required kidney replacement therapy (P<0.001). The hazard ratio of doubling of serum creatinine among patients with the collapsing variant was 14.57 (95%CI: 1.87 – 113.49) as compared to patients with the tip variant.

CONCLUSION

Conclusions: In conclusion, histological variants of FSGS are predictive of response to treatment with immnosuppressants and progressive kidney failure in adults in our setup.
Key Words: Adults; Columbia classification; Focal segmental glomerulosclerosis; Histological variants; Kidney failure; Kidney failure with replacement therapy


Core Tip: Core tip: Focal segmental glomerulosclerosis (FSGS) is one of the most common glomerular diseases throughout the world and a leading cause of kidney failure requiring kidney replacement therapy. FSGS is a heterogeneous disorder with many causes and varying pathogenesis. The clinical course is also heterogeneous. Columbia classification identified five histological variants of FSGS. The prognostic significance of these has remained controversial. Initial studies found a good correlation of the FSGS variants with clinical presentation, treatment responses, and final outcomes. However, a more recent series from Japan found no prognostic value of the variants in isolation. This present study one of the largest studies on FSGS variants and its results will help clinicians in making informed decisions regarding the treatment and prognosis of morphological variants of FSGS.

INTRODUCTION

Introduction:
Focal segmental glomerulosclerosis (FSGS) is a histological pattern of glomerular injury rather than a specific diagnosis. It can occur either in a primary or idiopathic form or may be associated with various systemic diseases, including autoimmune diseases, infections, drugs, and structural kidney diseases. The key pathological finding of FSGS on light microscopy is the obliteration involving a portion (segmental) of glomerular tufts of some (focal) but not all glomeruli by increased mesangial matrix. The underlying cause of idiopathic FSGS is uncertain although a putative circulating permeability factor is supposed to play a major role in its pathogenesis [1].
FSGS is one of the leading causes of glomerular diseases, particularly those presenting with nephrotic syndrome (NS), accounting for 20 to 40% of the pathological lesions in adult patients undergoing kidney biopsy for the evaluation of idiopathic NS [2-4]. It is also one of the most common glomerular diseases leading to kidney failure (KF) and KF requiring replacement therapy (KFRT) [2].

The classification of patients with FSGS is both challenging and controversial due to the broad variety of underlying etiologies, limited understanding of the pathogenesis, and the poor correlation between morphological lesions and response to treatment and clinical outcomes. The Columbia classification of FSGS provided a novel and pragmatic approach to classify FSGS based on histological features on kidney biopsy. This classification was supposed to help the clinicians in the assessment of the prognosis of the disease and its response to therapy. The classification, first proposed by D’Agati et al, in 2004, envisioned five mutually exclusive histological variants of the disease, based entirely on light microscopic (LM) features [5, 6]. These variants include collapsing (COL), not otherwise specified (NOS), tip (TIP), perihilar (PHI), and cellular (CEL) variants [2]. Since then, many studies of these variants have been conducted worldwide and have demonstrated a correlation of the variants with distinct clinical characteristics and prognostic and therapeutic outcomes [6-12]. The response rates are generally lowest for the COL variant, intermediate for the NOS variant, and highest for the TIP variant. On the other hand, the reported rates of KFRT are highest in the COL variant, intermediate in the NOS, and lowest in the TIP variant [13-16].

Other more recent studies have found no differences among these variants with respect to treatment responses and outcomes. A recent study from Japan by Kawaguchi et al observed that FSGS variants alone have no significant impact on kidney outcomes after five years, while proteinuria remission was predictive of improved kidney prognosis irrespective of any variant. They suggested that specific strategies and interventions to achieve proteinuria remission for each variant should be implemented for better kidney survival [17].
We previously reported that whatever the histological variant of FSGS, timely treatment with immunosuppressive drugs of all patients who fulfill the criteria is very important to achieve remission, either complete or partial remission (PR). Those patients who achieved remission did not progress to KF nor they required replacement therapy in the medium-term follow-up period [2, 18].

As steroids and cyclosporine (CsA)-induced remission is associated with better long-term survival, it is important to study which type of FSGS variants are more likely to respond to steroids and CsA treatment and whether such treatment affects kidney survival [2, 14, 17].

This study aimed to determine the relative frequency, clinicopathologic presentations, and outcomes of histological variants of FSGS in our population.

