Implications of tubulo-interstitial epithelial and mesenchymal relation in chronic kidney disease

OFELIA JERCAN¹, M. PENESCU², DIANA MARIA TRAŞCĂ³

¹University of Medicine and Pharmacy of Craiova
²"Carol Davila" Nephrology Hospital, Bucharest
³IVth Department, Medical Specialties II, University of Medicine and Pharmacy of Craiova

Abstract

Introduction: Several studies have shown the prognostic value of markers detecting interstitial infiltration, epithelial-mesenchymal transition (EMT) and tubulo-interstitial damage in chronic kidney disease evolution. Aim of our investigation was to further evaluate the pathological correlation of such parameters in a population with chronic kidney disease in early stages. Materials, Methods and Results: In a population of 16 patients, with a prior diagnosis of chronic kidney disease in early stages, that underwent a biopsy procedure for clinical indication, there were evaluated the expression in kidney tissue of mesenchymal, epithelial and proliferation markers. Material remaining after routine light microscopy and immunofluorescence was stained for mesenchymal markers such as vimentin, epithelial markers such as cytokeratin and E-cadherin. Quantitative evaluation was conducted by electronic image analysis on consecutive low power fields, avoiding glomeruli, and estimated as percentage of the total area. The clinical and biochemical characteristics evaluated during the hospitalization period showed the prevalence of multiple cardiovascular risk factors such as: arterial hypertension (68%), abnormal blood lipid levels (32%), obesity (27%), diabetes (19%). The histopathological characteristics of chronic kidney dysfunction was related with higher expression of mesenchymal markers ($p<0.001$) and a decrease expression of epithelial markers ($p=0.003$). Conclusions: The interrelation of epithelial and mesenchymal tubulo-interstitial markers was demonstrated even in early stages of chronic kidney dysfunction.

Keywords: epithelial-mesenchymal transition, tubulo-interstitial damage, chronic kidney disease.

Introduction

Several studies have shown the importance of tubulo-interstitial fibrosis as the final step in end-stage renal disease evolution, regardless of the initiating cause for the renal pathology [1–4]. The pathologic features for chronic kidney disease in the fibrotic stage are showing important deposition of extracellular matrix, myofibroblasts accumulation, glomerulosclerosis, tubular atrophy and peritubular capillary reduction [4–7].

Assessment of interstitial fibrosis has been proved to relate with the renal function outcome in several studies [8, 9]. Interstitial fibrosis quantification is a predictive marker for the kidney graft outcome [10].

Cellular interchanges between mesenchymal and epithelial phenotype by activating or deactivating specific genes represents a widely accepted concept that characterizes the embryonary processes [11].

Recent studies that analyze the pathological expression of needle biopsy in native kidneys have proved the importance of epithelial-mesenchymal transition (EMT) in tubulo-interstitial fibrosis development [12]. Phenotypic changes that appear throughout the fibrosis development are characterized by the differentiation into myofibroblasts that is considered one of the essential early cellular events that initiates the development of organ fibrosis and loss of cytokeratins [12, 13]. Given the complexity of cell lines, fibroblasts renal origin remains controversial, currently the most common theory prioritizes local interstitial cells but other authors claim that migrated leukocytes derived from local fibroblasts may be responsible for the fibroblasts expression and extracellular matrix deposition [14, 15].

In recent studies performed in cultured cells and in experimental models of nephropathy has been proposed that tubular epithelial cells through epithelial-mesenchymal transition become collagen-producing cells [15]. According to this hypothesis, epithelial cells should undergo several stages such as proliferation and phenotypic changes to eventually synthesize extracellular matrix. Thus, after injury to tubular epithelial cells various processes initiate such as cell death via apoptosis or necrosis, initial morphological characteristics recovery or the expression of mesenchymal characteristics as myofibroblasts presence and loss of epithelial phenotype characteristics.

Aim of our study was to evaluate the importance of interstitial infiltration, EMT and tubulo-interstitial damage in chronic kidney lesions description in a population with native kidney biopsy.

