

Original Research Article

HISTOPATHOLOGICAL SPECTRUM OF RENAL BIOPSIES IN NEPHROTIC SYNDROME-A DESCRIPTIVE STUDY IN THANJAVUR MEDICAL COLLEGE

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ABSTRACT

Background: In developing countries Glomerulonephritis is the most common cause of morbidity and mortality. Nephrotic Syndrome is a pentad of proteinuria more than 3.5g/24hr, hypoalbuminemia, edema, hyperlipidemia, and lipiduria. The present study was conducted to study the histopathological spectrum of nephrotic syndrome based on light microscopy and immunofluorescence findings.

Materials and Methods: Renal biopsies were taken from patients who presented with Nephrotic syndrome at Nephrology ward of Thanjavur Medical College and Hospital for a period of one year from 2020-2021. Based on inclusion and exclusion criteria the sample size calculated was 60 cases. All patients underwent routine biochemical tests concerned with nephrotic syndrome. Data were entered in the excel sheet and analyses were done using Graph pad Prism version 5 software. $p < 0.05$ were measured as statistically significant

Results: Majority of our study participants belongs to 16-29 years of age (66.7%). Female preponderance was observed (55%). 49(81.7%) of the study participants gets affected due to primary cause. The most common histopathologic finding was Minimal Change Disease (28.3%). Sex had a significant association with the etiology. Histopathological diagnosis has significant association with gender. Significant association was found between histopathological diagnosis and 24 hr urine protein gram per day.

Conclusion: The study concluded that the most common histopathological finding was Minimal Change Disease (28.3%) followed by Focal segmental glomerulosclerosis (21.7%). Among the secondary glomerular diseases, Lupus Nephritis was common and that too majority of patients belong to Class IV A and Class V Lupus Nephritis. Significant association was found between histopathological diagnosis and 24 hr urine protein gram per day.

Keywords: Proteinuria, Nephritis, Nephrotic syndrome, Hypoalbuminemia, Lipiduria.

INTRODUCTION

Glomerulonephritis is the frequent cause of morbidity and mortality in developing countries. The diagnosis and categorisation of glomerular diseases implies a great challenge to the pathologists because of its complexity and diversity of lesions. The renal biopsy procedure has helped in

simplifying the diagnosis of glomerular disease. The use of light microscopy alone is not enough to establish a probable diagnosis. Hence the usage of electron microscopy and Immunofluorescence have great role in the diagnosis of glomerular diseases.^[1] The glomerular diseases are classified based on combinations of significant expression of glomerular injury namely proteinuria, hematuria,

reduced GFR and derangement in sodium homeostasis that leads to edema and hypertension.^[2] Nephrotic Syndrome is a pentad of proteinuria more than 3.5g/24 h, hypoalbuminemia, edema, hyperlipidemia, and lipiduria.^[3] Most studies have relied on edema rather than hypoalbuminemia.^[4,5]

Diseases causing the Nephrotic Syndrome(NS) are generally classified into those that primarily involve the kidney - Primary glomerular diseases and those in which kidney involvement as a component of a systemic disorder - Secondary glomerular diseases. The incidence of NS in adults is three per 100,000 persons annually.^[6] More common in boys than girls in children, but there is no gender difference in adolescent age groups. Adult onset NS has a greater prevalence of hypertension, renal impairment and poor response to steroids, but decreased tendency to relapse. Hypertension in nephrotic syndrome appears more common in teenagers.

Approximately 90% of NS cases in adults are idiopathic.^[6] Focal segmental glomerulosclerosis (FSGS) is emerging as the primary cause of adult NS in the recent published data around the globe.^[7-9] But there are regional variations, with Minimal Change Disease being the commonest cause of adult NS and FSGS being much less common in studies from Korea and Denmark.^[10,11] Membranous nephropathy remains the most common cause in white patients, whereas Focal Segmental Glomerulosclerosis is the most common cause in black patients.^[12] Limited data from Indian subcontinent on adult NS too show FSGS as the commonest cause.^[13]

Secondary glomerulopathy, such as lupus nephritis, Henoch Schonelein purpuric glomerulonephritis and amyloidosis constitute 10% of cases of Nephrotic syndrome^[14]. In developed countries electron microscopy and immunofluorescence are used in the evaluation of renal biopsies. The immunoperoxidase technique is also used in the detection of immune deposits in glomerular lesions which have greater sensitivity. The electron microscope and immunoperoxidase studies are costlier than immunofluorescence and is routinely used in reporting of kidney biopsies especially in developing countries.^[13,15]

The present study is being conducted to study the histopathological spectrum of nephrotic syndrome based on light microscopy and immunofluorescence findings.