**MATERIALS AND METHODS**

**Materials and Methods**

This retrospective observational study was conducted at the Department of Nephrology, Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan. The study population comprised of all adult patients (≥16 years) of either gender who were diagnosed with primary FSGS between January 1995 and December 2017. Secondary causes of FSGS were excluded. We did not analyze PHI and CEL variants in detail because of the small number of patients. Patients with missing information and irregular or erratic follow-up were also excluded.

All patients underwent ultrasound-guided percutaneous native kidney biopsies, which were processed and prepared according to standard guidelines, as described in detail in our previous studies (2, 18, 19). The histological variants were diagnosed as per the criteria of the Columbia classification [5, 6]. Briefly, the TIP variant was diagnosed when at least one segmental lesion involved the tip domain (outer 25% of the tuft next to the origin of the proximal tubule). It required the exclusion of COL and PHI variants. The tubular pole needs to be identified in the defining lesion. FSGS, NOS, was diagnosed when at least one glomerulus showed a segmental increase in the mesangial
matrix obliterating the capillary lumina, with or without segmental glomerular capillary wall collapse but without overlying podocyte hyperplasia. It required the exclusion of PHI, CEL, TIP, and COL variants. The COL variant was diagnosed when at least one glomerulus showed segmental or global collapse and overlying podocyte hypertrophy and hyperplasia (Figure 1). The biopsies were reported by two experienced kidney pathologists and evaluated by LM, immunofluorescence (IF), and electron microscopy (EM).

As the criteria for genetic testing in adult patients with FSGS are still unclear, genetic testing was not done in this cohort of patients.

All patients with all variants of FSGS were treated in the same way. Briefly, unless contra-indicated, all patients were treated with prednisolone, 1 mg/kg/day for the first six weeks followed by 0.75 mg/kg/day for the next six weeks. If no remission was achieved by the end of 12 wk, prednisolone was tapered over the next four weeks and stopped. If remission occurred at any time during treatment, the same dose of steroids was given for two more weeks before slow tapering. We did not employ different treatment protocols for different variants or different patients included in this study.

The steroid-resistant cases or those in which steroids were contra-indicated were treated with CsA at a starting dose of 4 mg/kg/day. If a complete or partial response occurred, CsA was continued for at least one year. In case no response occurred by the end of two months, the use of CsA was discontinued.

Complete remission (CR) was defined as proteinuria ≤0.2 g/day or when the urine dipstick was negative for proteins with a stable serum creatinine concentration (<50% increase from the baseline). PR was defined as proteinuria between 0.21-2.0 g/day with at least a 50% reduction in proteinuria from the baseline or albumin detected on dipstick (+1 to +4). Relapse was defined as proteinuria >3 g/day after prior reduction of proteinuria to less than 2 g/day or albumin detected in dipstick (+1 to +3 or +4). Hypertension was diagnosed when patients were treated with antihypertensive drugs or with systolic blood pressure (BP) ≥140 mmHg or diastolic BP ≥90 mmHg. Elevated serum creatinine was defined as an increase of serum creatinine to >1.4 mg/dL in male
and >1.2 mg/dL in female patients. KF was defined as a sustained increase of serum creatinine concentration >50% from the baseline (at the time of kidney biopsy) or eGFR <60 mL/min/1.73 m² according to the Modification of Diet in Renal Disease (MDRD) equation. KFRT was defined as the need for continuous dialysis or kidney transplantation.

Data was collected by reviewing the medical record files of all selected patients. Data was collected over the period from diagnosis of primary FSGS to the last follow-up. The demographic characteristics (age, gender), BP measurements, and laboratory investigations including proteinuria, serum albumin, serum creatinine, and urine dipstick results for albumin/red blood cells (RBCs) on initial and last visits were noted. Drug information and side effects of steroids were gathered. The outcome of all patients regarding sustained remission, or progression to KF/KFRT, and death was noted.

Data was entered and analyzed in Statistical Package for the Social Sciences (SPSS) software 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used for summarizing the continuous and categorical variables. Continuous variables like age, BP, serum creatinine, serum albumin, 24-hour proteinuria, and drug dosages, were presented as mean± standard deviation (SD) and median interquartile range (IQR), as appropriate. Categorical variables, i.e., gender, sustained remission, KFRT, death, CR, PR, relapse, and hypertension were reported as frequencies and percentages. Mean differences for continuous variables and proportion differences for categorical variables between groups were compared using the student’s t-test and Chi-square or Fisher’s exact tests, as appropriate. Multivariate analysis for significant factors on univariate analysis was done by logistic regression method, while hazard ratios for risk factors of kidney outcome were calculated by using the Cox regression model. Response rates, doubling of serum creatinine, and KFRT rates were compared between histological variants using ANOVA or Kruskal Wallis, and Chi-square tests as appropriate. P-value ≤0.05 was considered as significant.