Materials and Methods

The study was performed in kidney tissues obtained from 16 patients that underwent a biopsy procedure during 2009–2011. The protocol was approved by
the Ethic Committee of “Carol Davila” Nephrology Hospital and University of Medicine and Pharmacy of Craiova, and was conducted according to the ethical principles of the Helsinki Convention.

Medical history and biochemical characteristics during the hospitalization period were recorded from the patient files as well as the renal function in prior examinations. The informed consent for using confidential data was obtained from each patient. The biopsies were performed according to the approved local procedure, with a 16 Gauge needle and under ultrasound control. The biopsies were performed on various clinical indications, which were as follows: isolated proteinuria; isolated reduced renal function (RF), assessed by increased serum creatinine >25% compared with previous presentations; association of both proteinuria and reduced RF. Estimated glomerular filtration rate (eGFR) was determined using the Modification of Diet in Renal Disease (MDRD) formula [16]. Renal function evaluation investigated the following parameters: serum creatinine and eGFR, proteinuria / 24 hrs., during the hospitalization period and in previous ambulatory presentations. Chronic kidney disease was defined as the reduction in glomerular filtration rate calculated with the MDRD formula, according to current diagnosis procedures [17].

The exclusion criteria represented biopsies with acute injury or active proliferative lesions such as: proliferative glomerulonephritis, interstitial nephritis, acute tubular necrosis, thrombotic microangiopathies or vasculitis.

Tissue samples, fixed in 4% buffered paraformaldehyde and embedded in paraffin, were processed following the international guidelines and examined by light microscopy and immunostaining. Material remaining after routine light microscopy was stained for mesenchymal markers such as vimentin, epithelial markers such as cytokeratins and E-cadherin.

The immunohistochemical processing was made on sections using the LSAB+ System-HRP (Dako). The antibodies used, clone, dilution and antigen retrieval are shown in Table 1.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin</td>
<td>V9</td>
<td>1:50</td>
<td></td>
</tr>
<tr>
<td>E-cadherin</td>
<td>NCH-38</td>
<td>1:50</td>
<td>Citrate buffer, pH 6</td>
</tr>
<tr>
<td>Cytokeratins</td>
<td>MNF116</td>
<td>1:75</td>
<td></td>
</tr>
</tbody>
</table>

Images were acquired by a Nikon Eclipse (Nikon, Apidrag, Romania) microscope, equipped with a 5-megapixels CCD digital videocamera. Consecutive images, avoiding glomeruli, were recorded from the whole renal biopsy tissue at ×200 magnification. An optical threshold followed by filtering was applied to all images, and the staining for both epithelial and mesenchymal markers was calculated as percentage of the total scanned area.

Statistical analysis was performed using the SPSS 10 software. In all statistical analysis significance was set for p-values <0.05. Continuous variables were expressed as average values ± SD. Differences among percentages were determined by χ² test and Fisher exact test.

Results

In this study, there were investigated the clinical and pathological characteristics of 16 patients that underwent a biopsy procedure for clinical indication. The most common indication for biopsy was represented of decreased RF assessed by increased serum creatinine >25% compared with previous presentations (63%) followed by association of both proteinuria and reduced RF (27%) and isolated proteinuria (10%). The clinical and biochemical characteristics of the population at the biopsy moment are resumed in Table 2. It was observed a decrease in creatinine and proteinuria levels in different time points after the renal biopsy (p<0.05).

<table>
<thead>
<tr>
<th>Variable Value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>Age [years]</td>
</tr>
<tr>
<td>Sex [% males]</td>
</tr>
<tr>
<td>Diabetes [%]</td>
</tr>
<tr>
<td>Creatinine [mg/dL]</td>
</tr>
<tr>
<td>Creatinine at follow-up [mg/dL]</td>
</tr>
<tr>
<td>eGFR [mL/min./1.73 m²]</td>
</tr>
<tr>
<td>Proteinuria / 24 hrs. [g/24 hrs.]</td>
</tr>
<tr>
<td>Proteinuria / 24 hrs. at follow-up [g/24 hrs.]</td>
</tr>
<tr>
<td>Antihypertensive therapy [%]</td>
</tr>
<tr>
<td>Steroid therapy [%]</td>
</tr>
</tbody>
</table>

¹Some values are expressed as mean ± std. dev.

