ABSTRACT

The nephrotic syndrome (NS) is a clinical syndrome complex characterized by number of renal and extra renal features and is defined by a urinary protein level exceeding 3.5 gm per 1.73 m^2 of body-surface area per day. The aim was to study clinical profile, the histopathological spectrum of renal biopsies in NS and to correlate histopathological spectrum with clinical profile. **Material and methods:** Total 120 patients of all age groups diagnosed clinically with NS and subjected to renal biopsy were included in study while those with insufficient biopsy material were excluded from our study. **Results:** In the present study patient’s age ranged from 6 to 79 years with the mean age of 36.73 ± 15.81 years. Male: Female ratio was 2:1. Overall most common etiology of NS was Minimal change disease (MCD) and in pediatric age group was Focal and segmental glomerulosclerosis (FSGS). Most common cause of primary NS was MCD while Diabetic nephropathy (DN) was most common cause of secondary NS. Most common clinical presentation was pedal edema. Elevated serum creatinine was observed in 21 patients. Hypercholesterolemia was detected in 89 patients. Microscopic hematuria was present in 47 patients. The most common complication in our NS study was urinary tract infection (UTI) followed by acute kidney injury (AKI), chronic kidney disease (CKD), pneumonia and thrombosis. All patients had hypoalbuminemia. All patients were seronegative for HIV, HBsAg and HCV. All 6 patients with LN had ANA and
dsDNA positive. **Conclusion:** MCD is the most common cause of NS in adults while FSGS in pediatric age group. In view of changing histological spectrum of NS it is essential to maintain a national renal biopsy registry data which would help to obtain accurate knowledge of spectrum, presentation, incidence and complications.

**KEYWORDS:** Nephrotic syndrome, renal biopsy, spectrum of renal disease.

**INTRODUCTION**

The nephrotic syndrome (NS) is a clinical syndrome complex characterized by number of renal and extra renal features and is defined by a urinary protein level exceeding 3.5 gm per 1.73 m² of body-surface area per day.¹ NS presents as adult and pediatric kidney disease. Incidence of NS is 2-7 per 100,000 children and of every three new cases per 100,000 each year in adults.² The syndrome manifest as proteinuria (adult > 3.5g/day, child >40mg / hour/ m³³⁴), hypoalbuminemia(<3.5g/dl), generalized edema, hyperlipidemia, lipiduria,⁵ hypercoagubility.⁶ Other features which are not part of syndrome but may occur along with NS are hematuria, hypertension, azotemia.⁷ Various complications like infections (urinary tract, respiratory tract), acute kidney injury (AKI), thrombosis (Deep vein thrombosis, Renal vein thrombosis), bone diseases are observed in patients with NS.⁸ The exact underlying causes of NS are not known but various implicated causes include infectious agents, autoimmunity, drugs, inherited disorders, environmental agents. NS is usually due to a glomerular disease and is currently categorized into primary nephrotic syndrome (PNS) / idiopathic nephrotic syndrome (INS) and secondary nephrotic syndrome (SNS). In PNS, the cause is not associated with any underlying disease while in SNS kidney involvement is a part of systemic disorder.⁵⁶⁸ Patient may be nephrotic with or without impaired renal function. Prolonged NS may progress to renal failure. Some episodes NS are self-limiting while few respond completely to specific treatment but in most of cases it’s a chronic condition. The NS is associated with a spectrum of primary glomerular disease (PGD) and secondary glomerular diseases (SGD). The most common PGD causing NS in children is minimal change disease (MCD) and in adult is membranous glomerulonephritis (MGN).⁶ However, changing trends have been reported in etiology of NS in last few decades. Focal segmental glomerulosclerosis (FSGS) is most important cause of NS in adults and children.⁸⁶ Lupus nephritis (LN) is most common cause of SGD world wide.⁹ It is necessary to differentiate among the etiologies of the NS as all these different glomerular lesions have their distinct clinical courses, treatments, and prognosis. In the present study our
aim was to study and correlate clinical profile and histopathological spectrum of renal biopsies in patient with NS.

