



Immunostaining of podocyte associate markers in renal biopsies; a valuable adjunct in characterisation of podocytopathies

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Received 14 January 2016

Accepted 3 March 2016

Published online 20 March 2016

Keywords: Nephrotic syndrome, Podocytopathies, Podocin, Nephrin, Minimal change disease, Focal segmental glomerulosclerosis

Citation: Badwal S, Kumar A, Singh S, Dutta V. Immunostaining of podocyte associate markers in renal biopsies; a valuable adjunct in characterisation of podocytopathies. *Immunopathol Persa.* 2016;2(2):e19.

Abstract

Introduction: Glomerular diseases comprise a wide spectrum of histopathological appearances within a clinically defined diagnosis of nephrotic syndrome (NS). Many of glomerular diseases are the result of genetic mutations encoding podocytic markers.

Objective: Our aim in this study was to offer a framework that integrates renal morphology with podocytopathies to facilitate management strategies and prognostic information.

Materials and Methods: Descriptive study of 50 cases of NS along with 50 controls wherein a combination of histopathological examination, morphometric studies, immunohistochemistry, direct and indirect immunofluorescence (IFF) were employed for evaluation of podocyte markers (podocin and nephrin) in renal biopsies. The clinical and demographic profile, management received and follow up was recorded for each patient and correlated with renal biopsy.

Results: All control renal tissues (n=50) exhibited linear glomerular basement membrane (GBM) staining. To maintain homogeneity we analyzed patients of minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), mesangial hypercellularity and diffuse mesangial sclerosis (DMS) and we grouped them as 'podocytopathies' (n=38). All cases (n=38) exhibited evidence of podocyte injury as evident by change of linear GBM positivity to granular pattern or complete absence of podocin (07/38, 14%) or nephrin (3/38, 7.8%). No histopathological variable predicted clinical response ($P=0.260$). The integrated diagnosis formulated by incorporating glomerular morphology and podocyte molecular phenotype strongly correlated with steroid resistance and outcome. ($\chi^2=26.437$, $P<0.001$ and $\chi^2=25.73$, $P<0.001$).

Conclusion: Evaluating podocytopathies in a more systematic manner by incorporating podocyte markers in the work up will facilitate more planned approach to diagnosis and management.

Introduction

Nephrotic syndrome (NS) is associated with a wide spectrum of primary and secondary glomerular diseases with varied histopathological features. Diverse glomerular lesions have different clinical courses, treatments, and outcomes (1).

Podocytopathies are group of disorders characterized by common element of podocyte injury. The clinical syndromes with podocyte dysfunction include minimal change disease (MCD), diffuse mesangial sclerosis (DMS), collapsing glomerulonephropathy, immune and inflammatory glomerulonephropathies, focal segmental glomerulosclerosis (FSGS), hypertensive nephropathy, diabetic nephropathy and age-associated glomerulonephropathy. Together these con-

Key point

Evaluating podocytopathies in a more systematic manner by incorporating podocyte markers in the work up will facilitate more planned approach to diagnosis and management.

ditions account for 90% of end-stage renal disease (ESRD) (1). Recent advances have identified molecular basis of these disorders where in a presence of structural defect in the glomerular barrier explains resistance to various management protocols. Mutations of NPHSI, NPHS2, WT-1 and ACTN4 are some of the causes for defective expression of podocyte associated proteins (nephrin, podocin and alpha actinin-4) (1).



Podocytopathies may appear at any age with varied implications for long term renal function. Efforts to develop a systematic approach to these diseases largely depends on histopathology. However different etiologies may exhibit similar histopathological features, accounting for heterogeneous clinical outcomes when managed on the basis of histology alone. Till date clinical response to steroids is the single most important determinant of prognosis.

Objectives

Our intent was to define the heterogeneous spectrum of NS by performing immunohistochemical staining for podocin and nephrin. We have also tried to offer a framework that integrates renal morphology with etiology to facilitate more clinician friendly information to select the optimal therapy for these often challenging diseases.

