Original Article

Pattern of morphology in renal biopsies of nephrotic syndrome patients. Correlation with immunoglobulin and complement deposition and serology

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Abstract

Objective: To determine the morphological pattern in renal biopsies from nephrotic patients and their correlation with immunoglobulin and complement deposition as detected by immunofluorescence microscopy (IMF) and serology to arrive at correct diagnosis of glomerulonephritis (GN).

Methods: The study was carried out at the departments of Immunology and Histopathology, Sindh Institute of Urology and Transplantation (SIUT) from April 2007 to March 2008. A total of 200 patients, including children and adults were included. All patients presented with nephrotic syndrome (NS). Clinical and laboratory investigations including serology were noted, renal biopsies performed and studied by light and immunofluorescence microscopy (IMF).

Results: Of 200 patients, 74 (37%) were children (≤ 18 years) and 126 (63%) adults (≥ 19 years). Mean age of children was 11.34 ± 4.85 years (range 3 - 18 years) and that of adults was 35.44 ± 11.4 years (range: 19-70 years). The total percent change in L/M diagnosis after serological tests was 11.5% and after IMF studies, 23.5%. Combined serologic and IMF studies lead to 35% change in L/M diagnosis of the renal biopsies in nephrotic syndrome patients.

Conclusion: Our results demonstrate that the ancillary techniques of IMF microscopy and serology are indispensable in the elucidation of final specific diagnosis causing nephrotic syndrome in a substantial number of cases and these should be employed routinely in the pathologic evaluation of renal biopsies. The study emphasizes the importance of combined approach in the investigation of renal biopsies in nephrotic syndrome (JPMA 59:540; 2009).

Introduction

Nephrotic syndrome (NS) is a clinical syndrome resulting from a variety of primary and secondary glomerulonephritides (GN).[1-3] Making an accurate diagnosis of underlying GN requires a combination approach including clinical data, serologic tests and complete pathologic evaluation of renal biopsy by light microscopy (L/M), and immunoflourescence (IMF).[4-10] A series of serologic tests are undertaken before biopsy as integral component of the diagnostic evaluation of patients with NS.[11] They provide information that assist in diagnosing systemic disease of which the renal manifestation is NS. The tests include lupus serology [antinuclear antibody (ANA), anti-double stranded DNA (anti-dsDNA)], complements components (C3, C4), hepatitis B surface antigen and hepatitis C. It has been suggested that these serologic investigations add to the diagnostic accuracy and also help in differential diagnosis when used in combination with L/M and IMF studies.[11] In developed countries this combination approach is a routine practice while evaluating renal biopsy.[1,2,4,7,8] However, in developing countries renal biopsy diagnosis is often based on L/M, a practice that often leads to over diagnosis of some lesions and under diagnosis of others.[12-15] To our knowledge, there is no study in local population where L/M, IMF and serological assessment has been employed in all patients with NS. This study evaluated renal biopsy in patients with NS by L/M, IMF and serological investigation and the impact of this three tier evaluation on the final diagnosis.

Patients and Methods

This was a prospective study carried out at the Departments of Immunology and Histopathology, Sindh Institute of Urology and Transplantation (SIUT) from April 2007 to March 2008. The study was approved by the ethical review committee of SIUT. Two hundred consecutive patients of NS, both children and adults, were included in the study. Patients' demographic and laboratory data including age, sex, renal function and 24 hour urinary protein were collected.

Serology:

Serologic tests included; complements C3 and C4, Hepatitis B surface antigen and anti HCV antibody, antinuclear antibodies (ANA) by indirect fluorescent

antibody (IFA) using Hep 2 cells, anti ds-DNA by IFA test. The established definition of abnormal (i.e.positive) serologies were used[11] as follows: C3 low (<0.7 g/L); C4 low (<0.16g/L); positive HBsAg reactive (> 0.05 IU), Anti-HCV reactive (>1.00 IU); ANA positive (≥ 1:40); anti ds-DNA positive as 1+ or greater fluorescence in the Crithidia kinetoplast or the nucleus.

Biopsy:

Renal biopsy was undertaken in all cases using Tru-cut needle under ultrasound guidance. Two cores were obtained in each case, one for L/M and other for IMF. Native renal tissues were processed for light microscopic examination according to established protocols.[4,16,17]

IMF:

Tissue specimens were snap-frozen, sections cut and stained by Fluorescence isothiocynate (FITC) conjugated antisera specific for IgG, IgA, IgM, C3 and C1q (Dako, Glostrup, Denmark). Staining reactions were graded semiquantitatively as 0 to ++++ and distribution described as membranous or mesangial in a granular or linear pattern.

Final Diagnosis:

This was based on renal biopsy investigation L/M, IMF and correlation of these pathologic studies with serological results. Established diagnostic criteria were used for the diagnosis of specific glomerular diseases.[18]

Data analysis:

Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 10 computer program. Descriptive statistics such as mean ± SD for continuous variables and percentages were used for categorical data.