Aim and Objectives

1. To study the histopathological spectrum of renal biopsies in Nephrotic Syndrome in Thanjavur Government Medical College.
2. To study the role of special stains and immunofluorescence in nephrotic syndrome.

3. To analyse the histologic types of glomerulonephritis with nephrotic presentations.
4. To correlate the clinicopathological findings of various types of glomerulonephritis.

MATERIALS AND METHODS

Source of data: Renal biopsies were taken from patients who presented with Nephrotic syndrome at Nephrology ward of Thanjavur Government Medical College Hospital for a period of one year from 2020-2021. The present study is with a sample size of about 60 cases. All patients underwent routine biochemical tests concerned with nephrotic syndrome.

Inclusion Criteria

- All patients clinically diagnosed as nephrotic syndrome.
- >15 years of age group

Exclusion Criteria

- Non glomerular diseases.
- <15 years of age.

Statistical Analysis

Data were entered in the excel sheet and the variables were coded. The analyses were done using Graph pad Prism version 5 software. Data were presented as mean with Standard deviation for normal distribution and were presented as frequency with proportion n (%) for categorical data. Fisher's exact test was used to evaluate the frequencies between the groups. $p < 0.05$ were measured as statistically significant.

Method

Renal tissue was taken by percutaneous automated spring loaded biopsy gun with a 16 to 18 gauge needle under ultrasound guidance after local administration of 2% lignocaine. Two cores of biopsy tissue taken and one was fixed in 10% buffered formalin solution for Light microscopy and another in Michel's solution for Immunofluorescent analysis.

Formalin preserved biopsies were processed and 12-15 sections each measuring 3 microns thickness were obtained and stained with hematoxylin and eosin. For certain cases special stains like PAS, Jones silver stain, Masson's trichrome stain were used. MTS was used to highlight the basement membrane thickening and PAS to demonstrate the increase in mesangial matrix, mesangial hypercellularity, basement membrane changes and tubular casts. Immunofluorescent staining was performed with polyclonal fluorescein isothiocyanate conjugated with antibodies against IgG, IgA, IgM, C3, C1q, kappa, lambda.

RESULTS

The results of the study was as discussed below

Table 1: Demographic Profile characteristics

Variable	N	%
Age Category		
16-29 years	40	66.7
30-59 years	18	30
≥60 years	2	3.3
Sex		
Female	33	55
Male	27	45
Serum Creatinine		
<1 mg/dl	33	55
1 – 1.49 mg/dl	15	25
≥ 1.5 mg/dl	12	20
HPE		
Minimal change disease(MCD)	17	28.3
Focal segmental glomerulosclerosis	13	21.7
Lupus nephritis	10	16.7
Membranous nephropathy(MN/MGN)	8	13.3
Membrano-proliferative glomerulonephritis(MPGN)	7	11.7
IgA nephropathy	2	3.3
Diffuse proliferative glomerulonephritis	1	1.7
Collapsing glomerulopathy	1	1.7
C3 nephropathy	1	1.7
Etiology		
Primary cause	49	81.7
Secondary cause	11	18.3

Majority of our study participants belongs to 16-29 years of age 40(66.7%). Female preponderance was observed 33(55%). Almost 33(55%) of the study participants have <1 mg/dl 33(55%). 49(81.7%) of

the study participants gets affected due to primary cause. The most common HPE finding was minimal change disease 17(28.3%) followed by focal segmental glomerulosclerosis 13(21.7%).

