Results. In the clinical parameters, patients with IMGN-FSGS had a significantly higher incidence of hypertension, raised serum creatinine and microscopic haematuria. The mean 24-h urinary protein excretion was higher in the group with IMGN-FSGS (7.4 ± 1.36 g) as compared to IMGN alone (3.85 ± 0.7 g, P < 0.001, Mann–Whitney test). On light microscopy, biopsies with IMGN-FSGS frequently had mesangial hypercellularity and more extensive tubulo-interstitial disease than those with IMGN alone. Stereological analysis showed that the non-sclerosed glomeruli in biopsies with IMGN-FSGS had a higher mean cross-sectional area (18546.7 ± 32493.3 μm²) and higher estimated volume (855200 ± 152640 μm³) as compared to glomeruli in cases with IMGN alone (76000 ± 14719.2 μm² and 576666.7 ± 131233.3 μm³, respectively).

Conclusion. The present study is probably the first systematic analysis of stereologic parameters in renal biopsies of IMGN with FSGS. Our results objectively demonstrate the glomerular enlargement in the non-sclerosed glomeruli in cases of IMGN with FSGS. This detection of enlarged glomeruli may serve to alert the renal pathologist to the possibility of coexisting FSGS, which is a poor prognostic factor in IMGN.

Keywords: focal segmental glomerulosclerosis; glomerular cross-sectional area; glomerular volume; idiopathic membranous glomerulonephritis; stereology

Introduction

Idiopathic membranous glomerulonephritis (IMGN) represents ~20–30% of cases of adult nephrotic syndrome [1]. The clinical course of IMGN is highly variable with ~15–50% of patients progressing to chronic renal failure in 10–15 years after diagnosis [2,3]. Various features including male sex, heavy proteinuria, renal insufficiency at presentation, hypertension, age and degree of interstitial fibrosis have been shown to predict unfavourable course in patients...
with IMGN [4–6]. A few earlier studies in the literature have stressed the prognostic implication of coexistence of focal segmental glomerulosclerosis (FSGS) in patients with IMGN [4,7–10]. However, none of the previous studies have examined the morphometric and stereologic parameters in renal biopsies of IMGN with FSGS.

This study was aimed at evaluating the clinical, pathologic and stereologic parameters in cases with IMGN-FSGS and comparing them with patients of IMGN alone.

Materials and methods

Patient selection

From the group of IMGN patients (excluding lupus membranous nephritis, hepatitis B, hepatitis C, drugs like gold, penicillamine, neoplastic conditions, systemic involvement by diabetes, sarcoidosis, sickle-cell anaemia, autoimmune conditions) diagnosed during a 3-year period (2006–2008), 23 patients whose renal biopsies showed a superimposed FSGS lesion were selected as the study group. From the remaining 156 biopsies, 35 age- and sex-matched patients of IMGN without FSGS lesion were selected for comparison.

Clinical and laboratory data

For each patient, age, sex, duration of symptoms before biopsy, blood pressure, serum creatinine level, 24-h urinary protein excretion and urine sediment examination were recorded. Hypertension was defined as a diastolic blood pressure of 95 mmHg or greater and/or a systolic pressure of 140 mmHg or greater. A serum creatinine of >1.5 mg/dl was considered as abnormal.

Pathologic evaluation

Renal biopsies were processed for light and immunofluorescence (IF) microscopy using the standard methods. Biopsies with less than nine glomeruli were excluded from analysis in the IMGN group. For detecting the presence of FSGS and tubulo-interstitial fibrosis, all available slides including haematoxylin and eosin (H&E), periodic acid Schiff (PAS)- and methenamine silver-stained sections were screened.

