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PATTERN OF GLOMERULAR DISEASES IN NORTH EAST INDIA: LIGHT MICROSCOPY & IMMUNOFLUORESCENCE STUDY

Pathology

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ABSTRACT

Introduction: The spectrum of glomerular diseases varies significantly in different parts of the world and they are the leading cause of end-stage renal disease world wide. In the light of paucity of data from north eastern part of India, we studied the spectrum of glomerular diseases in a tertiary care centre at Shillong, Meghalaya and compared the spectrum in defined age group and sex.

Methods: All the kidney biopsies from January 2012 to June 2016 were analyzed with clinical, laboratory data including light microscopy and immunofluorescence study.

Result: A total of 192 renal biopsies were analyzed. 70 were males and 122 were females with a M:F ratio of 0.57:1. The age of patients ranged from 10 months - 65 years. The number of pediatric patients was 81 and adult patients were 111. Primary glomerular disease comprised 117 (60.93%) cases and secondary glomerular disease comprised 60 (31.25 %) cases. The most common presentation of patients was Nephrotic syndrome (52.6%). Minimal Change Disease (25.6%) and Focal Segmental Glomerulosclerosis (24.7%) were the most common types of primary whereas Lupus nephritis (80%) was the most common type of secondary glomerular disease.

Conclusion: This study suggests that there is a need to incorporate the data from North East in the national biopsy registry to obtain accurate knowledge about incidence, spectrum, and distribution of biopsy proven glomerular disease in our racially heterogenous country.

KEYWORDS

Glomerular diseases,primary,secondary,light microscopy.

Introduction

Glomerular diseases are the leading cause of end-stage kidney disease worldwide. The glomerulus is affected by a number of environmental insults and systemic disorders.[1] Immunological mechanisms are responsible for a majority of glomerulonephritis, irrespectively of primary or secondary causes.[2]

Histopathological examination of renal biopsies including light, immunofluorescence and electron microscopic examination, along with a correlation of clinical features and biochemical parameters, is necessary to establish an accurate diagnosis in order to initiate proper treatment and to prognosticate the disease. [1,2]

The spectrum of renal diseases is influenced by geographical, environmental, socioeconomic factors and prevalence of infectious diseases in that region. Therefore, it is important to identify the glomerular disease pattern in any given geographical location to apprehend the pathobiology of the disease in the region. It also helps to study the incidence and identify risk factors in the development and progression of the glomerular diseases. [1,3] In the light of paucity of data from north eastern part of India, we studied the spectrum of glomerular diseases in a tertiary care centre at Shillong, Meghalaya and compared the spectrum in defined age group and sex.

Materials and Methods:

All the kidney biopsies performed in our institute from January 2012 to June 2016 were analyzed. Inadequate biopsies (those containing < 6 glomeruli), and neoplastic diseases were excluded from the study. Ethical clearance was taken from the Institute Ethics Committee.

We recorded the relevant clinical and laboratory data for each patient: name, age, sex, indication for renal biopsy, clinical diagnosis, serum creatinine, BUN, 24-hour urinary protein, urine R/E, virology (HBsAg, anti-HCV, HIV), serology [antinuclear antibody (ANA) and ANCA] and histopathological diagnosis.

Renal needle biopsy were obtained under ultrasound guidance using 16 or 18 gauge Bard's bioptic gun depending on the age of the patient, and renal tissues ranging from 1-2 cm was obtained. All renal biopsy specimens obtained were prepared as per the standard protocol and examined by the same group of pathologists of our institute. Analysis included light microscopy (LM) and immunofluorescence (IF). However, electron microscopy (EM) was not performed as this facility was not available. For LM, sections were stained with Hematoxylin and Eosin, periodic acid Schiff, Masson's trichrome, and Jones silver methanamine. Special stains were used when required. IF study was done by using polyclonal antisera (FITC conjugated Rabbit Antihuman Antisera) against human IgG, IgM, IgA, C3, kappa and lambda light chains.

The indications for renal biopsy were categorized into six clinical syndromes: nephrotic syndrome (NS), acute nephritic syndrome (ANS), non-nephrotic proteinuria (NNP), isolated hematuria, acute renal failure (ARF) and chronic renal failure (CRF). Standard

definitions of the clinical syndrome were used.4 In CRF, renal biopsy was performed for unexplained renal failure if kidney sizes were within normal limit with intact corticomedullary differentiation.