Table 2: Gender Distribution

Age category	Female (n=33)		Male (n=27)		P value
	n	%	n	%	
16 – 29 years	23	69.7	17	36	0.281 (NS)
30 – 59 years	10	30.3	8	29.6	
≥60 years	0	0	2	7.4	

There is no statistical significant association found between age groups and gender

Table 3: Association of Gender with Primary and Secondary Cause

Gender	Primary cause (n=49)		Secondary cause (n=11)		P value
	n	%	n	%	
Gender					0.001*
Female	22	44.9	11	100	
Male	27	55.1	0	0	
Age cat					0.757 (NS)
16 – 29 years	32	65.3	8	72.7	
30 – 59 years	15	30.6	3	27.3	
≥60 years	2	4.1	0	0	
Creatinine level					0.975 (NS)
<1 mg/dl	27	55.1	6	54.5	
1 – 1.49 mg/dl	12	24.5	3	27.3	
≥ 1.5 mg/dl	10	20.4	2	18.2	

Sex had a significant association with the etiology. Secondary etiology is seen only in females 11(100%). 27(55.1%) of males have Nephrotic syndrome due to primary cause or etiology.

Table 4: Comparison of HPE diagnosis with respect to gender noted in the study

S. No	HPE Diagnosis	Female (n=33)		Male (n=27)		P value
		n	%	n	%	
1	C3 nephropathy	0	0	1	3.7	0.001*
2	Collapsing glomerulopathy	0	0	1	3.7	
3	Diffuse proliferative glomerulonephritis	1	3	0	0	
4	Focal segmental glomerulosclerosis	6	18.2	7	25.9	
5	IgA nephropathy	0	0	2	7.4	
6	Lupus nephritis	10	30.3*	0	0	
7	Membrano-proliferative glomerulonephritis	2	6.1	5	18.5	
8	Membranous nephropathy	2	6.1	6	22.2	
9	Minimal change disease	12	36.3	5	18.5	

Chi-square value 21.53; degree of freedom = 9

HPE Diagnosis has significant association with gender.

Table 5: Comparison of HPE diagnosis with respect to age noted in the study

S. No	HPE Diagnosis	16-29y (n=40)		30-59y (n=18)		≥60 y (n=2)		P value
		n	%	n	%	n	%	
		1	C3 nephropathy	0	0	1	5.6	
2	Collapsing glomerulopathy	1	2.5	0	0	0	0	
3	Diffuse proliferative glomerulonephritis	1	2.5	0	0	0	0	
4	Focal segmental glomerulosclerosis	7	17.5	5	27.8	1	50	
5	IgA nephropathy	1	2.5	1	5.6	0	0	
6	Lupus nephritis	8	20	2	11.1	0	0	
7	Membrano-proliferative glomerulonephritis	7	17.5	0	0	0	0	
8	Membranous nephropathy	3	7.5	4	22.2	1	50	
9	Minimal change disease	12	30	5	27.8	0	0	

HPE Diagnosis has no significant association with the age

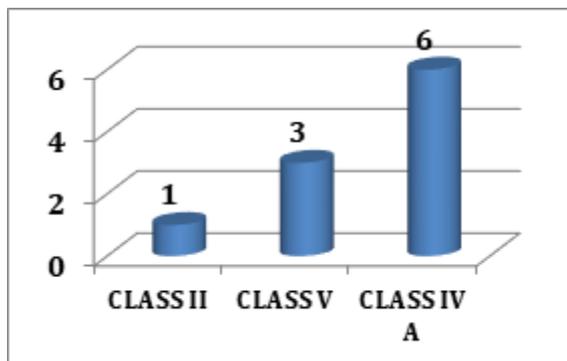


Figure 1: Lupus Nephritis

Among the patients with Lupus Nephritis, majority of patients 6 have Class IV A.