For each biopsy, the following features were recorded: the number of total glomeruli, the number of obsolescent glomeruli, mesangial proliferation (more than three nuclei per mesangial stalk in a 3-µm thick section) and segmental glomerulosclerosis. The FSGS lesions were noted for the number of glomeruli involved and the type of FSGS, according to the Columbia classification of FSGS [11]. Tubulo-interstitial damage and fibrosis were graded semi-quantitatively from 0 to 3 using the rating of Wakai and Magil [9]. Grade 1 indicates interstitial fibrosis of 5% or more but <25%; grade 2 indicates fibrosis of 25% or more but <50%; and grade 3 indicates fibrosis of 50% or more. MGN lesions were staged by light microscopic examination as follows: stage I (no thickening of glomerular basement membrane (GBM) by light microscopy, but IF shows capillary wall granular deposits), stage II (GBM thickened, spikes shown on silver methenamine) and stage III (GBM thickened, a complex appearance of rosary or chain-like appearance in a silver methenamine-stained section) [12].

Morphometry and stereology

Stereologic analysis was performed using the StereoInvestigator software (Microbrightfield Inc., Williston, VT, USA) under a light microscope with a dedicated digital camera. The software utilized the Cavalieri principle in volume estimation [13]. The measurements were done on 3-µm serial sections stained by haematoxylin and eosin (H&E) stain. The procedure briefly involved tracing the tuft of each glomerulus on serial step-sections (minimum of 12 steps in each case) and estimating the area and volume of the glomerulus (Figure 1). A minimum of four glomeruli were traced in each case.

Fig. 1. Photomicrograph showing the tracing of a glomerulus and filling with a marker in the StereoInvestigator™ software.
Table 1. Clinical and laboratory findings in patients with IMGN and IMGN-FSGS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IMGN-FSGS</th>
<th>IMGN</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>19–60 (31.5 ± 13.5)</td>
<td>17–65 (36.5 ± 10.6)</td>
<td>0.208</td>
</tr>
<tr>
<td>Male: female ratio</td>
<td>19:4</td>
<td>28:7</td>
<td>0.05</td>
</tr>
<tr>
<td>Duration of disease prior to biopsy (months) (mean ± SD)</td>
<td>3–12 (7.27 ± 3.28)</td>
<td>3–10 (5.71 ± 2.22)</td>
<td>0.109</td>
</tr>
<tr>
<td>Patients with hypertension (%)</td>
<td>60.8</td>
<td>9.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Patients with raised serum creatinine (%)</td>
<td>52.2</td>
<td>17.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Patients with microscopic haematuria (%)</td>
<td>65.2</td>
<td>28.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Statistical analysis
The chi-square test, Mann–Whitney U-test, Student’s t-test and one-way ANOVA were used where appropriate. A P-value of < 0.05 was considered as statistically significant.

Results
The clinical and laboratory data for both groups (IMGN and IMGN-FSGS) are presented in Table 1. Hypertension at presentation, deranged renal function tests (abnormal serum creatinine) and microscopic haematuria were more frequent in the IMGN-FSGS group than in the IMGN group. The 24-h urinary protein excretion was significantly higher in IMGN-FSGS group (7.4 ± 1.36 g) than in the IMGN group (3.85 ± 0.7, P < 0.001, Mann–Whitney test). However, there was no significant difference between the two groups with respect to the mean age and gender distribution of the cases or the duration of renal disease before biopsy. None of the patients with hypertension were on antihypertensive therapy at the time of biopsy.

The histologic and immunofluorescence features characteristic of membranous glomerulonephritis were observed in all the biopsies of both groups. In addition to these characteristic changes, the biopsies in the IMGN-FSGS group also showed glomerular lesions of FSGS in a variable number of non-obsolescent glomeruli (Figure 2).

The total number of glomeruli varied from 7 to 23 (13.4 ± 6.07) in the IMGN-FSGS group and from 9 to 21 (14.3 ± 4.18) in the IMGN group. In the biopsies with IMGN-FSGS, the proportion of glomeruli involved by segmental sclerosis ranged from 6.7 to 27.2% of the total glomeruli. Using the Columbia classification for type of FSGS lesion, the most common lesion was ‘not otherwise specified (NOS)’ seen in 20 (87%) biopsies. Perihilar and tip lesions were seen in two (8.6%) and one (4.4%) biopsies, respectively. No collapsing or cellular FSGS lesion was noted in our study.