Histological categories were classified as follows: I) primary glomerulonephritis (PGN) which included minimal change disease (MCD), FSGS, membranous nephropathy (MGN), IgA nephropathy (IgAN), membranoproliferative glomerulonephritis (MPGN), crescentic glomerulonephritis (CresGN), acute post streptococcal glomerulonephritis (APSGN), diffuse mesangiosclerosis and mesangioproliferative glomerulonephritis; II) secondary glomerulonephritis (SGN) included lupus nephritis (LN), diabetic nephropathy (DN), Henoch–Schönlein purpura (HSP), hypertensive nephropathy (HTN), hemolytic uremic syndrome (HUS) and light chain deposition disease (LCDD) ; III) Chronic glomerulonephritis (CGN); IV) End stage renal disease (ESRD) V) Tubulointerstitial disease.

Simple descriptive statistics such as mean, ratio and percentage were used for analysis.

Results

A total of 192 renal biopsies were analyzed in the study period from January 2012 to June 2016. Among them, 70 were males and 122 were females with a M:F ratio of 0.57:1. The age of patients ranged from 10 months - 65 years (mean age 24.21±14.03 years). The number of pediatric patients was 81 and adult patients were 111.Tables 1&2 demonstrates the glomerular disease spectrum according to age and sex.

The most common presentation of patients with glomerular diseases was NS (52.6%), followed by ANS (19.79%), NNP (16.15%), CRF (6.77%), isolated hematuria (2.6%) and ARF (2.08%). Among the glomerular diseases presenting with nephrotic syndrome, MCD and FSGS were the most common type of PGN and LN was the most common type of SGN. Table 3 summarises the various glomerular histologies in each clinical syndrome.

Primary glomerular disease (PGD) comprised 117 (60.93%) cases, secondary glomerular disease (SGD) accounted for 60 (31.25 %) cases, end-stage renal disease (ESRD) for 11 (5.73%) cases, chronic glomerulonephritis for 3 (1.56%) cases and tubulointerstitial disease for 1 (0.52%) cases. Among the PGD cases, MCD (25.64%) was the leading cause followed by FSGS (24.79%) and IgAN (22.2%). The most common secondary glomerulonephritis was lupus nephritis (80%), followed by HSP nephritis (8.3%).

In the pediatric age group (n=81), PGD comprised 59 (72.84%) cases, SGD accounted for 17 (20.99%) cases and end-stage renal disease for 5 (6.12%) cases. Among the PGD, FSGS was the most common type followed by IgAN and MCD. Among the SGD, LN was the most common type.

Among adults (n=111), PGD and SGD accounted for 58 (52.25%) cases and 43 (38.74%) cases respectively, whereas end-stage renal disease accounted for 6 (5.40%) cases. MCD and FSGS were the most common PGN. Among the SGN, LN was the most common type.

Female predominance was seen in MCD, IgAN, CresGN, LN, HSP nephritis, DN, HTN and ESRD. In rest of the types male predominance was seen.

MCD was seen in 30 patients with a M:F ratio of 0.76:1. 66.67 % of cases occurred in <30 years of age. The most common presentation was NS (90%) followed by NNP (6.67%). 10 % cases had microscopic hematuria. Deranged RFT and HTN were seen in 2 (6.67%) of the cases.

There were 29 cases of FSGS with a M:F ratio of 1.07:1. It was seen more in the age group of <18 years (55.2%) and the most common presentation was NS (86.2%). Hypertension was seen in 24.1% cases, deranged renal function was seen in 34.48% of the cases and hematuria was seen in 24.1% cases. The most common of FSGS according to Columbia classification was FSGS, NOS (75.86%) followed by cellular variant (20.69%) and tip variant (3.44%). No cases of collapsing or hilar variant were seen in the study.

There were total 26 cases of IgAN with a M:F ratio of 0.86:1.It occurred more commonly in 11 – 20 years of age group (42.3%) and the most common presentation seen were NS (38.46%) and NNP (34.61%). Hematuria was seen in 80.76% of the cases (21 cases). Hypertension and deranged renal function was seen in 38.46% of the cases. According to Haas classification the most common class of IgAN was class IV (14/26) followed by class II (5/26). We could review 23 cases according to Oxford/MEST classification and found mesangial proliferation in 17/23, endothelial proliferation in 6/23, segmental sclerosis in 17/23, tubular atrophy/ interstial fibrosis in 4/23 and crescents in 6/23 cases.

