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Evaluation of fibrosis in renal biopsies by morphometric analysis and visual analysis and its correlation with renal function

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Abstract

Introduction: Evaluation of interstitial fibrosis is an essential component of any kidney biopsy report. This requires the degree of fibrosis to be accurately measured to validate these therapies. Unfortunately, however, there is little comparative information on the relative advantages of the various techniques to quantify fibrosis. The commonly used method for assessment of fibrosis traditionally has been a visual assessment. Morphometric assessment of fibrosis promises to be a tool which could quantify fibrosis better.
Objectives: We undertook this study to compare visual assessment with a morphometric method, in relation to serum creatinine levels.
Patients and Methods: A total sample size of 40 was calculated on the basis of prevalence studies. Evaluation of fibrosis of the cases by visual method was performed by an experienced renal pathologist on the Masson Trichrome stained slide and the result was expressed as a percentage of the total biopsy area. Evaluation of fibrosis was subsequently performed by image based computer assisted morphometric analysis using Biowizard 4.2 software.
Results: Correlation was carried out between visual and morphometric assessment of fibrosis and a moderate degree of correlation was obtained. Each of the methods was correlated against the serum creatinine levels and both showed a mild correlation.
Conclusion: We showed that there is moderate correlation between visual assessment of fibrosis on a trichrome stained slide and morphometric evaluation on the same slides.

Introduction

It is well-known that any chronic renal disease, whether glomerulonephritis, interstitial nephritis or pyelonephritis will lead to fibrosis involving the tubulointerstitial compartment. This fibrosis takes the form of accumulation of the extracellular matrix in the interstitial area and usually is accompanied by tubular atrophy (1). Hence evaluation of interstitial fibrosis is an essential component of any kidney biopsy report (2). There is a lot of research currently on therapeutic inhibition of fibrosis (3). This requires the degree of fibrosis to be accurately measured to validate these therapies. Unfortunately, however, there is little comparative information on the relative advantages of the various techniques to quantify fibrosis.

The commonly used method for assessment of fibrosis traditionally has been a visual assessment (4), however it has been reported to be poorly reproducible (5). Morphometric assessment of fibrosis promises to be a tool which could quantify fibrosis better. Considering the inherent limitations in the currently available methods to detect fibrosis, we undertook this study to compare visual assessment with a morphometric

Key point

Evaluation of interstitial fibrosis is an essential component of any kidney biopsy report. We undertook this study to compare visual assessment with a morphometric method, in relation to serum creatinine levels. We showed that there is moderate correlation between visual assessment of fibrosis on a trichrome stained slide and morphometric evaluation on the same slides.

method, in relation to serum creatinine levels.

Patients and Methods

The study was carried out at the Department of Pathology at a Tertiary Care Institute. A total sample size of 40 was calculated on the basis of prevalence studies. The cases were collected over a 4-month period and included consecutive cases undergoing renal biopsy. Serum creatinine was available in all the cases. In all the cases, two cores of renal tissue were obtained, one for routine light microscopy in formalin and the other for immunofluorescence studies in saline. Tissue for light microscopy was routinely processed and studied with hematoxylin and eosin, periodic acid–Schiff (PAS) and silver stains. Masson's trichrome stain was performed in all cases for assessment of fibrosis. Tissue for immunofluorescence microscopy (IF) was processed for staining with a panel of antibodies to IgG, IgA, IgM, C3 and C1q for diagnostic purposes.

Evaluation of fibrosis of the cases by visual method was performed by an experienced renal pathologist on the Masson's trichrome stained slide and the result was expressed as a percentage of the total biopsy area. Evaluation of fibrosis was subsequently performed by image based computer assisted morphometric analysis using Biowizard 4.2 software. The fibrotic area was traced using the free and tool and the total fibrotic area was expressed as a percentage of the total biopsy area (Figure 1).

Ethical issues

The research followed the tenets of the Declaration of Helsinki; informed consent was obtained from all the participants of the study; and the research was approved by the institutional review board, Armed Forces Medical College, Pune, India.