Results

Of the 200 patients, 74 (37%) were children (≤ 18 years) and 126 (63%) adults (≤ 19 years). The mean age of children was 11.34 ± 4.85 years (range 3 - 18 years) and that of adults was 35.44 ± 11.4 years (range: 19-70 years). Of children, 38 (51.4%) were males and 36 (48.6%) females (M:F ratio: 1.65:1). Among adults, 75 (59.5%) were males and 51 (40.5%) females (M:F ratio: 1.47:1).

Laboratory Findings:

The mean 24 hrs urinary protein in children was 4.73 ± 0.84 gm/ 24 hrs (range: 3.30-6.9gms/24 hrs). The values for adults were 5.44 ± 1.16gm/24 hrs (range: 3.80-10.1gm/24hrs). ANA was positive in 66 (33%) cases, anti-dsDNA in 8 (4%). Low C3 was observed in 60 (30%), while low C4 was seen in 21 (10.5%) cases. HBsAg was positive in 13 (6.5%) cases, while anti-HCV was positive in 8 (4%).

The common patterns observed on L/M in 200 patients with NS are shown in Table-1.

Table 1: Light Microscopic findings in 200 patients with Nephrotic Syndrome.

L/M patterns	Frequency	Percentage
Focal and segmental scarring	60	30
Minor changes	41	20.5
GBM thickening	38	19
Mesangioproliferative pattern	34	17
Mesangiocapillary pattern	12	6
Hyalinosis	8	4
Chronic sclerosing glomerular pattern	3	1.5
Diffuse proliferative and exudative pattern	2	1
Crescentic pattern	2	1

GBM: Glomerular basement membrane.

Table 2: Final diagnosis based on morphology, IMF and serology in 200 patients with nephrotic syndrome.

Final diagnosis	Number	Percentage
FSGS	56	28
Minimal Change Disease	39	19.5
Membranous GN	37	18.5
IgM Nephropathy	15	7.5
Lupus Nephritis	13	6.5
Mesangioproliferative GN	10	5
Mesangiocapillary type I	8	4
Amyloidosis	6	3
IgA Nephropathy	5	2.5
Post Infectious GN (Resolving)	4	2
Diabetic Nephropathy	2	1
Others	5	2.5

FSGS: Focal segmental glomerulosclerosis; GN: Glomerulonephritis.

Final specific GN diagnosed with combined L/M, IMF and serology are given in Table-2. Out of 60(30%) L/M diagnoses of FSGS, 56 (93%) were idiopathic FSGS, while 2(3%) of healed class III lupus nephritis and one case each (2%) of healed necrotizing GN and IgA nephropathy (IgAN) were seen.

Of 41(20.5%) L/M diagnoses of minor changes, 39 (95%) were diagnosed as minimal change disease (MCD). The remaining 2(5%) turned out to be non-MCD lesions, one each of pre-spike MGN and IgAN, showing diagnostic IMF patterns.

Out of 38(19%) cases of GBM thickening on L/M, 36(95%) were idiopathic MGN, while 2 (5%) consisted of lupus nephritis class V on the basis of positive lupus serology and full house IMF pattern. One case of idiopathic MGN was picked up in stage I (prespike stage) with the help of IMF, the L/M of this case showed minor changes.

Of 34 (17%) L/M diagnoses of mesangioproliferative pattern, 15(44%) cases were diagnosed as IgM nephropathy

(IgMN), 10 (29%) as idiopathic MesPGN, 4 (12%) as resolving postinfectious GN, 3 (9%) as lupus nephritis class II and 2 (6%) as IgAN by their characteristic IMF and serologic findings.

Of 12 (6%) L/M diagnoses of mesangiocapillary pattern, 8 (67%) were classified as idiopathic mesangiocapillary GN (MPGN) type I and 4 (33%) as lupus class IV by IMF and serologic studies.

Serology and IMF were also useful in the definitive diagnosis of 2 (100%) cases of diffuse proliferative and exudative pattern. These turned out to be lupus class IV with full house positivity on IMF and positive lupus serology.

Table-3 shows percent change in the L/M diagnosis after IMF and serologic studies. Total percent change in L/M diagnosis after serological tests was 11.5% and after IMF studies 23.5%. Overall change in L/M diagnosis was seen in 35% of the biopsies.

Table 3: Magnitude of change in L/M findings (in numbers and percentages) with the addition of serology and IMF.

Light Microscopic findings	Total	Serology	IMF	Overall
Focal Segmental Scarring	60	2(3.3%)	4(6.6%)	6(10%)
Minor changes	41	1(2.4%)	2(4.81%)	3(7.3%)
GBM Thickening	38	2 (5.2%)	2(5.2%)	4(10.4%)
Mesangial Proliferation	34	3(8.82%)	24(70.58%)	27(79.41%)
Mesangiocapillary Pattern	12	12(100%)	12(100%)	12(100%)
Hyalinosis	8	0	0	0
Chronic Sclerosing Lesions	3	0	0	0
Crescents	2	1(50%)	1(50%)	2(100%)
Diffuse proliferative and exudative Pattern	2	2(100%)	2(100%)	2(100%)
Total	200	23(11.5%)	47(23.5%)	70(35%)

GBM: Glomerular basement membrane; IMF: Immunoflourescence.