In the study LN was the most common glomerular disease and comprised 48 cases. 95.83% of the cases occurred in females. 22/48 cases (45.83%) occurred in the 21 – 30 years of age group. The most common presentation was ANS (37.5%) followed by NS (35.42%). Hematuria, HTN and deranged RFT was seen in 62.5%, 50% and 37.5% of the cases respectively. According to ISN/RPS Classification the most common class of LN was class IV (50%) followed by class III (21.4 %). ANA was positive in all the cases.

Immunofluorescence studies were done for all the cases. In 5 cases no glomeruli were seen so were considered inadequate for IF. IgG, IgA, IgM, C3, kappa and lamda were used in all the cases. Full house positivity was seen in 31/47 cases of LN in which IF were done.

Discussion

This study was carried out in a single tertiary care centre in north-eastern part of India for a period of four and half years and provides information about the demographic, indication of renal biopsy and pattern of various biopsy proven glomerular disease in this part of the country. But since the number of cases in the present study was less so it might not be actual representative of the spectrum. Secondly electron microscopy was not done in all of the cases which could have helped in the better diagnosis.

A comparison of the basic data and some common diseases in our series with those of other published studies from India and various parts of the world is given in Table 4 & 5. It is obvious from these two tables that the spectrum of glomerular disease in our study did not correspond to other Indian series. Most of these studies are multicentric.

In most of the studies done across the world and India, it was found that glomerular diseases occurred more commonly in males than females.[3,5,6,15,16,17] However, in the present study it was observed that females predominated over the males with a M:F ratio of 0.57:1. Similar observation was made in studies done in Egypt, Iran and Jordan.[18,19,20] The observed female predominance over males in the current study may be due to the high proportion of lupus nephritis cases, which is much higher in females than in males. Nearly one quarter of patients in our study (25%) had LN.

In our study nephrotic syndrome was the most common presentation of glomerular diseases at all ages. This was similar to that reported in various studies across the world including India, China, Romania, Australia and Jordan.[5,6,16,21,22] 52.6% of cases presented with nephrotic syndrome and it was comparable with other studies where the percentage of patients presenting with nephrotic syndrome ranged from 34% to 67%.[5,6,16,21,22]

Spectrum of PGD is quite variable across the world and even within various parts of the same country. Although there is no central renal biopsy registry in India, there are however many published data across various parts of the country, findings of various PGD of which are summarized in Table 6.

Conclusion

There is a wide variation in the spectrum of various primary and secondary glomerular diseases in different parts of the world and even within the same country. The spectrum in the north eastern part of India is different from other parts of India except Kolkata and is more comparable with countries like China and Nepal, this is possibly due to ethnically identical population. MCD and FSGS were found to be occurring with equal frequency in our series of biopsies, unlike the rest of India where MCD is the most common. However, recent studies on the spectrum of GD from various parts of the country have shown an increase in the incidence of FSGS. Moreover, the incidence of Lupus

nephritis is also very high in this part of the country compared to any other region.

As India is a racially heterogenous country, a national biopsy registry data may be established to address these regional differences in the spectrum of GD. There is a need to incorporate the data from north east in the national biopsy registry to obtain accurate knowledge about incidence, spectrum, and distribution of biopsy proven GD in our country.

Table 1: Glomerular disease spectrum according to the age of presentation.

Biopsy diagnosis	Age category (years)						
	0-10	11-20	21-30	31-40	41-50	51-60	61-70
MCD (30)	5	11	7	3	2	1	1
FSGS (29)	3	14	7	3	1	1	0
IgAN (26)	5	10	6	4	0	1	0
MPGN (12)	1	2	3	2	3	1	0
MGN (09)	0	2	1	2	2	2	0
APSGN (06)	1	5	0	0	0	0	0
Crescentric GN (03)	0	1	1	0	1	0	0
Diffuse mesangiosclerosis (1)	1	0	0	0	0	0	0
Mesangioproliferative GN (01)	0	1	0	0	0	0	0
LN (48)	2	17	23	4	2	0	0
HSP (05)	4	0	1	0	0	0	0
DN (03)	0	0	0	0	0	3	0
HTN (02)	0	0	0	0	0	2	0
HUS (01)	1	0	0	0	0	0	0
LCCD (01)	0	0	0	0	0	1	0
CGN (03)	0	0	2	1	0	0	0
ESRD (11)	0	6	3	0	0	1	1
TI (01)	0	0	0	0	1	0	0
	23	69	54	19	12	13	2