MATERIAL AND METHODS
The present study was carried out for two years in pathology department of Central Indian tertiary care hospital. Total 120 patients of all age groups diagnosed clinically with NS and subjected to renal biopsy were included in study while those with insufficient biopsy material were excluded from our hospital based cross sectional study. Detailed history and physical examination was conducted for all patients. Parameters like age, sex, any family history of nephrotic syndrome, hypertension, type 2- diabetes mellitus were recorded along with presenting complains like pedal edema, facial puffiness, generalized weakness, loss of appetite, difficulty breathing. Blood was analyzed for routine hemoglobin levels. Serum was examined for creatinine, urea, albumin, cholesterol, anti nuclear antibody (ANA), anti-double stranded deoxyribonucleic acid antibodies (anti- dsDNA antibodies), antibody against the human immunodeficiency virus (HIV), hepatitis B surface antigen, hepatitis C virus antigen. Urine was examined for presence of any red blood cells (RBC), white blood cells (WBC), casts, urine albumin and 24 hour urine protein levels were recorded. Various complications like urinary tract infection (UTI), acute kidney injury (AKI), pneumonia, thrombosis, chronic kidney disease (CKD) were also recorded. Ultrasound was done to evaluate each kidney. After administrating local anesthesia, under continuous ultrasound guidance the biopsy material was obtained using automated spring loaded biopsy gun with 16 (adults) or 18 (pediatric patients) gauge needle. The biopsied patients were observed for 24 hours for biopsy related complication. At the time of biopsy two cores were obtained whenever possible. One core was fixed in 10% formal saline and embedded in paraffin, 4-5 μm thick sections were cut and then stained with Hematoxylin & Eosin (H&E), Periodic acid Schiff (PAS), Silver-methenamine (Jones), and Masson’s trichrome. For immunofluorescence (IF) biopsy core was fixed in phosphate buffer saline at pH 7.2 and temperature of -4°C till the core was processed. Frozen sections of 3 μm thick were taken with help of cryostat. These were alcohol fixed. Fluorescent tage of IgG, IgA, IgM, C3, κ and λ light chains were added. The sections were then studied under microscope and were evaluated. The biopsy was considered adequate if at least 5 glomeruli are present or even single glomeruli showing changes which are diagnostic and pathognomic of the lesion. The clinical and laboratory data along with histopathology results were obtained and entered in proforma and then studied together.
OBSERVATION AND RESULTS

Table 1: CLINICOPATHOLOGICAL FINDINGS.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>No. of Patients</th>
<th>Male</th>
<th>Female</th>
<th>Pediatric age group</th>
<th>Adult age group</th>
<th>Anemic</th>
<th>Elevated creatinine</th>
<th>Hypercholesteremia</th>
<th>Microscopic hematuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPGN</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>IgA</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>FSGS</td>
<td>31</td>
<td>21</td>
<td>10</td>
<td>7</td>
<td>24</td>
<td>26</td>
<td>6</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>MCD</td>
<td>47</td>
<td>30</td>
<td>17</td>
<td>4</td>
<td>43</td>
<td>42</td>
<td>4</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>MGN</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>DN</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>LN</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>120</td>
<td>80</td>
<td>40</td>
<td>15</td>
<td>105</td>
<td>107</td>
<td>21</td>
<td>89</td>
<td>47</td>
</tr>
</tbody>
</table>

In the present study age of the patients ranged from 6 to 79 years with the mean age of 36.73 ± 15.81 years. Male: Female ratio was 2:1. Overall most common etiology of NS was MCD followed by FSGS. Most common cause of primary NS was MCD while DN was most common cause of secondary NS. Male predominance was observed in all etiology of NS except for LN. Out of 120 cases, 15 were of pediatric age group and 105 of adult age group. Most common etiology in pediatric age group was FSGS. Most common clinical presentation was pedal edema followed by facial puffiness. Difficulty in breathing was rare presentation. At time of presentation 12 patients had type 2-diabetes mellitus and hypertension was in 27 patients. Hypertension was more associated with MPGN (80%) followed by MGN (55.5%) and least associated with amyloidosis and MCD (6%). Out of 120 patients 107 (89.16%) were anemic in present study.