Materials and Methods

Study design

In this cross-sectional observation study, 50 cases of nephritic syndrome, diagnosed between October 2011 to May 2013, at the departments of pathology and nephrology of a tertiary care hospital were studied. All freshly diagnosed cases of NS that underwent renal biopsy as a part of their diagnostic workup were included in the study. Patients with inadequate follow up data and quantitatively inadequate material were excluded from the study. The relevant demographic and clinical data was accrued from the data maintained in the ward and outpatient register.

Histopathological evaluation

Informed consent was obtained from all patients and from the parents in the case of neonates and children. A minimum of two cores of renal biopsy were obtained. One core received in 10% formalin was processed for paraffin embedding and stained as per standard institutional protocol for evaluation of renal biopsy. The second renal biopsy core received in saline was processed for direct (DIF) utilizing FITC tagged IgG, IgM, IgA, C3c, C1q and for indirect immunofluorescence (IIF).

Immunohistochemistry

The primary antibodies used were NPHS2, dilution 1:50 (abcam, catalogue number ab 82108), NPHS1 dilution 1:50 (abcam, catalogue no 72908) and alpha actinin dilution 1:50 (abcam catalogue no 9465). Secondary detection system used was poly-HRP reagent conjugated to a biotinylated anti-rabbit secondary antibody with DAB as chromogen. Positive control used was renal tissue from autopsies, where death was not due to a specific renal pathology

Evaluation of immunohistochemistry

Evaluation was done on the basis of positivity, pattern, and intensity of antibody. Linear membranous pattern staining of glomerular basement membrane (GBM) was considered positive.

(i) Intensity

Absence of staining or localisation to cytoplasm or nucleus: 0, Staining intensity intermediate between positive control and negative control: 1, Staining equal to positive control: 2.

(ii) Pattern: Linear or granular/interrupted

(iii) Quantification of staining

Quantification was carried-out using digital image analysis software. Images of the glomeruli in the immunohistochemically stained sections were taken at a $\times 400$ magnification using a Dewinter trinocular research microscope with an inbuilt CCD camera and further analyzed using Dewinter Biowizard version 4.2 image analysis software. The glomerular area stained was calculated by drawing a region of interest around the glomerulus in which the amount of staining was measured as a percentage of glomerular area stained within a colour spectrum specific for the diaminobenzidine staining was determined. In each section, five images of individual glomeruli were recorded at $\times 400$ magnification. The quantification was carried out for control biopsies and patient biopsies. Mean percentage of glomerular area stained (mean of 05 glomeruli photographed) for each biopsy was determined.

Indirect immunofluorescence

IIF for nephrin (NPHS1 catalogue no 82108) and podocin (NPHS2 catalogue no 50339) was carried out. Frozen sections prepared from renal biopsy were washed with phosphate buffer saline (3 washes in 20 minutes). Slides were then incubated with primary antibody (dilution 1: 50) for 60 minutes. After incubation slides were again washed with PBS (3 washes in 20 minutes). Slides were then incubated with FITC tagged secondary antibody (abcam FITC tagged goat antibody to rabbit IgG catalogue no 97050 dilution 1:80) for 45 minutes. Slides after incubation were again washed with phosphate-buffered saline (PBS) three times, mounted with 90% glycerine and stored in dark at 4°C until observation with fluorescent microscope. The images so generated were evaluated in similar fashion as immunohistochemistry. (Control used was IIF photograph which was generated by carrying out IIF on fresh autopsy tissue). Relevant cases where additional tissue was available was taken for electron microscopic evaluation.

Ethical issues

The research followed the tenets of the Declaration of Helsinki; informed consent was obtained from all the participants of the study; and the research was approved by the institutional review board, Armed Forces Medical College, Pune, India.