MCD-Minimal Change Disease, FSGS-Focal Segmental Glomerulosclerosis, IgAN-IgA Nephropathy, MPGN-Membranoproliferative Glomerulonephritis, MGN – Membranous Glomerulopathy, APSGN – Acute post streptococcal glomerulonephritis, LN-Lupus nephritis, HSP- Henoch Schonlein purpura ,DN- Diabetic Nephropathy, HTN-Hypertensive Nephropathy, HUS-Hemolytic Uremic Syndrome, LCCD- Light Chain Deposition Disease, CGN – Chronic Glomerulonephritis ,ESRD – End Stage Renal Disease, TI- Tubulo Interstitial disease.

Table 2: Glomerular disease spectrum according to sex.

Biopsy diagnosis	MALE	FEMALE
MCD (30)	13	17
FSGS (29)	15	14
IgAN (26)	12	14
MPGN (12)	6	6
MGN (09)	5	4
APSGN (06)	4	2
Crescentric GN (03)	1	2
Diffuse mesangiosclerosis (1)	1	0
Mesangioproliferative GN (01)	1	0
LN (48)	2	46
HSP (05)	2	3
DN (03)	0	3
HTN (02)	0	2
HUS (01)	1	0
LCCD (01)	1	0
CGN (03)	2	1
ESRD (11)	4	7
TI (01)	0	1
	70	122

MCD-Minimal Change Disease, FSGS-Focal Segmental Glomerulosclerosis, IgAN-IgA Nephropathy, MPGN-Membranoproliferative Glomerulonephritis, MGN – Membranous Glomerulopathy, APSGN – Acute post streptococcal glomerulonephritis, LN-Lupus nephritis, HSP- Henoch Schonlein purpura ,DN- Diabetic Nephropathy, HTN-Hypertensive Nephropathy, HUS-Hemolytic Uremic Syndrome, LCCD- Light Chain Deposition Disease, CGN – Chronic Glomerulonephritis ,ESRD – End Stage Renal Disease, TI- Tubulo Interstitial disease.

Table 3: Spectrum of various glomerular histologies in each clinical syndrome.

Biopsy diagnosis	NS	ANS	NNP	CRF	Isolated hematuria	ARF
MCD (30)	27	1	2	0	0	0
FSGS (29)	25	2	1	1	0	0
IgAN (26)	10	2	9	1	2	2
MPGN (12)	7	2	2	0	0	1
MGN (09)	7	0	2	0	0	0
APSGN (06)	0	4	1	0	0	1
Crescentric GN (03)	1	2	0	0	0	0
Diffuse mesangiosclerosis (1)	1	0	0	0	0	0
Mesangioproliferative GN (01)	1	0	0	0	0	0
LN (48)	17	18	11	1	1	0
HSP (05)	0	1	2	0	2	0
DN (03)	2	0	0	1	0	0
HTN (02)	0	2	0	0	0	0
HUS (01)	0	1	0	0	0	0
LCCD (01)	1	0	0	0	0	0
CGN (03)	1	1	0	1	0	0
ESRD (11)	1	1	1	8	0	0
TI (01)	0	1	0	0	0	0
	101	38	31	13	5	4

MCD-Minimal Change Disease, FSGS-Focal Segmental Glomerulosclerosis, IgAN-IgA Nephropathy, MPGN-Membranoproliferative Glomerulonephritis, MGN – Membranous Glomerulopathy, APSGN – Acute post streptococcal glomerulonephritis, LN-Lupus nephritis, HSP- Henoch Schonlein purpura ,DN- Diabetic Nephropathy, HTN-Hypertensive Nephropathy, HUS-Hemolytic Uremic Syndrome, LCCD- Light Chain Deposition Disease, CGN – Chronic Glomerulonephritis ,ESRD – End Stage Renal Disease, TI- Tubulo Interstitial disease. NS-Nephrotic syndrome, ANS- Acute Nephritic Syndrome, NNP-Non Nephrotic Proteinuria, CRF – Chronic Renal Failure, ARF – Acute Renal failure.

Table 4: Comparison of some clinical data and common renal diseases in our series with other studies.