Statistical analysis

Correlation was performed between the two methods of assessment and the serum creatinine levels and correlation coefficient (Spearmen's Rho) was calculated. Mean± SD of variables and frequency distribution of demographic data was obtained using SPSS version 10 and P value below 0.05 was considered significant.

Results

Of the 40 patients included in the study, 24 were males and 16 were females. The average age of the subjects was 31 years. The mean serum creatinine level was 1.7 mg/dL with a range from 0.8 to 4.0 mg/dL. Mean percentage of fibrosis by visual assessment was 18.38% with a range from 0% to 75%. The mean area of fibrosis by morphometric assessment was 17% with a range from 0% to 83.01%.

Correlation was carried out between visual and morphometric assessment of fibrosis and a significant degree of correlation was obtained with a correlation coefficient of $\rho = 0.915$, $P < 0.001$ (Figure 2A). Each of the methods was correlated against the serum creatinine levels (Figures 2B and 2C) and both showed a significant correlation ($\rho = 0.454$, $P = 0.003$ for visual method and $\rho = 0.324$, $P = 0.041$ for morphometric method).

Discussion

The renal interstitium is situated in the space between the basement membranes of the epithelial cells and of the peritubular capillaries. This compartment of the kidney is of extreme functional importance in the normal and diseased kidney. The interstitial compartment of the renal cortex, including its cellular elements, particularly the fibroblasts are often overlooked in routine microscopic slide preparations of normal kidneys. In diseased kidneys, especially when there is inflammatory injury, the interstitial space becomes expanded with cells and later as chronicity develops, with fibrosis.

There are many different morphological patterns of interstitial fibrosis and these different patterns in all probability do not have similar causes or functional effects. Destructive processes like pyelonephritis and renal infarcts usually result in large geographic scarred areas with severe loss of renal proximal and distal tubules. This pattern comes across as a kind of "wound healing" response to severe destructive injury. It usually helps in getting rid of the infection, but results in an irretrievable loss of renal function. In sharp contrast to this pattern, is another pattern of interstitial fibrosis and tubular atrophy which is commonly seen on kidney biopsies for medical renal disease and is diffuse or patchy and fine, characterised by thickening and wrinkling of the tubular basement membranes, wide separation of the tubules and accumulation of fibrosis in the intervening areas, and associated with either diffuse or focal disease of glomeruli, tubules, or vessels, best appreciated on Masson trichrome and PAS stains (2,6).

An interesting study was undertaken by Farris et al (7) on evaluation of fibrosis in renal biopsies by direct visual and morphometric techniques. They used Masson trichrome stained slides and performed assessment of fibrosis by four morphometric methods and evaluated them against two methods of direct visual fibrosis scoring on renal biopsies. Of the four morphometric methods they had included, two of them involved preparation of digital images of

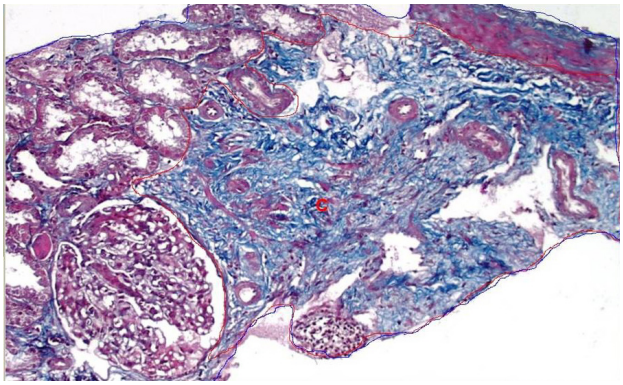

Figure 1. Morphometric evaluation of images using Biowizard 4.2 software using free hand tool. Area enclosed by red line indicates fibrotic area and area enclosed by blue line indicates total area.