Elucidation of diagnosis and statistical analysis

An excel data sheet was generated to analyse the complete data and was analyzed on SPSS version 17. The continuous variables were evaluated using paired student *t* test and one way analysis of variance (ANOVA). The categorical variables were assessed using chi-square analysis. For all, *P* value of 0.05 or less was considered significant.

Results

Demographic and clinical profile (n=50)

Age of the patients varied from 1 month to 61 years with mean of 24 years and a median of 26 years. Male patients (38/50, 76%) outnumbered female patients (12/50, 24%). Mean proteinuria was 4.1 gm/ 24 h. Microscopic haematuria was evident in 11/50 (22%) patients. Urine sediments in rest of the patients were bland. Mean serum creatinine was 1.15 mg/dL. 21/50 patients had hypertension at presentation.

Histopathological diagnosis, immunohistochemistry and immunofluorescence on renal biopsy (n=50)

16/50 (32%) cases were MCD followed by FSGS 15/50 (30%), membranous nephropathy 7/50 (14%), mesangial hypercellularity 5/50 (10%), membranoproliferative glomerulonephritis 5/50 (10%) and DMS 2/50 (4%). Seven (14%) patients showed absence of podocin expression, 38 (76%) patients had reduced staining intensity and 5 (10%) patients had intensity equal to positive control. All controls exhibited linear GBM staining while all cases (n=50) displayed granular or uneven basement membrane pattern. Nephtrin immunostaining exhibited preserved staining equal to positive control in 30/50 cases, reduced staining in 17/50 cases with granular pattern and completely absent in 3/50 cases (Figures 1-5).

To maintain homogeneity of statistical analysis, we took patients of MCD, FSGS, mesangial hypercellularity and DMS into consideration. As all these cases exhibited effacement of podocyte foot processes, these diseases were grouped as 'podocytopathies' (n=38).

Demographics and clinical profile

There was preponderance of male 32/38 (84.2%) over females 6/38 (15.8%). The age was categorized as represented in Table 1. 89.5% (34/38) of patients had received

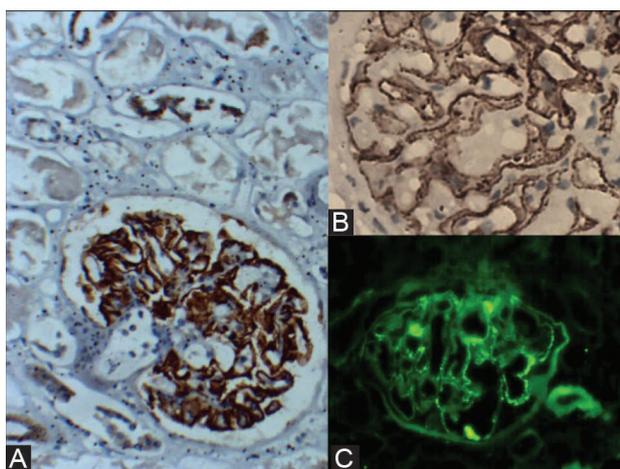


Figure 1. (A) Podocin control with linear pattern. Immunoperoxidase stain with DAB staining, magnification 200x. (B) Minimal change disease (MCD). Podocin staining with granular pattern. Immunoperoxidase stain with DAB staining, magnification 400x. (C) MCD. Indirect immunofluorescence (IIF) for podocin exhibiting granular GBM staining, magnification 200x.