	Present study	South India4	Kolkata5	CMC Vellore3	Pakistan6	Nepal7	China8
Duration	2012-16	1990-2008	2010-12	1990-2001	1995-2008	2001-07	1979-2002
Age (yrs)	22.7	32.27±18.4	28±14.6	>15	32.9±12.8	30.6	32.7±12.2
M:F	0.57:1	1.5:1	1.05:1	-	1.6:1	1.6:1	1.3:1
No of cases	192	2401	666	4035	1793	137	13519
NS (%)	52.6	49	61.56	-	49.9	81.6	-
PGD (%)	60.93	69.1	79.13	-	73	-	68.6

SGD	31.25	18.2	20.87	-	10.9	-	24.8
MCD	15.6	15.1	20.12	10.8	5.8	10.2	0.60
FSGS	15.1	10.5	18.02	16.8	21.2	8.0	4.1
MGN	4.68	7.0	12.01	9.5	17.2	42.3	6.7
IgAN	13.5	4.4	8.1	8.4	1.5	2.9	31.0
MPGN	6.25	3.9	5.25	2.9	1.1	21.9	2.3
APSGN	3.1	5.6	4.95	13.5	-	2.2	-
Crescentric GN	1.56	4.5	7.51	3.5	3.9	-	1.3
DN	1.56	1.2	0.15	2.8	0.9	0.7	1.6
HSP	2.6	-	0.3	-	-	-	5.0
LN	25	14.6	5.23	6.9	4.9	1.5	13.5
Amyloidosis	-	1.5	1.2	0.5	4.6	1.5	0.6
ESRD	5.73	2	-	4.2	-	-	-
CGN	1.56	6.7	3	-	11.6	2.2	-
TI	0.5	6.7	-	3.6	11.6	1.5	3.4

PGD – Primary Glomerular Disease, SGD- Secondary Glomerular Disease, MCD-Minimal Change Disease, FSGS-Focal Segmental Glomerulosclerosis, IgAN-IgA Nephropathy, MPGN-Membranoproliferative Glomerulonephritis, MGN – Membranous Glomerulopathy, APSGN – Acute post streptococcal glomerulonephritis, LN-Lupus nephritis, HSP- Henoch Schonlein purpura ,DN- Diabetic Nephropathy, HTN-Hypertensive Nephropathy, HUS-Hemolytic Uremic Syndrome, LCCD- Light Chain Deposition Disease, CGN – Chronic Glomerulonephritis ,ESRD – End Stage Renal Disease, TI- Tubulo Interstitial disease.

Table 5: Comparison of some clinical data and common renal diseases in our series with other studies.

	Serbia9	Czech 10	Korea11	Brazil 12	Spain13	Bahrain 1	Hongkong 14
Duration	1987-2006	1994-2002	1987-2006	1993-2007	1994-99	1990-2002	1993-97
Age (yrs)	35±13.4	-	36	-	-	-	41.0
M:F	1.1:1	1.4:1	1.02:1	-	-	-	-
No of cases	1626	4004	1818	9617	7016	490	1413
NS (%)	53.6	39.3	-	-	36.6	-	27.4
PGD (%)	63.6	59.8	74.0	51	66	44.8	-
SGD	23.05	25.4	11.8	22.6	20	33.6	-
MCD	6.2	12.5	15.5	15.5	7.8	30	8.8
FSGS	18.7	10.8	5.6	24.4	10	23.8	4.7
MGN	23.8	9.3	12.3	20.7	9.7	13.5	8.3
IgAN	10.7	34.5	28.3	20	15.2	0.4	23.9
MPGN	13.3	4.6	4	4.2	4.3	14.3	-
APSGN	2.2	-	-	0.7	-	3	-
Crescentric GN	6	3.2	-	1.7	-	2.7	0.6
DN	-	-	2.0	10.1	-	31.9	5.7
HSP	-	5.7	1.0	-	-	-	0.5
LN	53.3	23	8.7	42.4	8.8	38.9	20.5
Amyloidosis	6.7	9.9	-	6.2	4	2.7	0.5
ESRD	-	1.2	-	-	-	-	-
CGN	-	3.2	-	-	-	-	-
TI	3.0	4.4	-	2.2	-	13.1	-

NS-Nephrotic Syndrome,PGD – Primary Glomerular Disease, SGD- Secondary Glomerular Disease, MCD-Minimal Change Disease, FSGS-Focal Segmental Glomerulosclerosis, IgAN-IgA Nephropathy, MPGN-Membranoproliferative Glomerulonephritis, MGN – Membranous Glomerulopathy, APSGN – Acute post streptococcal glomerulonephritis, LN-Lupus nephritis, HSP-Henoch Schonlein purpura ,DN- Diabetic Nephropathy, LCCD-Light Chain Deposition Disease, CGN – Chronic Glomerulonephritis ,ESRD – End Stage Renal Disease, TI- Tubulo Interstitial disease.