Table 2: Clinical Presentation.

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedal edema</td>
<td>70</td>
</tr>
<tr>
<td>Facial Puffiness</td>
<td>61</td>
</tr>
<tr>
<td>Generalized weakness</td>
<td>14</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>9</td>
</tr>
<tr>
<td>Difficulty breathing</td>
<td>2</td>
</tr>
</tbody>
</table>

Elevated serum creatinine was observed in 21 patients. Out of these 19 were adults and 2 were of pediatric age group. Hypercholesterolemia was detected in 89 patients. 11 out of 15 pediatric cases and 78 out of 105 adult cases had hypercholesterolemia in our study of
Microscopic hematuria was present in 47 patients. 83.33% of patients with LN had microscopic hematuria. The most common complication in our NS study was UTI followed by AKI, CKD, pneumonia and thrombosis.

### Table 3: Complications and Etiology At Presentation.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>UTI</th>
<th>AKI</th>
<th>Pneumonia</th>
<th>Thrombosis</th>
<th>CKD</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPGN</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IgA</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>FSGS</td>
<td>13</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>MCD</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>MGN</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DN</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>LN</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Most of the complications were associated with FSGS. Thrombosis was seen in patient with MGN. Patients with amyloidosis did not present with any complications.

All patients had hypoalbuminemia. Serum albumin ranged from 1.82 - 3.5gm/DL. Serum urea ranged from 16 – 280 mg/Dl. Urine albumin ranged from 1+ to 5+. Urinary cast were identified in only 7 patients and pus cells in 26 patients.

It was observed that all patients were seronegative for HIV, HBsAg and HCV. All 6 patients with LN had ANA and dsDNA positive. Immunofluorescence (IF) was done on 16 biopsies while Electron microscopy (EM) was not done on any biopsy.

**DISCUSSION**

Nephrotic syndrome is best known presentations of both adult and pediatric kidney disease. If left undiagnosed or untreated it progresses to renal failure.

In our study, there were total of 15 pediatric cases (age ≤ 18 years) and 105 adult cases (> 18 years) of nephrotic syndrome. Age ranged from 6 to 79 years with the mean age of 36.73 ± 15.81 years and with male to female ratio of 2:1 in all disease category except LN were ratio is reversed. Das U et al[9] also observed female predominance in LN. Majority of patients presented in age group of 21 -30 years (25.83%) similar to study conducted by Singh GK et al.[6]

Glomerular disease is one of the most common forms of renal diseases and can have different clinical presentations.[10] They are also important cause for end stage renal disease.[11]
In the present study, glomerular pathologies based on renal biopsy and clinical features were classified as.

Primary glomerular diseases (PGD) - MCD, FSGS, IgA nephropathy, MGN and MPGN
Secondary glomerular diseases (SGD) - DN, LN and Amyloidosis.

The various complication observed in present study were, UTI, AKI, CKD, pneumonia and thrombosis.

The histological spectrum of glomerular disease is different in adults as compared to children as well as in tropical as compared with temperate countries.[11,12]

Out of total 120 cases in this study, PGD accounted for 102 cases (85%) while SGD accounted for 18 cases (15%). The most common cause of nephrotic syndrome in this study was MCD (39.17%) followed by FSGS (25.83%), MGN (7.50%), IgA nephropathy (8.33%), DN (5.83%), LN (5%), MPGN (4.17%) and amyloidosis (4.17%). In adults most common cause of nephrotic syndrome in present study was MCD (40.95%) followed by FSGS (22.85%). Agarwal SK et al[13] reported MCD to be most common cause of nephrotic syndrome in adults. Other studies in adults report FSGS to be most common cause of nephrotic syndrome.[12,14]

Data is found to be different in different countries across the globe. Study from Pakistan and Nepal reports MGN to be most common cause for nephrotic syndrome. While study from Korea and China showed IgA nephropathy to be most common cause of nephrotic syndrome. Data from West (USA) demonstrate increasing incidence of FSGS as cause of end stage renal disease. And data from Italy and Spain reports MGN to be most common cause of nephrotic syndrome in adults. While data from Denmark, Czech republic and Romania report most common cause of nephrotic syndrome as MCD, IgA nephropathy and MPGN respectively.[12,15-23]

