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**ORIGINAL ARTICLE****Spectrum of Secondary Glomerular Diseases in Adult Nephrotic Syndrome***Devarasetty Shashank<sup>1</sup>, Mohini Singh<sup>1\*</sup>, S.R. Ramakrishnan<sup>1</sup>**<sup>1</sup>Department of General Medicine, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai-600116 (Tamil Nadu) India***Abstract:**

*Background:* In developing countries like India, the causes for end stage renal failure has been changing with glomerular diseases occupying an important place. The trend of various primary and secondary causes of Nephrotic Syndrome (NS) over the past few decades has been debatable especially with reference to geographical locations. *Aim and Objectives:* This study was intended to find out the histologic patterns of glomerular disease associated with NS in South India and analyze the corresponding clinical and biochemical abnormalities associated with these conditions. *Material and Methods:* One hundred eighty eight patients in the age group of 18-80 years with NS who underwent renal biopsy between the period from 1<sup>st</sup> January 2018 to 30<sup>th</sup> September 2019 were included in this study. Baseline investigations and other investigations related to renal profile evaluation including complement levels and HIV antibody were done. *Results:* Focal Segmental Glomerulosclerosis (FSGS) accounted for the majority (25.5%) among primary glomerular diseases while among the secondary glomerular diseases Lupus Nephritis (LN) (51.19%) constituted the majority and a substantial contribution from Human Immunodeficiency Virus Associated Nephropathy (HIVAN). LN was seen only in female patients with a mean age of  $33.36 \pm 10.74$  years. Among LN, Class III was the most predominant while Class IIIC was less frequent. C3 and C4 complement levels were less than normal in Class IV and Class variants of LN. *Conclusion:* This study highlights the spectrum of Lupus Nephritis and HIV associated nephropathy as a cause for adult NS in a tertiary care center in South India.

**Keywords:** Nephrotic Syndrome, Primary Glomerular disease, Secondary Glomerular disease, Lupus Nephritis, HIV Associated Nephropathy

**Introduction:**

Nephrotic Syndrome (NS) is defined as a condition characterized by edema and hypoalbuminemia arising from loss of excess proteins in urine, and is associated with complications such as thromboembolism, altered carbohydrate and lipid metabolism, increased susceptibility to infections and losses of binding proteins of the urine [1]. In developing countries like India, NS as a cause for chronic kidney disease has been assuming a great importance especially following increased diagnostic and laboratory criteria available for classification of glomerulonephritis. There exists a vast difference among glomerular diseases seen in tropical countries from those in temperate countries in terms of epidemiology, etiology, natural history, socio economic conditions, race and age [2]. Primary Glomerulonephritis (PG) comprises of those conditions affecting only the kidney without any systemic cause for glomerular lesion while secondary glomerular diseases are those in which an underlying cause can be established. Though several studies from around the world have shown that during 1970s among PG, membranous nephropathy was the most common cause of adult NS accounting for 35% to 50% of cases, it has been replaced by Focal Segmental Glomerulosclerosis

(FSGS) in recent times [3-6]. Among Secondary Glomerulonephritis (SG) causing adult NS infections are seen commonly in developing countries while Diabetes Mellitus (DM) and Systemic Lupus Erythematosus (SLE) account for the majority among developed countries[7]. Lupus Nephritis (LN) encompasses immune complex-mediated renal injury and its sequelae characterized by diverse patterns of renal disease including glomerular, tubulointerstitial, and vascular pathology [8-9]. However, recent developments in the treatment of HIV has led to substantial increase in the survival period of affected patients. This increase has led to occurrence of HIV Associated Nephropathy (HIVAN) at an increased frequency especially in developed countries. HIVAN is characterized histopathologically by collapsing focal segmental glomerulosclerosis with microcystic tubular dilatation and interstitial inflammation [10]. Other forms of renal diseases such as IgA nephropathy, immune complex nephritis and interstitial nephritis are commonly associated with HIV infection [11]. Though studies on adult NS exist, these are limited in number especially when focused on a national level but are virtually nonexistent when it comes to regional level. Similar to world scenario, few studies from Indian subcontinent have shown FSGS as a major cause among PG for adult NS [12-13]. However, the other histologic variants of glomerulonephritis especially the immune complex glomerulonephritis and HIVAN has not been under the focus of any of the studies in India. This study is intended to analyze the various histologic patterns of adult NS with particular focus on secondary glomerular diseases like LN

and HIVAN from a Tertiary Care Institution in South India. The aim of the study was to analyze the distribution of the primary and secondary glomerular diseases causing adult onset NS on the basis of histopathologic patterns.