Table 6: Spectrum of various PGD in India.

Place	Spectrum of PGD
South India (Das et al.)4	MCD > FSGS
Kolkata (Golay et al.)5	MCD > FSGS
North India (Mannan et al.)23	MGN >Mesangioproliferative GN
Kashmir (Reshi et al.)24	MCD > FSGS
AIIMS, Delhi (Agarwal et al.)25	MCD > MGN
Haryana (Aggarwal et al.)26	MCD > MPGN
CMC, Vellore (Balakrishnan et al.)3	MCD > FSGS

PGD – Primary Glomerular Disease, MCD-Minimal Change Disease, FSGS-Focal Segmental Glomerulosclerosis, MPGN-Membranoproliferative Glomerulonephritis, MGN – Membranous Glomerulopathy,

Fig 1a:20x H&E: lupus nephritis with Grade Iv diffuse glomerulonephritis and Fig 1b: DIF 40x: full house positivity in lupus nephritis.

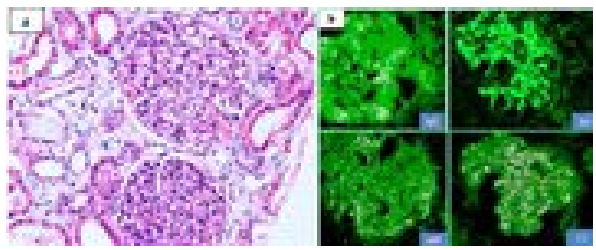


Fig 2 a:20x H&E: Acute poststreptococcal glomerulonephritis: showing enlarged hypercellular glomeruli with neutrophilic extravasation. b.DIF 40x,C3: Granular capillary wall & mesangial 3+ deposit in APSG

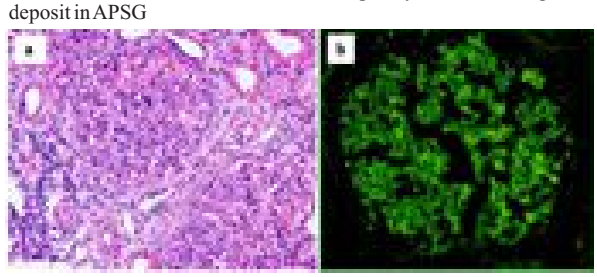


Fig 3a: 20x PAS: showing IgA nephropathy with M1E1S1T0 score. b. DIF 20x,IgA : 3+ Mesangial deposits in IgA nephropathy

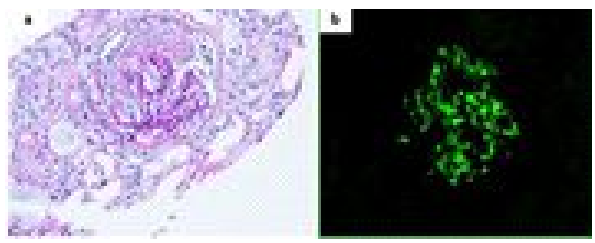
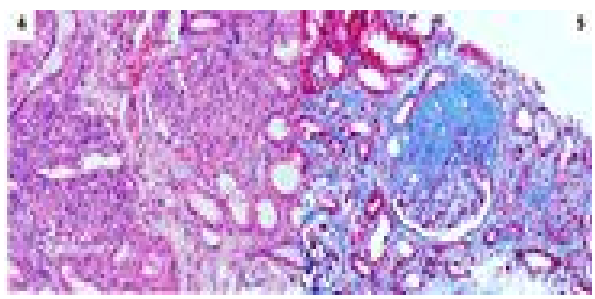


Fig 4:20x H&E: membranoproliferative glomerulonephritis with lobular accentuation

Fig5: 20x MT:showing focal segmental glomerulosclerosis



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