In this study DN (6.66%) was most common cause of SGD followed by LN (5.71%) and amyloidosis (4.76%). Study conducted by Reshi AR et al[24] and Agarwal SK et al[13] also reported DN to be most common cause of SGD causing nephrotic syndrome. Golay V et al[10] reported LN (6.58%) as the most common cause of nephrotic syndrome. While in study of Varshney et al[11] both LN and amyloidosis accounted for 5.8% each.
High incidence of DN in our study may be due high incidence of diabetes in population, late presentation in tertiary care hospital and fear towards renal biopsy procedure. Few studies from Pakistan and India have reported that high incidence of amyloidosis occur due to high prevalence of tuberculosis and other infectious diseases. In present study we performed renal biopsy only on non suspected cases of amyloidosis and found it to accounted for 4.76% of cases. In suspected cases biopsies were taken from other locations like gum, rectum etc.

In pediatric age group most common cause of nephrotic syndrome in present study was FSGS (46.67%) followed by MCD (26.66%), LN (13.33%), MPGN (6.66%) and IgA nephropathy (6.66%).

MGN is known to be rare in childhood and was not found in any of the pediatric biopsies in the present study.

Obiagwu PN et al and Safaei A et al also found most common cause of pediatric nephrotic syndrome as FSGA followed by MCD. Kumar J et al reported FSGS (38%) to be most common cause of nephrotic syndrome followed by MCD (32%) in Indian children. Gulati S in 1999 and 2001 have also reported FSGS to be increasing in Indian children. In 2015, Vijayalaxmi P et al in their study observed MCD (77.78%) to be more common cause of nephrotic syndrome followed by FSGS (16.67%). The reason for increase in frequency of FSGS in past two decades is unclear. Nephrotic syndrome is characterized by proteinuria, which in turn causes fall in serum albumin, and if liver fails fully to compensate for urinary protein losses by increased albumin synthesis, plasma albumin concentrations decline, leading to edema formation. Most common clinical presentation in present study was edema.

Most studies have relied on edema as manifestation of nephrotic syndrome and have not strictly defined hypoalbuminemia. The presence of edema is variable even when the serum albumin is below 3gm/Dl. In our study all patients had hypoalbuminemia. Pedal edema was present in 70 patients (58.33%). Facial puffiness was present in 61 patients (50.83%).

Apart from edema other presentations were, generalized weakness in 14 patients, loss of appetite in 9 patients and difficulty breathing in 2 patients. Many patients had more than one presentation.
In study conducted by Singh GK et al[6], 92% patients had swelling of feet and puffiness of face, 23.1% of patients presented with weakness, loss of appetite. Varshney A et al[11] reported 98.8% of patients had edema in their study, 57.1% had weakness and 80.9% had loss of appetite. Reshi AR[24] reported 99% patients had facial puffiness and 91% had pedal edema.

Out of 120 patients in our study only 27 patients (22.5%) had hypertension. 80% of patients with diagnosis of MPGN had hypertension. 55.56% of patients with MGN, 33.33% of patients with LN, 30% of patients with IgA nephropathy, 28.57% of patients with DN, 22.58% of patients with FSGS, 20% of patients with amyloidosis and only 6.38% of patients with MCD had hypertension.

Hypertension was least associated with minimal change type of nephrotic syndrome in present study and similar result was observed by Singh GK et al.[6]

Only 2(13.33%) out of 15 of pediatric cases had hypertension at time of presentation. One was diagnosed as FSGS and other had MPGN as underlying etiology of nephrotic syndrome.

Obiagwu PN et al[25] in his study based on nephrotic syndrome in children reported that the presence of hypertension on initial diagnosis could be strong pointer of a non-minimal change type nephrotic syndrome. The same was observed in our study.

Anemia in nephrotic syndrome occurs as a result of transferrin loss due to proteinuria and decreased renal synthesis of erythropoietin. In our study anemia was observed in 107 cases (89.16%). Varshney A et al[11] observed 92.8% of their cases had anemia.