There was no significant difference between the two groups with respect to the proportion of obsolescent glomeruli (P-value 0.08, Student’s t-test). The various histological parameters are tabulated in Table 2. The IMGN-FSGS group showed higher frequency of mesangial...
hypercellularity (86.9%) as compared to the IMGN group (40%, P-value < 0.001, chi-square test). Tubular atrophy and interstitial fibrosis were more frequent and of a higher grade in the IMGN-FSGS group as compared to the IMGN group (Figure 2). In the IMGN-FSGS group, the most common stage of MGN was III (56.5%) followed by stage II (39.13%) and stage I (4.34%). In comparison, the IMGN group showed stage II to be the most common (68.57%) followed by stage I (17.14%) and stage III (14.29%). This difference was statistically significant (P-value < 0.001).

Among the stereologic parameters, the mean glomerular cross-sectional area and mean glomerular volume (estimated using Cavalieri principle) were significantly higher in the IMGN-FSGS group as compared to the IMGN group (P < 0.001, Mann–Whitney test). These results are summarized in Table 2 and Figures 3 and 4.

All patients in both groups received angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers. Follow-up information was available in 11 of IMGN-FSGS patients and 15 of IMGN patients. Nine patients in the IMGN-FSGS group had reduction in proteinuria; however, none of them achieved partial or complete remission. Two patients in this group had an increase in the 24-h protein excretion and were started on immunosuppressive therapy (endoxan or tacrolimus). In comparison, patients with IMGN had a significant reduction in proteinuria with complete remission in five patients and partial remission in another four patients, while the remaining six patients required immunosuppressive therapy.

**Discussion**

A number of prognostic factors, including age, sex, degree of proteinuria, extent of tubulo-interstitial changes, hypertension and stage of glomerular disease, have been identified in IMGN patients [4,9]. In recent times, the association of FSGS with IMGN has attracted the attention of renal pathologists. An extensive literature search yielded few such reports [4,7–10,14,15]. In the report by Iwahashi, patients with IMGN-FSGS had higher systolic blood pressure, longer duration of proteinuria with advanced stage of glomerular lesion and more severe tubular atrophy and interstitial fibrosis [8]. Similar findings have been demonstrated by other authors as well [4,7,10]. Studies by Heeringa et al. and Troyanov et al. showed that FSGS did not have an independent prognostic importance in the overall renal survival in patients of IMGN with...
superimposed FSGS [14,15]. However, even in the study by Heeringa et al., patients with IMGN-FSGS had higher serum creatinine and more advanced stage of glomerular lesion as compared to the IMGN group [14]. Also in the present study, the group of IMGN-FSGS had significantly higher incidence of hypertension at presentation, raised serum creatinine and microscopic haematuria on urinalysis. The degree of 24-h proteinuria was also significantly higher in the IMGN-FSGS group as compared to the IMGN group. These findings are in concurrence with the earlier reported studies in the literature and suggest that FSGS in patients with IMGN is associated with adverse clinical parameters.

Morphometric measurement of the glomerular cross-sectional area in biopsies with IMGN and IMGN-FSGS was earlier attempted by Wakai and Magil [9]. They reported that the IMGN-FSGS group had a higher mean glomerular cross-sectional area as compared to those with IMGN alone ($P = 0.032$, Student’s $t$-test). However, they did not attempt further morphometric assessment in their study [9]. In another study, Lee and Koh determined a mean glomerular area using the point counting method. They failed to find a significant difference in the glomerular area between IMGN and IMGN-FSGS groups [10]. However, this could be due to the relative insensitivity of the point counting method, if the number of points counted is few. In the study by Lee and Koh, mesangial expansion and relative interstitial volume were higher in the IMGN-FSGS group [10]. To the best of our knowledge, this is the first study evaluating the stereologic parameters, including glomerular area and volume, in IMGN with and without FSGS. Our study shows that in biopsies of IMGN with associated FSGS, the non-sclerosed glomeruli had a significantly higher mean glomerular cross-sectional area and estimated glomerular volume as compared to those with IMGN alone ($P < 0.001$). These results suggest and support the hypothesis of glomerular hyperperfusion and hyperfiltration as one of the causative mechanisms in the development of the FSGS lesion in IMGN. These changes are similar to the results in minimal change disease progressing to FSGS as reported earlier [16]. In FSGS following unilateral nephrectomy, glomerular hypertrophy has been shown to be indicative of glomerular hypertension, which over time, leads to capillary collapse and segmental sclerosis [17].