#### **Material and Methods:**

One hundred eighty eight adult patients diagnosed with NS based on standard definitions [14] and subjected to renal biopsy at Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, India during the period of 1<sup>st</sup> January 2018 to 30<sup>th</sup> September 2019 were included in the study. Written informed consent was obtained from all the study participants. This observational prospective study was done after obtaining necessary permission from Institutional Ethics Committee and was performed in compliance with ethical standards of human experimentation in accordance to the Helsinki Declaration. Hemoglobin, clotting parameters, renal profile, lipid profile, serum albumin, Antinuclear Antibody (ANA), Hepatitis B surface Antigen (HbsAg) and anti-Hepatitis C Virus (anti HCV) were estimated in all patients. Additional investigations such as anti-double stranded deoxyribonucleic acid, HIV, CD4 counts, C3 and C4 levels were done in indicated patients. Biopsies were performed after baseline investigations by nephrology / general medicine residents under supervision. The biopsies were done under ultrasound guidance (real time) using the Bard Max Core Disposable Core Biopsy Instrument with 18G × 16 cm needle. Two core biopsies were obtained and were subject to Histopathological Examination (HPE) and immunofluorescence microscopy. Staining with both Hematoxylin and

Eosin / Periodic Acid Schiff stains was used for HPE while for selected cases Masson's trichrome staining and Jones silver staining were done. Cases were classified into PG and SG diseases based on clinicopathological correlation. Data entry were done using microsoft excel sheet and descriptive analysis was done using Software Package for Social Sciences version 17.0 (SPSS, IBM, USA).

### Results:

#### Demographic Profile of the Selected Subjects:

During the 18-month period a total of 188 subjects who underwent renal biopsy at our center were studied comprising of 59 (31.38%) males and 129 (68.62%) females with a male to female ratio of 1:2.2. The study group aged between 18 to 71 years and the mean age at presentation  $36.17 \pm 11.86$  years. Pedal edema was seen among 171 (91%) patients, while facial puffiness (26.6%) and abdominal distension (11.2%) were noted less frequently. Twenty four (12.8%) patients had DM while 26 (13.8%) patients had hypertension. Eight (4.3%) patients had a history of fever before the admission period and 3 (1.6%) patients were diagnosed with a cardiac problem. The laboratory investigations in these subjects included nephrotic range proteinuria as evidenced by mean 24-hour protein excretion of  $4.78 \pm 15$  g (range 3.6–10.92 g), mean serum albumin was  $2.13 \pm 0.57$  g/dL, mean serum creatinine was  $1.418 \pm 1.22$  mg/dL, mean protein creatinine ratio of  $6.61 \pm 3.02$ , mean cholesterol of  $271.45 \pm 110.63$  mg/dL. Five patients were positive for HIV and were on anti-retroviral therapy. Hepatitis B surface antigen was positive in 1 patient while 28 patients were positive for anti-neutrophilic antibody.

#### Primary and Secondary Glomerular Diseases:

Table 1 shows the distribution of various histologic patterns of glomerular diseases among adult NS patients as well as the PG and SG disease types. 55.32% of patients belonged to PGD while 44.68% were classified as SGD. Among all the histologic types FSGS accounts for the majority (25.5%) with while among the SG diseases LN (51.19%) constitute the majority.