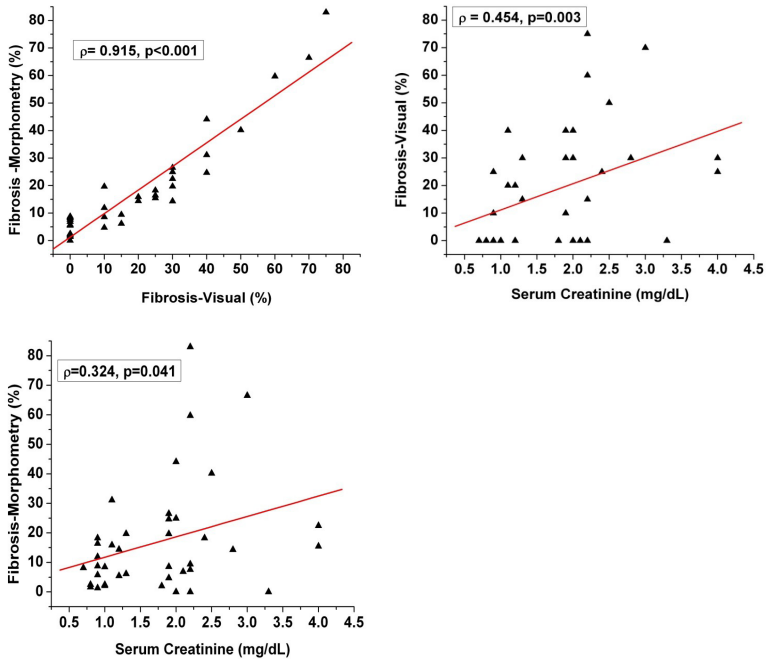

Figure 2. (A) Correlation of assessment of renal fibrosis by morphometry (Y axis) with assessment of renal fibrosis by visual assessment (X axis). (B) Correlation of assessment of renal fibrosis by visual assessment with serum creatinine. (C) Correlation of assessment of renal fibrosis by morphometric assessment with serum creatinine.

the entire slide with immunohistochemical staining for collagen type III or preparation of digital images of the entire slide with a new technique using Masson trichrome and PAS subtraction morphometry. They also included 2 other methods that involved Sirius red staining and its interpretation with and without the use of polarisation on multiple fields after digitisation. The merit of the study was that they evaluated multiple serial sections from different kidney biopsies with a wide spectrum of extent of interstitial fibrosis and tubular atrophy with differing final histopathological diagnoses. These assessments were done on duplicate sections, each with a separate method on different days. The visual scoring was performed by a total of three pathologists on the digitised images of the complete slides. The direct visual and morphometric methods showed good to excellent inter-assay reproducibility and inter-observer reproducibility. In general, morphometric methods showed less variation between observers than those involving direct visual assessment. Of the different methods studied, the ones which showed best correlation with each other were immunohistochemistry for collagen III, Sirius Red staining with assessment without use of polarized microscopy non-polarized, and the direct visual scores. After taking into account various factors that are important in evaluation of a technique, like efficiency, reproducibility, and functional correlation, two current techniques stood out as potentially the best for clinical trials: collagen III morphometry and visual assessment of trichrome-stained slides. In our study, we mainly compared the visual based assessment on trichrome stains with morphometric evaluation on the same trichrome stained slides. We also found that visual assessment on trichrome stained slides gave a good result, comparable with image based computer assisted morphometric evaluation.

We also tried to investigate as to why visual assessment of fibrosis performed so well against image based computer assisted morphometric evaluation. The only explanation we could think of was that the visual assessment was performed by an experienced renal pathologist.

Conclusion

Assessment of renal fibrosis forms an important cornerstone of a renal biopsy report. Morphometric assessment has proven to be more accurate over the years. However, it is time consuming and requires software. Visual assessment, on the contrary can be performed right at the time the slide is being evaluated for diagnostic abnormalities. We showed that there is a good correlation between visual assessment of fibrosis on a trichrome stained slide and morphometric evaluation on the same slides.

Limitations of the study

This is a single center study with a limited sample size. A multi-centric study with a larger sample size and incorporation of molecular analysis will enable validation of the results.

Authors' contribution

All authors contributed to the manuscript equally. They read and signed the final paper.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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