2 RESULTS
Results:

A total of 401 patients were diagnosed with primary FSGS during the study period. Among these, 352/401 (87.7%) had a designated Columbia histological variant. Among the latter, NOS was the commonest variant, found in 185 (53.9%) followed by the TIP variant in 100 (29.1%) patients. COL, CEL, and PHI variants were seen in 58 (16.9%), 6 (1.5%), and 3 (0.7%) patients, respectively. The three most common morphologic variants of FSGS (TIP, NOS, COL) comprised 343/352 (97.4%) and were included in the final analysis (Figure 2). The main demographic, clinical, and laboratory characteristics along with treatment information of these patients are shown in Table 1. There was no statistically significant difference in the mean ages of the three variants ($P = 0.7$). A statistically significant difference was observed in the diastolic BP where the COL variant showed a higher value of 87.6±14.1 mmHg compared to other histologic variants ($P = 0.04$). Initial proteinuria was nephrotic-range in all patients with a median (IQR) of 3774 (2216-5900) mg/24 h and there was no significant difference among the variants ($P = 0.418$). The initial estimated glomerular filtration rate (eGFR) was 83.9 (55.9-127.4) ml/min/1.73 m² in all with no significant difference among the three variants ($P = 0.463$). Elevated serum creatinine at presentation was found in 82 patients: of these, 58 were males and 24 were females ($P = 0.25$).

The mean follow-up duration of all patients was 36.5±29.2 months with no significant difference among the three variants ($P = 0.114$).

Out of 343 patients, 302 (88%) received treatment with immunosuppressive agents combined with angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) as shown in Figure 2. All patients received steroids as initial therapy as per our treatment protocol. The total duration of treatment of steroid therapy and total dose of steroids are shown in Table 1. The total dose of CsA is also shown in Table 1. The remaining patients were treated with ACE inhibitors or ARBs only due to some reasons like uncontrolled diabetes, intolerance of steroids, risk of osteoporosis, and other complications of immunosuppressive drugs.
Table 2 shows the details of pathological findings in the three morphological variants of FSGS. Upon review of the histopathological findings, a statistically significant difference was found among the three most common variants with respect to the number of glomeruli with global sclerosis \((P = 0.001)\), number of glomeruli with segmental collapse \((P = 0.05)\), and mild and moderate tubular atrophy \((p < 0.001)\).

The treatment responses and clinical outcomes of all selected study subjects about complete and PR, or no remission, as well as progressive KF/ KFRT, doubling of serum creatinine at last follow-up, final eGFR, relapse, and death of the patients are shown in Table 3. Out of 302 patients, 84 (27.8%) patients achieved CR [Figure 2]. Of these, 42 belonged to the TIP variant, with a CR rate of 42/89 (47.2%), 40 belonged to the NOS variant with a CR rate of 40/160 (25%), and 2 belonged to the COL variant with CR of 2/53 (3.7%) \((p < 0.001)\). A total of 49/302 (16.2%) patients achieved PR. Among these, 11 belonged to the TIP variant with a PR of 11/89 (12.4%), 27 belonged to the NOS variant with a PR of 27/160 (16.8%), and 11 to the COL variant with a PR of 11/53 (20.8%) \((P = 0.005)\). The highest percentage of no remission was noted in the COL variant at 40/53 (75.5%) followed by the NOS variant, 93/160 (58.1%) \((p<0.001)\) (Figure 3). The COL variant showed a marked decline in final eGFR and this was significant \((p<0.001)\) (Figure 4).

Among all treated patients, a doubling of serum creatinine was noted in 30 (9.9%) patients. Of these, 16/160 (10.0%) were of the NOS variant, 1/89 (1.1%) of the TIP variant, and 13/53 (24.5%) of the COL variant \((p < 0.001)\).