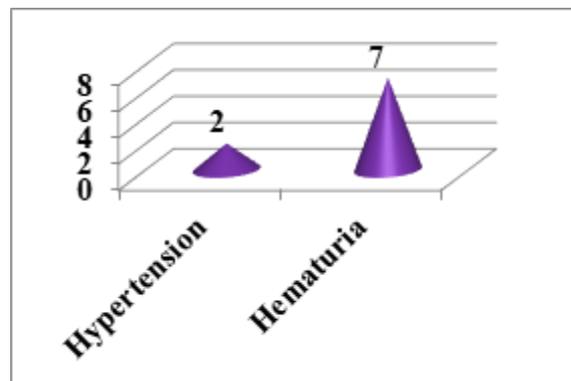


Figure 2: Clinical and Biochemical Features

Table 6: Comparison mean values of various parameters based on the type of lesions noted in the study

S. No	HPE Diagnosis	Age in years		24hr Urine protein g/day		Sr. Creatinine (mg/dl)	
		Mean	SD	Mean	SD	Mean	SD
1	Focal segmental glomerulosclerosis (n=13)	33.1	16.4	4.3	0.68	1.18	0.55
2	Lupus nephritis (n=10)	23.3	7.4	3.9	0.23	0.96	0.41
3	Membrano-proliferative glomerulonephritis (n=7)	21.1	4.5	4.75	0.44	1.17	0.44
4	Membranous nephropathy (n=7)	33.8	16.3	5.15*	0.87	0.9	0.34
5	Minimal change disease (n=17)	26.3	11.1	5.56*	0.92	1.01	0.58
6	Others (n=7)	30.7	12.8	4.17	0.58	0.97	0.35
	F value	1.589		9.07		2.259	
	Df1, df2	5, 54		5, 54		5, 54	
	p value	0.179 (NS)		0.001*		0.061(NS)	

Significant association was found between HPE diagnosis and 24 hr urine protein gram per day.

Table 7: Immunofluorescence study

No of cases	HPE Diagnosis	If Findings
17	Minimal Change Disease	Negative staining
11	FSGS	Negative staining
2	FSGS	Segmental IgM,
8	MN	IgG, C3 granular positive over capillary wall
7	MPGN	IgG,IgM,IgA,C3,C1q positive
2	IgA NEPHROPATHY	IgA positivity in the mesangium
1	C3 GLOMERULOPATHY	Granular to pseudolinear positivity over capillary wall
1	DPGN	Granular staining
10	LUPUS NEPHRITIS	IgG,IgA,C3,C1q granular positive over capillary wall and mesangium
1	COLLAPSING GLOMERULOPATHY	Negative staining

Negative staining was observed in patients with Collapsing glomerulopathy, Minimal change and FSGS disease.

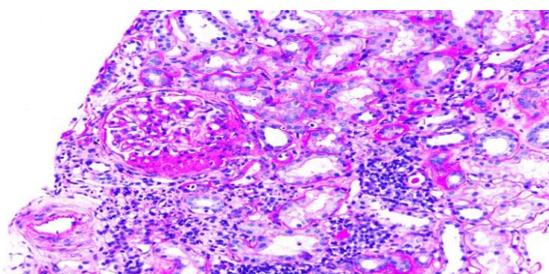


Figure 3: FOCAL SEGMENTAL GLOMERULOSCLEROSIS PAS, X 400

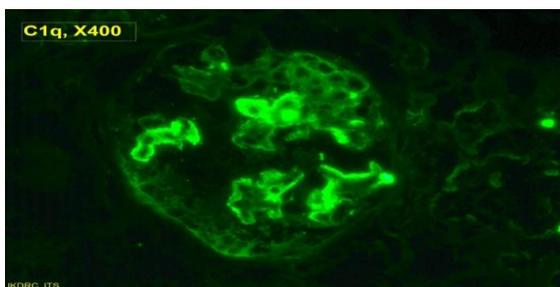


Figure 4: Wire loop lesion on IF- LUPUS NEPHRITIS

DISCUSSION

Glomerular diseases are common cause of end stage renal diseases. Histopathological examination of renal biopsies plays an important role in the diagnosis and management of nephrotic syndrome. They not only give diagnostic information, but also the information regarding disease progression and also in the management of the patient concerned. Kidneys are the ultra filter and the urinary protein loss is around 150mg/day in normal adult. Around 60% of the excreted proteins are filtered by glomeruli and the remaining portion –Tamm Horsfall protein derived from tubular secretions. It is important to recognize that nephrotic syndrome is associated either with primary or secondary glomerular diseases.