#### Secondary Glomerular Diseases:

LN was classified based on the International Society of Nephrology / Renal Pathology Society (ISN/RPN) classification [15]. In our study classes III, IIIA, IIIC, Class IV and Class V were seen with Class III (40.5%) contributing to majority. All the patients were females and the mean age was  $33.36 \pm 10.74$  years. All the classes had predilection for age groups less than 40 years. Other co-morbidities like diabetes in Class III and hypertension among Class IV were also present. Only Class III patients had elevated creatinine values while all other classes had serum creatinine within normal limits. Mean serum creatinine levels among patients with LN were  $1.126 \pm 1.094$  mg/dL. But among different classes except for Class III the creatinine values were within normal limits. Low complement C3 and C4 levels were noted in Class IV and Class V groups of LN. Patients were positive for HIV 2.65% and had HIVAN characterized by FSGS on histopathology and increased renal cortical echoes on ultrasonography. The mean age of those with HIVAN was 31.8 years and among these 40% were males and 60% were females. Mean 24-hour urinary protein excretion was ( $5208.06 \pm 1725.42$ ), protein creatinine ratio 7.61, C3 level of ( $135.18 \pm 24.87$ ) and C4 level of ( $33.92 \pm 24.07$ ).

**Table 2: Distribution of Primary and Secondary Glomerular Diseases among Adult Nephrotic Syndrome**

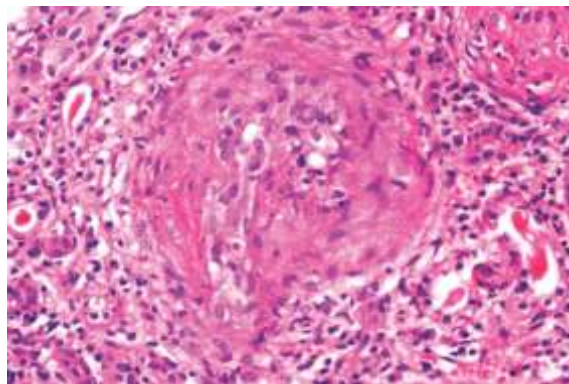
Pathology	Overall frequency n (%)	PGD n (%)	SGD n (%)
FSGS	48 (25.5)	39 (37.5)	9(10.71) *
MGN	22 (11.7)	16 (15.38)	6 (7.14) **
MPGN	2 (1.0)	1(0.9)	1 (1.19) ***
MCD	22 (11.7)	21 (20.19)	1 (1.19) ****
IgAN	14 (7.44)	14 (13.46)	0
ATN	5 (2.65)	5 (4.8)	0
AIN	10 (5.31)	8 (7.6)	2 (2.3)
PIGN	9 (4.78)	0	9 (10.71)
LN	43 (22.87)	0	43 (51.19)
DN	11 (5.85)	0	11 (13.1)
HTN	2 (1.06)	0	2 (2.3)
<b>Total</b>	<b>188</b>	<b>104 (55.32)</b>	<b>84 (44.68)</b>

PGD: Primary glomerular diseases SGD: Secondary glomerular diseases FSGS: Focal segmental glomerulosclerosis MGN: Membranous glomerulonephritis MPGN: Membrano proliferative glomerulonephritis MCD: Minimal change disease IgAN: Immunoglobulin A nephropathy ATN: Acute Tubular Necrosis AIN: Acute Interstitial Nephritis PIGN: Post infectious glomerulonephritis LN: Lupus nephritis DN: Diabetic Nephropathy HTN: Hypertensive Nephrosclerosis, \* 5(6%) had HIVAssociated Nephropathy (HIVAN), \*\* Malignancy (Lung Ca, Prostrate Ca) and Malaria, \*\*\* Cirrhosis of Liver, \*\*\*\* Hodgkin's Disease

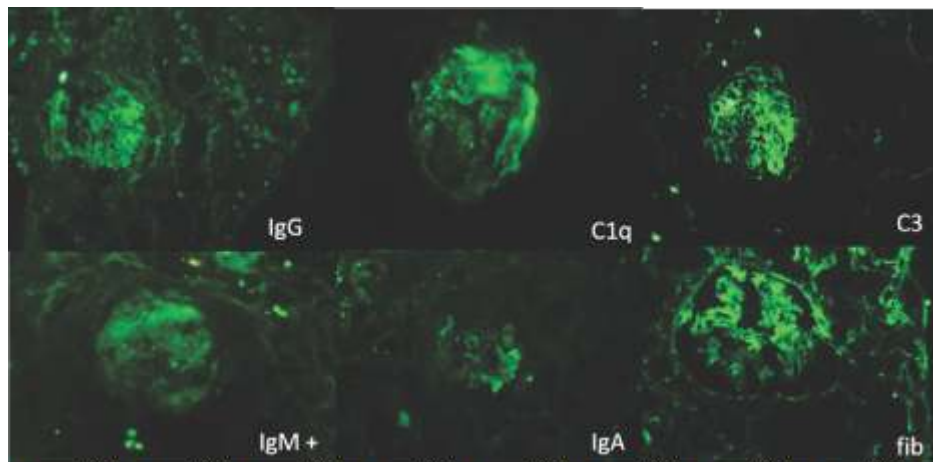
**Table 2: Various Parameters among Different Classes of Lupus Nephritis**