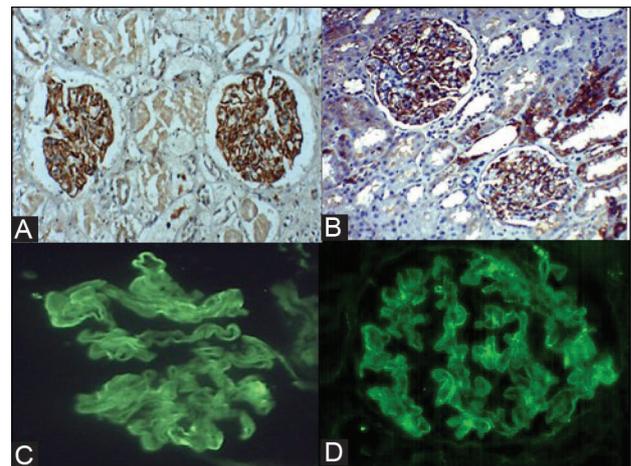


Figure 2. (A) Minimal change disease (MCD) podocin, immunoperoxidase stain with DAB, magnification 200x; (B) Nephtrin, immunoperoxidase stain with DAB, magnification 200x; (C & D) Indirect immunofluorescence (IIF) for podocin and nephrin exhibiting linear GBM staining, magnification 400x.

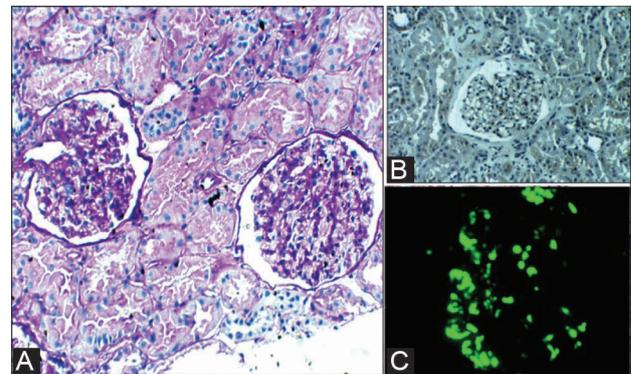


Figure 3. Mesangial hypercellularity (A) PAS magnification 100x; (B) podocin (intensity=0) immunoperoxidase stain with DAB, magnification 200x; (C) Direct immunofluorescence exhibiting mesangial pattern for FITC tagged IgM, magnification 400x.

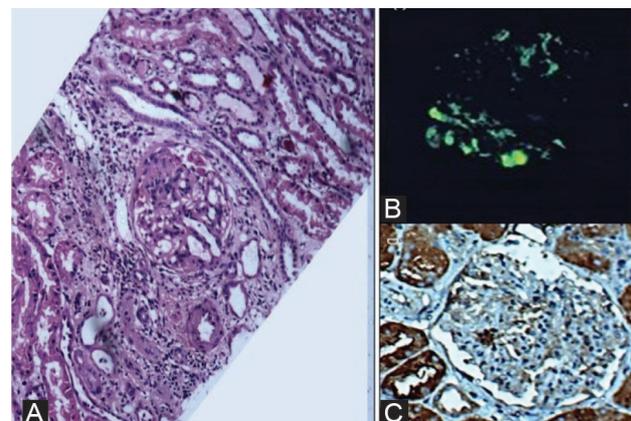


Figure 4. Focal segmental glomerulosclerosis (A) Glomerulus exhibiting tuft sclerosis with synechia formation. H&E magnification 100x; (B) Direct immunofluorescence FITC labelled IgM, mesangial positivity, magnification 400x; (C) Podocin negative (intensity=0). Immunoperoxidase stain with DAB, magnification 200x.

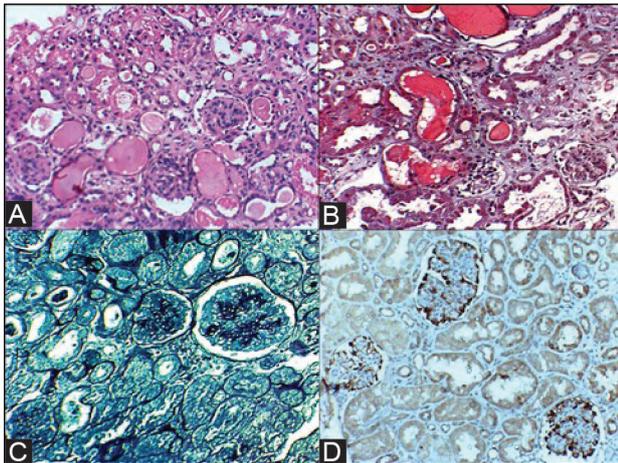


Figure 5. Diffuse mesangial sclerosis (DMS) with dilated tubules exhibiting hyaline casts. (A) H&E magnification 200× (B) MT stain magnification 200×; (C) PASM stain magnification 200×; (D) Podocin proliferated at the periphery and absent in the central tuft of glomerular capillaries. Immunoperoxidase stain with DAB, magnification 200×.