A total of 60 renal biopsies conducted between 2020-2021 in Thanjavur Medical college were included for analysis. Specimens were subjected to light microscopy and immunofluorescence to arrive at a final diagnosis.

Comparison of age range

Authors	Year	Age range
Kazi et al. ^[9]	2009	16-78
Zhou et al. ^[16]	2011	14->60 years
Vishal golay et al. ^[17]	2013	16- >60 years
M Rathi et al. ^[19]	2014	18-60 years
Present study	2019	16- 73years

In the present study, the age of the adult patients range from 16 years to more than 60 years had a good correlation with other studies.

Comparison of mean age of presentation

In the present study the mean age is 28 years which is which is lower than studies done by Zhou et al,^[16] and Vishal golay et al,^[17] where the mean age is 37 years and 33 years and this can be attributed to bias in population as well as sample size in our study population.

Comparison of gender distribution

In the present study, out of 60 cases 33 (55%) of cases occurred in female and 27 (45%) cases in

males. This was contrast to study done by Vishal golay,^[17] probably because of population bias as well as prevalence of lupus nephritis.

Comparison of frequency of primary lesion

In the present study primary cause for nephrotic syndrome constituted 81.7 % of the cases which was in concordance with other studies.^[17]

Comparison of frequency of secondary lesion:

In the present study, secondary lesions constituted 18.3% of cases which is correlated with the studies by Vishal golay et al.^[17]

Comparison of most common primary lesion

Authors	Year	Common Lesion
A.R.Reshi et al	2008	Minimal Change Disease
Vishal golay et al, ^[17]	2013	Membranous nephropathy
M Rathi et al, ^[19]	2014	FSGS
Present study	2019	Minimal Change disease

In the present study Minimal change disease constituted the highest number of cases among the primary lesions and this is similar to studies done by A.R.Reshi et al^[18] . In India the pattern varies corresponding to the demographic location, mesangioproliferative GN is the most common cause of nephrotic syndrome in South India and Minimal Change Disease is the commonest lesion in northern India. In our present study the dominant lesion on histopathology was Minimal change disease.

Comparison of histopathological findings with studies done in other countries:

The pattern of glomerular diseases are different in different countries and are changing with time within the same country, due to better infection control, changes in environmental pollution, increased awareness of the disease and changes in life expectancy. Studies done in USA have clearly demonstrated increasing incidence of FSGS

particularly in African-Americans making it the most common cause of nephrotic syndrome in their adult population. Studies done from Italy and Spain have shown MGN to be the most common cause of adult nephrotic syndrome, while those from Denmark, the Czech republic and Romania have shown MCD, IgAN and MPGN respectively to be the most common lesions. There has been considerable heterogeneity in the histologic spectrum of the nephrotic syndrome.

Comparison of most common secondary lesion:

In the present study Lupus nephritis was the commonest secondary cause which is similar to studies done by Vishal golay et al,^[17] and Rathi et al,^[19] study.

Comparison of frequency of lupus nephritis

In the present study Lupus nephritis constituted 16.7 % of the total number of cases which in not in concordance with other studies where it is 3.1%, 38.8% etc because of population bias.

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CONCLUSION

Our study concluded that majority of the study participants 16-29 years of age 40(66.7%). Female preponderance was observed 33(55%).Majority of the biopsies were of primary glomerular diseases. The most common cause of nephrotic syndrome accounts for Minimal change disease 28.3% followed by Focal segmental glomerulosclerosis 21.7%. Among the secondary glomerular diseases, Lupus Nephritis found to be commonest in patients presented with Nephrotic syndrome. The most common finding was minimal change disease 17(28.3%). Sex had a significant association with the etiology. Histopathological diagnosis has significant association with gender. Significant association was found between histopathological diagnosis and 24 hr urine protein gram per day.

Limitations: The sample size was small and the study was done in a single center, so the results cannot be generalized.

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