Elevated serum creatinine was present in 21 (17.5%) patients in present study. 44.44% of patients with MGN as underlying etiology had elevated serum creatinine and 8.51% of patients with MCD as underlying etiology had elevated serum creatinine. Rathi M et al[12] in their study found all patients with MCD had normal serum creatinine. While in the present study we observed MCD to be least associated with elevated serum creatinine.

Hypercholesterolemia was observed in 89 (74.16%) patients in our study and the serum cholesterol ranged from 149 – 480mg/dl. Singh GK et al[6] reported that hyperlipidemia is often present in nephrotic syndrome. Total cholesterol may be increased 10-fold.
In this study microscopic hematuria was observed in 47 (39.16%) patients. 83.33% of patients with LN had microscopic hematuria, 60% of patients with MPGN, 44% of patients with MGN, 43% of patients with DN, 42% of patients with FSGS, 23% of patients with IgA nephropathy and MCD each and 20% of patients with amyloidosis had microscopic hematuria.

Varshney A et al\cite{11} in study found 69.9% of patients had microscopic hematuria. Golay V et al\cite{14} reported 28.54% of patients had either microscopic or macroscopic hematuria but we had no patient with macroscopic hematuria.

Proteinuria, results from altered permeability of the glomerular filtration barrier for proteins, namely glomerular basement membrane, podocytes, and their slit diaphragm.\cite{6} In the present study nephrotic range proteinuria was defined as proteinuria >3.5 gm/24 hours in adults and >40 gm/hr/m\textsuperscript{2} in children.

The 24 hour urine protein excretion ranged from 3.52 – 10.7 g/dl. All patients in present study had nephrotic range proteinuria. Proteinuria more than 10 gm/24 hours was observed in 2 patients with FSGS as underlying etiology.

Singh GK et al\cite{6} also had similar findings. They reported 24 hour urinary protein excretion ranged from 3.5 – 10.8 gm/day. They also reported that the rate of progression of variety of renal pathological conditions is related to the state of proteinuria, it was also predicted that heavy proteinuria was the worst prognostic marker.

Complications of nephrotic syndrome are secondary to urinary protein loss.\cite{6} In this study, urinary tract infection was most common complication at presentation which occurred in 22 (18.33%) patients out of 120 patients. This was followed by acute kidney injury in 12 patients (10%), CKD in 3 patients (2.5%), pneumonia in 2 patients (1.66%) and thrombosis – deep vein thrombosis (DVT) in single patient (0.83%).

Safaei A et al\cite{7} observed in their study that 18% of patients had acute kidney injury. Varshney A et al\cite{11} reported 26.16% of patients had pneumonia in their study.

In our study, chronic kidney disease was present in 3 patients. Two of them had diabetic nephropathy and one had IgA nephropathy following which they developed chronic kidney disease. Rathi M et al\cite{12} in their study stated that with increase in diabetes mellitus, the
majority of chronic kidney disease patients in India have diabetic nephropathy as their underlying etiology. This was observed in the present study also.

In the present study all patients were seronegative for HBsAg, HIV and HCV. Most of the patients attending to tertiary care hospital were of low socioeconomic class and were not affording for expensive techniques like immunofluorescence and electron microscopy. There were no cases of thin basement membrane disease or Alport’s syndrome detected in the present study. This may be due lack of electron microscopy study on biopsies we received in our study.

CONCLUSION
Thus we conclude in this Central Indian study that nephrotic syndrome is more common in 2nd and 3rd decade of life. Males are more affected than females irrespective of underlying cause except in lupus nephritis. FSGS remains the most common cause of nephrotic syndrome in children while in adults it is MCD. Among secondary causes DN is the leading cause of nephrotic syndrome. Hypertension is more in patients with MPGN, while elevated creatinine is more in MN.

Biopsy together with electron microscopy, immunofluorescence and increase in awareness, manpower, and infrastructure will definitely decrease complications and will improve prognosis of patients. Due to considerable heterogeneity in histological spectrum of nephrotic syndrome, it is essential to maintain a national renal biopsy registry data which would help to obtain accurate knowledge of spectrum, presentation, incidence and complications.

REFERENCES