treatment in the form of steroids prior to biopsy. Steroid resistance was the indication for biopsy in 14/38 (36.8%) of cases. Other were frequent relapses 8/38 (21%), adult onset NS 8/38 (21%), clinically suspected FSGS 4/38 (11.5%), myasthenia gravis 1/38 (2.6%), clinically chronic kidney disease (CKD) 2/38 (5.2%), post-transplant NS 1/38 (2.6%).

Podocin and nephrin status

7/38 (18.8%) cases showed absent podocin expression, 26/38 (68.4%) cases showed reduced intensity and in 5/38 (13.2%) cases the intensity was similar to normal renal biopsies. Nephrin expression was lost in 3/38 cases and rest showed reduced staining with pattern similar to podocin. Results of indirect IIF were available in 15/38 cases (Table

Table 1. Age-wise distribution of ‘podocytopathy’ group (n=38)

Age group	Cases	Percent
0	7	18.4
1	7	18.4
2	22	57.9
3	2	5.3
Total	38	100.0

0 (less than 1), 1(>=1-15), 2 (15-50), 3 (more than 50).

Table 2. Results and interpretation of immunohistochemistry

Variables	All cases, n=50		Podocytopathy group, n=38		Controls, n=50
	Podocin	Nephrin	Podocin	Nephrin	Podocin & Nephrin
Intensity					
0	7	3	7	3	0
1	38	17	26	35	0
2	5	30	5	0	38
Pattern					
Negative	7	3	7	3	0
Linear	0	30	0	0	38
Granular	43	17	31	35	0

0 (Absent staining), 1(Staining intensity intermediate between positive control and negative control), 2 (Staining equal to positive control).

2). In 23 cases either tissue was suboptimal or staining was not satisfactory (all cases with background stain or strong interstitial/tubular stain was considered unsatisfactory). 4/15 cases were negative for podocin stain and rest exhibited granular GBM staining. For nephrin, IIF results were available in 18/38 cases wherein 2/18 cases were negative and rest showed granular GBM positivity. There was no case where there was disparity between markers evaluated by IIF or immunohistochemistry. The cases after morphological analysis, immunohistochemical studies, and clinical workup were categorised into entities as indicated by Barisoni et al (1) (Table 3).

Area of podocin expression stained; control versus patient

Mean percentage area stained for podocin in control and patient slides was tabulated and compared using paired student *t* test. The difference in the two means was found to be statistically significant with lower mean percentage area stained in patients as compared to the tissue controls (22.81 ± 9.30% versus 40.44 ± 2.08%, *t* = 8.22, *P* = 0.001).

Management and follow up

Patients were managed with steroids, immunosuppressant and other supportive measures. Mean follow up period was 1.2 years. 21/38 (55.3%) patients exhibited complete/partial remission while rest 17/38 (44.7%) showed no remission. Two patients went into ESRD and became dialysis dependent. One patient underwent medical nephrectomy followed by renal transplantation. However patient developed recurrence of nephrotic range proteinuria after two months. Repeat biopsy revealed MCD.

Analysis was carried within podocin group as very few cases exhibited absent nephrin staining. Though no statistical association was observed between podocin positivity and age categories 5/7 podocin negative cases were seen in less than 15 years of age as compared to 2/24 cases seen in age group of 15 years and above ($\chi^2 = 4.4, P = 0.035$). All cases, less than 1 year of age were steroid resistant. All cases with negative nephrin staining were less than 1 year and all were steroid resistant.