Discussion

Percutaneous needle biopsy of the kidney forms an indispensable element in the investigation of NS.[4] Optimum approach to laboratory investigation of renal biopsy includes light microscopy, immunohistochemistry and EM examination.[4-10] This is the routine approach taken in most laboratories in the developed world. On the other hand, situation in developing countries is quite different and renal biopsy diagnosis is often rendered solely on L/M examination.[4] L/M provides a morphological pattern, but not a specific diagnosis, in glomerular disease, as a range of morphological patterns is observed in NS. These include minor changes, focal and segmental scarring, glomerular basement membranous (GBM) thickening, mesangial proliferation, mesangiocapillary pattern, hyalinosis and a number of rare patterns that are not synonymous with disease entities.[1-3] Making an accurate diagnosis of glomerulonephritis causing NS requires integration of serological and clinical data, detailed investigation of renal biopsy by L/M, IMF, EM studies and a correlation of all the above findings.[4-11]

In our series, most common pattern on L/M was focal segmental scarring. This is a glomerular appearance, which represents the histological aspect of a final common pathway of injury, and not a disease entity.[19] On further study by IMF and serology, majority (93%) of these were classified as idiopathic FSGS. The incidence of idiopathic FSGS is on the rise both in children and adults with NS, and is the leading cause of NS in adults around the world.[19,20] Idiopathic FSGS showed focal positivity of IgM in 87.3% and C3 in 83.63% cases. A similar rate of positivity of IgM (80%) and C3 (100%) was noted in one local study in cases of FSGS in adults.[16] This is a nonspecific finding and represents trapping of heavy IgM molecules in sclerosed regions. Other local studies did not employ IMF in the evaluation of renal biopsies,[12,13,15] except two reported previously from Islamabad[16] and our center.[17] No serologic data has been given in local studies.[12,13,15,16]

Next frequent pattern observed by L/M consisted of minor changes seen in 41 (20.5%) cases. On further investigation by IMF and serology, majority (95%) were categorized as MCD. IMF showed negative results for immunoglobulins and complement in MCD. This is in agreement with results of IMF in a previous study from Pakistan.[16]

Third common pattern on L/M consisted of GBM thickening, seen in 38 (19%) cases. On further study by IMF and serology, majority (95%) of these cases were labeled as idiopathic MGN. All cases of idiopathic MGN showed positivity of IgG and C3 in granular membranous distribution on IMF study. This is similar to the findings of other studies, which showed positivity of IgG and C3 in all cases of MGN.[16-21] In one study of MGN from Pakistan, IMF was done in only 10 out of 176 cases, of which 8 cases of idiopathic MGN showed positivity of IgG and C3, while two cases showed full house positivity and were labeled as lupus nephritis, class V.[22]

Mesangial proliferative pattern on L/M was seen in 34 (17%) cases. IMF and serologic studies were very useful in the differential diagnosis of specific GN causing this pattern, as revealed by overall percent change in L/M diagnosis of 79.4% in this category. Idiopathic mesangioproliferative GN was diagnosed only when immunoglobulin or complement deposits were not seen on IMF.[16,23]

Mesangiocapillary pattern on L/M was seen in 12(6%) cases in this study. IMF showed full house

positivity in 4 (33%) cases of mesangiocapillary pattern, these cases being labeled as lupus nephritis, class IV. Remaining 8 (67%) cases of mesangiocapillary GN (MPGN), type I showed positivity of C3 and C1q in all cases and IgG in 7 cases. These results are similar to those of previously cited local and international studies.[16,24] Abnormal serology was seen in 75% of idiopathic MPGN, mostly in the form of low C3 and C4. This is in contrast with findings from a study from USA,[11] where abnormal serology was not detected in 2 cases of MPGN. This may be due to very low number of cases of MPGN in that study.

The pattern of immunoglobulin and complement deposition as detected by IMF was indispensable in the final diagnosis of 23.5% cases of GN. Similarly serology was useful in arriving at a final diagnosis in 11.5% cases; a diagnosis of lupus nephritis could be made on the basis of positive ANA and anti-dsDNA. Application of serologic and IMF studies to L/M pattern resulted in overall 35% change in L/M diagnosis. Our results show that the routine application of serologic and IMF testing when evaluating NS patients was helpful in diagnosing specific disease entities.

In conclusion, the findings of this study demonstrate that ancillary techniques of IMF microscopy and serology are indispensable in the elucidation of final specific diseases causing nephrotic syndrome in a substantial number of cases and these should be employed routinely in the pathologic evaluation of renal biopsies.

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