Parameter	LN Class				
	III	IIIa	IIIc	IV	V
Frequency n (%)	17 (40.5)	3 (7.1)	2 (4.8)	15 (35.7)	5 (11.9)
<b>Age Group</b>					
< 40 years	12 (70.6)	3(100)	1 (50.0)	11 (73.3)	4 (80)
≥ 40 years	5 (29.4)	-	1(50.0)	4 (26.7)	1 (20)
<b>Gender</b>					
Male	-	-	-	-	-
Female	17	3	2	15	5
Diabetes Mellitus	3 (17.6)	-	-	-	-
Hypertension	-	-	-	2 (13.3)	-
24hr urinary protein (g/dL)	4.68 ± 1.53	3.96 ± 0.48	4.05 ± 0.64	4.726 ± 1.295	4.79 ± 1.85
Albumin (g/dL)	2.32 ± 0.58	3.1 ± 0.79	2.95 ± 0.07	1.98 ± 0.53	1.96 ± 0.38
Creatinine (mg/dL)	1.54 ± 1.65	0.83 ± 0.28	1.1 ± 0.14	1.096 ± 0.41	1.08 ± 0.23
C3 level (mg/dL)	78.88 ± 40.07	71.33 ± 9.02	86.0 ± 38.18	54.13 ± 27.08	57.80 ± 22.76
C4 level (mg/dL)	18.53 ± 15.46	14.0 ± 5.29	17.0 ± 7.07	11.47 ± 6.91	9.80 ± 8.89

LN: Lupus Nephritis



**Fig.1: Lupus Nephritis International Society of Neurology and the Renal Pathology Society (ISN/RPS) Class IV: Glomerular form showing several wire loops (subendothelial deposits) and intraluminal hyaline thrombi (deposits) (Hematoxylin and Eosin stain × 500)**



**Fig. 2: Lupus Class IV:** Immunofluorescence shows full house effect

### Discussion:

The dynamic behavior of spectrum of glomerular kidney diseases in a particular region is influenced by various geographical, environmental and socioeconomic factors as a result of which economic differences justify different magnitudes among developing and developed countries. This also depends on the setting where the disease presents or where it is treated [16]. Very limited studies exist in India that discuss on the spectrum of glomerular diseases and these studies have not looked into the aspects of secondary glomerular diseases especially LN as a cause of NS in adults [13, 17-18]. Studies in India and in other parts of the world have shown a changing trend among primary glomerular diseases from Minimal Change Disease (MCD) to FSGS [17-18] and among SG diseases a shift from Diabetic Nephropathy (DN) to LN [19-20].

In our study, NS due to PG diseases was 55.32% and those due to SG diseases was 44.68%. This is similar to studies by Das *et al.* with PGD accounting for majority of NS cases 69.1% and SGD contributing to 18.2% cases [17]. An

increased prevalence of female patients was noted among SGD cases especially with LN and is in concordance with several studies done all over the world [21-22].

In Table 3, a summary of the various studies done in India over the past few decades showing the pattern of distribution of PG and SG diseases is presented. The studies show a shift in pattern from MGN to FSGS among PG diseases while among SG diseases there has been a shift from DN to LN as a cause for adult NS. The study done from Vellore in 1970's noted that among SGD, LN alone contributed to 50.39% [23].