Regarding the development of KF, 23/53 (43.3 %) patients of the COL variant developed progressive KF, 13/160 (18.1%) of the NOS variant, while none from the TIP variant progressed to KF during the mean follow-up period of 36.5 months (Figure 5). Similarly, 13/53 patients (24.5 %) of the COL variant, 5/160 (3.1%) of NOS, and none from the TIP variant developed KFRT \((p < 0.001)\). A total of four patients died, 1/53(1.8%) of the COL variant, and 3/160 (1.8%) of the NOS variant with no significant difference among the three variants.
The final kidney and patient outcomes of the three common FSGS variants are shown in Figure 3. Patients with the TIP variant achieved remission more frequently (59.5%) than NOS (41.8%), and COL (24.52%) variants (p<0.001). The hazard ratio (HR) of complete response among patients with the COL variant was 0.163 (95% CI: 0.039 - 0.67) as compared to patients with the NOS variant. The TIP variant showed an HR of 2.50 (95% CI: 1.611 - 3.894) for CR compared to the NOS variant. Overall, progressive KF was observed more frequently in COL variant at 43.4% (p<0.001). Among these, 24.53% of patients required kidney replacement therapy (KRT) (p <0.001). The HR of doubling of serum creatinine among patients with the COL variant was 14.577 (95% CI: 1.872 - 113.493) as compared to patients with the TIP variant.

**DISCUSSION**

**Discussion:**

This is the largest study with a cohort of 343 adult patients with biopsy-proven primary FSGS classified according to the Columbia classification from Pakistan. This study analyzed in detail the three most common morphological variants of primary FSGS, namely NOS, TIP, and COL variants, as the numbers of the remaining two variants were very small. The vast majority of patients included in this study received treatment with steroids and CsA along with ACE inhibitors and ARBs. The total number of patients with the NOS variant was 185; out of which, 160 (86.4%) patients received treatment. Likewise, there were 100 patients with the TIP variant; out of which, 89 (89%) received treatment. There were 58 patients with the COL variant; out of which, 53 (91.3%) patients received treatment.

There is marked variation among these variants with regard to their frequency, clinical presentation, response to treatment, and prognosis in different regions of the world. The exact reason for the paucity of PHI and CEL variants is not clear but there may be a misclassification of the CEL variant as the TIP variant because cellular lesions can exist within the tuft at the tubular pole as the tip location and intracapillary expansive foam cells can be observed in both variants.
This is one of the largest cohorts of patients with COL FSGS in the Asian population as other studies from Asia did not show such a large number of patients with this lesion. A study done on the Korean population of 111 patients with primary FSGS showed 63% NOS, 18% TIP, 15% PHI, 3% CEL, and only one patient of COL FSGS [8].

In general, there is a wide diversity in the prevalence of different variants of FSGS in different regions. In a Brazilian report, NOS was the most common variant, and COL was the second most common [7]. It was also observed that there was a substantial overlap of criteria for the NOS and PHI variants as well as for COL and CEL variants. With regard to the CEL variant, it has been claimed that it is merely a form of the COL variant, and histologically it is very difficult to differentiate between these two variants. In fact, a common pathophysiological pathway affecting cell cycle regulatory proteins has been suggested for both variants [7]. The literature review also shows that the COL variant is less common in Whites than in the black race as compared to NOS, TIP, PHI, and CEL variants, which are not common in the black race [6,8,12].

The literature review of the published studies on the Asian population shows that in China and India, TIP, NOS, and CEL variants are more frequent morphological variants with different outcomes to treatment [16,20-22]. On the contrary, we found different results in our population. Shakeel et al studied a large cohort of 184 patients from our center. They found that the COL variant was the second most common morphological variant of FSGS in our patients [19]. These results were different from the Indian cohort as described previously. A more recent study from our institute evaluated the long-term outcome of adults with primary FSGS and showed a large number of patients with the COL variants after NOS and TIP variants [2]. However, PHI and CEL variants were not common in our population as we did not find a significant number of patients of both these variants. A recent study from Japan shows a significant number of patients with PHI and CEL variants with no significant difference in outcome after treatment among different morphological variants of FSGS [17].