Histological features versus podocin status and steroid resistance

Renal biopsy was examined for mesangial hypercellularity,

Table 3. Integrated diagnosis based on morphology, IHC and clinical profile (n=38)

Morphology	Podocin	Nephrin	Integrated diagnosis	No.
MCD	+	+	Primary idiopathic	13
MCD (NSAID associated, Myasthenia gravis)	+	+	Secondary	02
MCD	-	+	Possibly NPHS2 mutation associated	01
FSGS	+	+	Primary idiopathic	08
FSGS (Posttransplant, CIN-CKD)	+	+	Secondary	02
FSGS	-	+	Possibly NPHS2 mutation associated	04
Mesangial hypercellularity	-	+	Possibly NPHS2 mutation associated	01
Mesangial hypercellularity	+	-	Possibly NPHS1 mutation	02
Mesangial hypercellularity	-	-	Possibly multiple mutations	02
DMS	+	+	DMS	02

Abbreviations: MCD, Minimal change disease; FSGS, Focal segmental glomerulosclerosis; DMS, Diffuse mesangial sclerosis.

Table 4. Association of histological features with podocin expression and podocin intensity

Serial No.	1	2	3	4	5	6	7
Histological Feature	Mes cell	Segscl	Gloscl	TA	IF	DIF	Histological category
Podocin expression (P value)	0.219	0.290	0.04	0.158	0.29	0.519	0.260
Podocin Intensity (P value)	0.257	0.119	0.06	0.04	0.119	0.012	0.07

Abbreviations: Mes cell: mesangial hypercellularity; Seg scl: segmental sclerosis; Glo scl: global sclerosis; TA: tubular atrophy; IF: interstitial fibrosis; DIF: direct immunofluorescence.

Table 5. Association of integrated histological diagnosis with steroid resistance

Steroid resistance ^a	Histological diagnosis (integrated) ^b					Total
	0	1	2	3	4	
0	1	1	6	4	2	14
1	14	9	1	0	0	24
Total	15	10	7	4	2	38

Pearson chi-square, $\chi^2=26.437$; df (4) $P=0.000$.

^aSteroid resistance (0: no remission, 1: incomplete or complete remission).

^bHistological diagnosis (integrated): 0 - MCD (Idiopathic/Secondary); 1- FSGS (Idiopathic/Secondary); 2- Possibly NPHS2 mutations; 3- Possibly other mutations/nephrin; 4 - DMS.

Table 6. Association of integrated histological diagnosis with outcome

Outcome	Histological diagnosis integrated					Total
	0	1	2	3	4	
0	1	3	7	4	2	17
1	14	7	0	0	0	21
Total	15	10	7	4	2	38

Pearson chi-square, $\chi^2=25.73$; df (4) $P=0.000$.

^aOutcome (0: no remission, 1: incomplete or complete remission).

^bHistological diagnosis (integrated): 0 - MCD (Idiopathic/Secondary); 1- FSGS (Idiopathic/Secondary); 2- Possibly NPHS2 mutations; 3- Possibly other mutations/nephrin; 4 - DMS.

segmental sclerosis, global sclerosis, tubular atrophy and interstitial fibrosis. The biopsies were also categorized as MCD, mesangial hypercellularity, FSGS and DMS. These features were assessed for steroid resistance and podocin positivity. No statistical association was found between histological features with podocin expression except for global sclerosis and tubular atrophy (Table 4). All 3 cases with absent nephrin staining revealed mesangial hypercellularity.