Similarly, studies from Hyderabad and Kolkata, also found LN to be responsible for more than 50% of secondary causes of NS [13, 17]. Recent studies done at Chandigarh and Kolkata also show a shift in trend among secondary causes of NS with DN showing a downward trend due to increased diabetic control and better medications [24]. LN patients especially Class IV and Class V had hypocomplementemia especially C3 and C4. Among LN Class III (40.5%) and Class IV

**Table 3: Indian Studies on Patterns of PG and SG as a Cause for ANS\***

Reference	Dates <i>et al.</i> [23]	Das <i>et al.</i> [17]	Rathi <i>et al.</i> [24]	Golay <i>et al.</i> [13]	Present study
<b>Year</b>	1971-85	1990-2008	2002-2007	2010-2012	2018-2019
<b>Place</b>	Vellore	Hyderabad	Chandigarh	Kolkata	Chennai
<b>Sample size</b>	1532	1615	364	410	188
<b>Primary</b>	1276 (83.3)	1278(79.1)	324(89)	361(88.1)	104(55.32)
<b>FSGS</b>	238(18.6)	195(15.2)	99(30.6)	99(27.4)	39 (37.5)
<b>MGN</b>	174(13.6)	129(10.1)	79(24.4)	89(24.6)	22 (11.7)
<b>MPGN</b>	177(13.9)	73(5.7)	58(17.9)	2.4(6.6)	1(0.9)
<b>MCD</b>	457(35.8)	279(21.8)	48(14.8)	98(27.1)	21(20.19)
<b>IgAN</b>	57(4.5)	177(13.8)	6(1.8)	29(8.1)	14 (13.46)
<b>Secondary</b>	256(16.7)	337(20.9)	40(11)	49(11.9)	84 (44.68)
<b>LN</b>	129 (50.39)	270 (14.6)	25 (62.5)	27 (6.58)	43 (51.2)
<b>DN</b>	103 (40.23)	22 (1.2)	1 (2.5)	2 (0.49)	11(13.1)

\* based on information provided in the publications some figures have been recalculated to maintain uniformity. FSGS: Focal segmental glomerulosclerosis MGN: Membranous glomerulonephritis MPGN: Membrano proliferative glomerulonephritis MCD: Minimal change disease IgAN Immunoglobulin A nephropathy PIGN: Post infectious glomerulonephritis LN: Lupus Nephritis DN: Diabetic Nephropathy

(35.7%) were common. This observation has been noted in similar studies undertaken by Moc C *et al.* in China with 25% accounting for Class III and 45% for Class IV of LN by the ISN/RPN classification [25]. Studies from Chezh registry of renal biopsies have shown a static trend of LN over the past decade [26] but studies from Spain has shown an increasing trend of LN especially an age shift from adolescent to middle age population similar to our study [27].

Several studies based on different screening methodology and patient population have

demonstrated overt proteinuria in 14-50% of HIV/AIDS patients [11, 28-30]. A prevalence of  $\geq 1+$  proteinuria in 22.4% patients with prevalence of persistent proteinuria as 14% was demonstrated by Crowley *et al.* [31]. In our study, higher percentage of patients with HIVAN (84.31%) was found to have proteinuria.

#### **Conclusion:**

To conclude, though the clinical spectrum of glomerular diseases has divergent patterns throughout the world, several attempts have been made to describe the same with regard to PG and

SG diseases. Time trend changes in occurrence of various forms of SG diseases in different geographical locations may be ascribed due to the following reasons.

1. Advances in medical diagnostic procedures like renal biopsy and immunohistochemical staining to classify PG and SG diseases.
2. Drugs used for treatment of chronic conditions which show different geographic distribution may trigger SLE.
3. Increase in *H. pylori* associated gastritis especially in South Indian population which has been postulated as increase in occurrence of SLE among general population.

In this study two of the less studied spectrum of SG diseases namely LN and HIVAN are analyzed in detail as the disease prevalence has increased because of latest diagnostic techniques and quality renal biopsy with grading. This study has thereby corroborated few variations that LN and HIVAN has brought to adult onset NS.

#### Limitations:

The limitation in our study was the smaller sample size and the non-availability of electron microscopic studies of the renal biopsies done. Further our center being a tertiary referral center in South India predominantly attracts population of the south, which therefore demographic and social practices may not be applicable to the general population throughout the world. Hence, we recommend that these results are should be validated in a larger sample of patients with NS with more participation from nephrologists throughout India so the changing pattern of adult NS is obtained regularly for better management of the disease. In addition, the changing scenario with HIVAN in those receiving anti-retroviral therapy has to be studied in detail.

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