In our study, the majority of patients were young with an average age of 29 years, and all presented with nephrotic-range proteinuria. The mean follow-up period of our study
was around 3 years. The primary outcome was the response to treatment and the secondary outcome was the composite outcome of the doubling of serum creatinine and KF with or without KRT. In addition, we also evaluated the improvement in eGFR as an additional outcome feature. We observed a good overall response to treatment in terms of achieving CR and PR. As in previous studies, this was highest in TIP, intermediate in NOS, and lowest in COL variants. In the TIP variant, there was a >34% increase of eGFR from the baseline after treatment which is a remarkable response to treatment in this variant, while in the NOS variant, eGFR increased to >10% and in the COL variant, there was a decrease of eGFR of 13% at the end of follow-up period. Only one patient developed a doubling of serum creatinine in the TIP variant with no patient developing KFRT or expired in this variant. There was a significant decline in eGFR in the COL variant with only two patients out of 53 achieving CR, and 40 patients out of 53 achieved no remission. This poor outcome of the COL variant is not different from previous studies and observations worldwide [23-28]. This variant has always remained aggressive with poor response to therapy [29-32]. However, a recent study from Japan showed almost similar outcomes for this variant compared to other variants except for the TIP variant. They proposed that this may be because of improved immunosuppressive treatment in recent years [17].

The poor outcomes of the COL variant in our study are alarming in the sense that we have a large number of patients with the COL variant which ultimately can result in an increased burden of KFRT in patients in the much younger population. In the NOS variant, we observed that 93/160 (58.1%) patients achieved no remission. Overall, the TIP variant showed a very good response to therapy in terms of primary and secondary composite outcomes.

Two of our recent studies showed that almost half of the adults with primary FSGS achieved sustained remission with steroids and immunosuppressants and consequently exhibited excellent short- to long-term kidney outcomes [2, 18]. Almost the same results were obtained in this study in that out of 302 patients, slightly less than half of the
patients achieved sustained remission while 169 patients achieved no remission with a wide diversity of responses to treatment in different variants.

There are certain strengths as well as limitations in this study. The strengths include a large sample size, homogeneous race and uniform treatment protocol in all patients. Meticulous and accurate classification of morphological variants by experienced nephropathologists is also a strong point of the study. We also noted some unique findings in this study. The prevalence of COL variant was quite high as compared with other regional studies. We also show that morphological variants, if accurately classified, do have therapeutic and prognostic importance. The limitations include a single-center and retrospective nature of the study. The follow-up duration was not very long. Two variants were not analyzed because of very small numbers. Moreover, genetic testing was not done in this cohort of patients, as currently the indications for genetic testing in adult patients with FSGS are unclear. A pilot project is in the pipeline for studying the role of genetics in adult nephrotic patients at our center and its results will be published in due course of time.

**CONCLUSION**

**Conclusion:**

The histological variants of primary FSGS according to Columbia classification are associated with different clinicopathological presentations and are predictive of response to treatment and progressive KF. There was a large number of patients with COL variant in this study, which is different from the rest of the Asian populations. These are results from a single center, and other studies are needed from our country so that we can compare the results and establish guidelines to effectively treat patients of primary FSGS with different morphological variants.

**ARTICLE HIGHLIGHTS**

*Research background*
The classification of focal segmental glomerulosclerosis (FSGS) is controversial and challenging. There is still a lack of a unified and consensus-based approach to classifying this disease, which is both practical and clinically useful.

**Research motivation**

This study addressed the clinical utility of the morphological classification of FSGS in real-world scenarios. We aimed to investigate the therapeutic and prognostic significance of the morphological variants of FSGS in a large cohort of adult patients.

**Research objectives**

This study aimed to determine the relative prevalence, clinicopathologic presentations, and outcomes of the morphological variants of FSGS in a large cohort of adult patients at a single center in Pakistan.

**Research methods**

This retrospective study included all consecutive adults (≥16 years) with biopsy-proven primary FSGS from January 1995 to December 2017. Studied subjects were treated uniformly with steroids and cyclosporine. The response rates and kidney outcomes were compared between histological variants using appropriate statistical tests. Data was analyzed in SPSS version 22.0. P-value ≤0.05 was considered significant.

**Research results**

The not otherwise specified (NOS) variant was the commonest, being found in 185 (53.9%) patients, followed by the tip variant in 100 (29.1%) patients. Collapsing, cellular, and perihilar variants were seen in 58 (16.9%), 6 (1.5%), and 3 (0.7%) patients, respectively. The response rates were higher in the tip variant and lowest in collapsing variant. Kidney outcomes were best in the tip variant and worst in the collapsing variant. NOS variant was intermediate.
Research conclusions
The morphological variants of FSGS are relevant and should be utilized to inform treatment and prognosis in individual patients. Combining these with other clinicopathological features to refine their predictive value needs to be studied in future studies.

Research perspectives
A holistic approach to disease categorization needs to be developed, which is practical and clinical-friendly.
## PRIMARY SOURCES

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