No statistical significance was obtained between histological features and steroid resistance. However significant statistical association was found between steroid resistance and podocin staining wherein 6/7 cases showing absent podocin expression exhibited steroid resistance ($\chi^2=8.808$,

$P=0.003$). Only 1/14 cases exhibiting steroid resistance displayed normal podocin intensity. All 13 cases either showed absent or reduced podocin intensity. When combined histological, DIF and immunohistological factors were taken together there was strong association between integrated diagnosis and steroid resistance (Table 5). Strong statistical association was found between podocin status and integrated histological diagnosis with remission achieved. Negative podocin status was strongly associated with 'lack of remission'. On management with immune suppressants, no case belonging to the category DMS and possibly mutation group achieved remission. Whereas all cases but one case belonging to the MCD group went into remission (Table 6).

Discussion

The spectrum of NS encompasses varied etiologies, histopathological features, variable sensitivity or high resistance to therapy. Recent developments in the molecular field suggest that traditional pathological descriptions are insufficient to classify these disorders. We studied the 50 cases of NS for podocin and nephrin expression by immunohistochemistry and IIF. Immunohistochemistry has the advantage of utilising archival cases and also applying control slide with every case processed. Hence standardisation was not problematic. IIF was convenient, fast with rapid results but could be performed only on fresh cases with adequate tissue core. It also had the constraint of not having fresh normal renal tissue as control with every case besides a long learning curve. However the results between immunohistochemistry and IIF were congruent with no evidence of gross unacceptable disparity between staining. 43/50 patients exhibited podocin and 47/50 patients exhibited nephrin expression with presence of interrupted/granular pattern as against strong linear basement pattern observed in renal biopsies from control group. This alteration of pattern from linear to granular occurred in all cases irrespective of diagnosis. This is in agreement with Arias et al, where all his cases comprising of MCD, FSGS and membranous nephropathy exhibited loss of normal linear pattern (2). This occurs because normal distribution of podocin and other proteins as nephrin, CD2AP is lost in cases of proteinuria. This suggests that the podocyte alteration found in these patients are directly related to the redistribution or loss of proteins associated with filtration diaphragm seen as effacement of foot processes under electron microscopy. This implies that loss of normal expression may or may not be associated with genetic alterations and is ubiquitous in cases of NS. Study by Koop et al, also found that the podocin levels were significantly decreased in disease categories with alteration in staining pattern as compared to controls (3).

The traditional glomerular disease classification encompasses a wide array of descriptive pathological entities. Mutations causing glomerular phenotypes reveal a simplified concept of glomerular diseases in which podocyte dysfunction, injury or loss is a common and determining factor (4). The group of 50 patients studied were an amalgam of variable underlying causes. Hence to maintain homogeneity of our cohort we selected 38 cases which by review of our literature, we knew were caused by podocyte damage or dysfunction. Our attempt was to categorise them so as to provide a more clinical and patient friendly diagnosis, using our tool of podocyte markers.

Podocin staining intensity and status

In 38 patients, 07 (18.4%) cases exhibited complete loss of podocin expression and 26 expressed reduction in intensity, percentage area stained and granular/interrupted pattern of podocin staining. We also found significant difference in reduction in area stained within the podocytopathy categories of MCD, FSGS and DMS. This was observed

by Barisoni et al, who elucidated that podocyte depletion and hence reduction in staining intensity and alteration in staining pattern occurs consequent to different mechanisms; direct podocyte depletion (FSGS), by a switch of the podocyte phenotype to one which cannot maintain normal glomerular structure and function (MCD) and podocyte injury, low proliferation with aberrant phenotype (DMS) (5).

Steroid resistance

Though patients of congenital and infantile NS are classically biopsied at the outset, the childhood NS is given a trial of steroids which is a therapeutic as well as a diagnostic measure for future outcome. We witnessed 34 of our 'podocytopathy' group patients were given steroids before biopsy. Attempts to find diagnostic morphological features which could predict the disease progression and response to treatment have been inconclusive. This is attributed to the fact that there are several heterogeneous disease entities under the morphology of MCD, FSGS, mesangial hypercellularity or DMS and steroid resistance defines their clinical phenotype and expression.