The occurrence of FSGS in IMGN patients has been shown to be associated with mesangial expansion and advanced stages of glomerular lesion [4,10]. In the present study too, the IMGN-FSGS group had a higher stage of the MGN lesion as compared to the IMGN group. We agree, however, that the stage of MGN, as determined by light microscopy, lacks the accuracy of ultrastructural determination. It has been suggested that subepithelial immune deposits in IMGN may lead to disruption of podocyte attachment to the GBM and contribute to segmental sclerosis [4]. However, this does not explain the occurrence of FSGS in some cases of stage I IMGN in a study by Dumoulin et al., as well as in the present study [4].

The present study, in conjunction with the earlier studies, shows that the presence of hypertension, marked proteinuria and elevated serum creatinine are certain clinical features associated with the frequent occurrence of FSGS in patients with IMGN. In addition, the presence of enlarged glomeruli in the first renal biopsy of such patients should alert the renal pathologist to the possibility of the presence of FSGS in association with IMGN. In such instances, serial step-sections and multiple PAS-stained sections need to be carefully examined to detect the segmental sclerosis lesion. This is particularly important since such patients may benefit from measures such as use of angiotensin-converting enzyme inhibitors, which have been shown to reduce proteinuria in these patients [18].

In conclusion, FSGS may be superimposed on idiopathic membranous glomerulonephritis and is frequently associated with adverse clinical features. The presence of enlarged glomeruli with an increased cross-sectional area and estimated glomerular volume in a kidney biopsy should serve to alert the renal pathologist to the likelihood of the presence of the segmental sclerosis lesion with membranous glomerulonephritis. Detailed examination of the renal biopsy with multiple sections and PAS stain would help in early detection of segmental sclerotic lesion in such cases.

Acknowledgements. Dr Ruchika Gupta and Dr Alok Sharma would like to acknowledge CSIR for the research grant support as senior research associates. The authors wish to thank Dr Sompal Singh for his assistance in statistical analysis and critical review of the manuscript.

Conflict of interest statement. The results presented in this paper have not been published previously in whole or part, except in abstract form in the proceedings of 57th Annual Conference of Indian Association of Pathologists and Microbiologists, Katankuluthur, November 2008.

References

Development and validation of GFR-estimating equations using diabetes, transplant and weight

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Abstract

Background. We have reported a new equation (CKD-EPI equation) that reduces bias and improves accuracy for GFR estimation compared to the MDRD study equation while using the same four basic predictor variables: creatinine, age, sex and race. Here, we describe the development and validation of this equation as well as other equations that incorporate diabetes, transplant and weight as additional predictor variables.

Methods. Linear regression was used to relate log-measured GFR (mGFR) to sex, race, diabetes, transplant, weight, various transformations of creatinine and age with and without interactions. Equations were developed in a pooled database of 10 studies [2/3 (N = 5504) for development and 1/3 (N = 2750) for internal validation], and final model selection occurred in 16 additional studies [external validation (N = 3896)].

Results. The mean mGFR was 68, 67 and 68 ml/min/1.73 m² in the development, internal validation and external validation datasets, respectively. In external validation, an equation that included a linear age term and spline terms in creatinine to account for a reduction in the magnitude of the slope at low serum creatinine values exhibited the best performance (bias = 2.5, RMSE = 0.250) among models using the four basic predictor variables. Addition of terms for diabetes and transplant did not improve performance. Equations with weight showed a small improvement in the subgroup with BMI <20 kg/m².

Conclusions. The CKD-EPI equation, based on creatinine, age, sex and race, has been validated and is more accurate than the MDRD study equation. The addition of weight, diabetes and transplant does not significantly improve equation performance.

Keywords: creatinine; development; estimating equation; glomerular filtration rate; validation

Introduction

Glomerular filtration rate (GFR) is an important indicator of kidney function, critical for detection, evaluation and management of chronic kidney disease (CKD). GFR cannot be practically measured for routine clinical or research purposes, and therefore, serum creatinine is often used to