In order to define the role of histopathology in prognostication and response to treatment we analyzed individual histological features of the renal compartment as well as morphological diagnostic categories with steroid resistance, podocyte marker status and outcome. No statistical correlation was observed except for global sclerosis and tubular atrophy reiterating the historical fact that however methodical the morphology is, it does not define the response to treatment, outcome and prognosis. This is in concurrence to the findings of Machdo et al, who stated that histological picture on renal biopsy did not allow prediction or response to therapy or relapse (6).

Presence of global sclerosis and tubular atrophy are indicators of advanced disease and according to us may not be useful indices for prediction of response. This fact further adds strength to studying podocyte marker expression especially in infants and childhood NS. It is apparent therefore that classification of the podocytopathies on the basis of morphology alone is inadequate to capture fully the essence of these disorders (5).

Integrated morphological diagnosis, steroid resistance and outcome

Barisoni et al, has proposed a working classification on the basis that MCN, FSGS, DMS, and CG manifest distinct combinations of podocyte protein expression and can be classified as primary idiopathic, secondary and genetic causes (4,5). Applying the proposed taxonomy we classified glomerular morphology with podocyte molecular phenotype utilizing podocin/nephrin immunostaining and clinical inputs. Since reduced intensity can also occur in acquired disorders we labeled cases of absent podocin/nephrin expression as possibly mutation associated.

Hence when combined histological, DIF and immunohistological factors were taken for making integrated diagno-

sis, a strong association emerged between integrated diagnosis, steroid resistance and outcome. This emphasises the fact that MCD, FSGS mesangial hypercellularity and DMS are morphological patterns that needs to be defined by mutation phenotype or by work up for secondary causes. Besides variable morphological features observed in MCD that found in first biopsy, which was followed by diagnosis of FSGS in second biopsy results in delay in specific management and outcome. Such cases if diagnosed as MCD with possibly NPHS2 mutation at the outset, would have avoided a label of benign disease. More scientific approach to management in the form of renoprotective modalities and planned transplant could have been instituted early. A highly significant association has been observed between podocin absence and steroid resistance. Frishberg et al (7) and Caridi et al (8), demonstrated that NPHS2 mutations are limited to steroid resistant NS. Ruf et al, found no podocin associated mutations in 124 children with steroid sensitive nephritic syndrome. It is therefore preferable to perform podocin expression at the outset in children presenting with NS (9). Ruf et al (9) and Frishberg et al (7) further go on to recommend that all children with NS should be tested for podocin mutation before therapy to avoid an unnecessary steroid course. Also instead of giving complicated histomorphological diagnosis alone, absence of podocin expression must be mentioned as it will directly connote lack of steroid responsiveness. There are cases where instead of total podocin loss there is structural alteration of protein (10-12). These cases can only be detected by NPHS2 mutation studies since podocin immunostaining will reveal reduced intensity with alteration in staining pattern. Besides podocin/nephrin, there are many other mutations of structural proteins of GBM (13,14). Hence a panel of markers will be more useful followed by specific mutational analysis. Incorporation of immunohistochemical markers will provide a valuable direction in the histological workup prior to molecular analysis which is critical for a more specific therapeutic approach.

Conclusion

Our study employed a combination of histopathological examination, DIF, immunohistochemical and IIF evaluation of podocyte markers to approach podocytopathies in a more systematic manner so as to express them in a language more explicable to the clinicians. This approach will not only spare the patient of unnecessary steroid induced morbidity but will also facilitate more planned approach to diagnosis and management in the form of mutation analysis, renoprotective modalities and intended transplant. Most importantly it will enable segregation of couples for genetic counselling, so that the disease burden can be controlled.

Limitations of the study

This is a single center study with a limited sample size. A multicentric study with a larger sample size and incorpo-

ration of molecular analysis will enable validation of the results.

Authors' contribution

All authors contributed to the manuscript equally. They read and sign final paper.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by authors.

Funding/Support

The study was funded by director general armed forces medical services.

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