The clinical and biochemical characteristics evaluated during the hospitalization period showed the prevalence of multiple cardiovascular risk factors such as arterial hypertension (68%), abnormal blood lipid levels (32%), obesity (27%), diabetes (19%). Chronic kidney disease stage III was the most common stadiization diagnosis (56%), followed by chronic kidney disease stage II (18%) and stage I (9%).

The most common histopathological diagnosis was primary and second forms of focal segmental glomerulosclerosis (n=5, 45%), followed by membranous nephropathy (n=4, 36%), diabetic nephropathy (n=3, 27%), primary chronic tubulo-interstitial nephritis (n=2, 18%), amyloidosis / monoclonal immunoglobulin deposition disease (n=1, 9%) and minimal change disease (n=1, 9%).

For statistical purposes, we evaluated the degree of glomerular sclerosis, tubular atrophy and interstitial fibrosis according to the following criteria: glomerular sclerosis was evaluated as the percentage of sclerotic glomeruli in each sample, conversely, tubular atrophy and interstitial fibrosis were qualitatively graded using a scale of 0–3 (0 – no pathology; 1 – <25% involvement, mild; 2 – 25–50% involvement, moderate; and 3 – >50% involvement, severe).

As regards the different types of histological lesions, glomerulosclerosis was present in 67% of biopsy specimens, involving 13±11% of glomeruli. A severe degree of tubular atrophy and interstitial fibrosis were observed in 19% and 23% of biopsy specimens, respectively. There were not observed any differentiations between histological characteristics and chronic kidney disease stages in our group.
Interrelations between histological and immunohistochemical characteristics are shown in Table 3.

**Table 3 - Interrelations between histological and immunohistochemical parameters**

<table>
<thead>
<tr>
<th>Group</th>
<th>Vimentin</th>
<th>Cytokeratin</th>
<th>E-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial fibrosis</td>
<td>34.5±22.1</td>
<td>10.7±8.4</td>
<td>7.2±4.3</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>22.5±11.4</td>
<td>3.1±2.7</td>
<td>5.9±6.7</td>
</tr>
<tr>
<td>Glomerular sclerosis</td>
<td>11.2±10.8</td>
<td>7.4±6.9</td>
<td>17.4±8.1</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± std. dev.*

In normal kidney, vimentin is present in glomeruli, arterioles, and interstitial fibroblasts but is not found in the tubule. Tubulo-interstitial expression of vimentin, expressed as percentage area was observed in this study to be within the range 0.2–79% with a mean of 27±5.9%. A percentage of 34% of the cases showed vimentin expression more than 27%. In the present study, however, when vimentin was found outside of usual areas, in tubules and the interstitial area it was associated with higher values of creatinine levels (*p*<0.001), this particularly expression was greater in areas with chronic tubulo-interstitial injury.

Regarding the particularity of E-cadherin and cytokeratins expression as epithelial markers, the association between the absence of epithelial markers expression and presence of vimentin as a mesenchymal marker was observed in this study (Figure 1, A–E).

In a multiple regression analyze the major determinants for the decrease in RF evaluated as increased serum creatinine >25% at biopsy moment was vimentin expression (*p*=0.003) and the association of more than two cardiovascular risk factors (*p*<0.001).

**Discussion**

The degree of interstitial remodeling, especially interstitial fibrosis, in native kidney biopsies has been shown to be associated with poor renal outcome defined by progression to end stage renal disease and initiation of renal substitution therapy [18, 19]. A major controversy regarding the use of surveillance biopsies in routine clinical practice relates to the clinical utility of the information derived from these tissues [20], in our study the decision for performing a renal biopsy was made based only on the clinical and biochemical patients outcome.

Fibroblasts cells are principal effectors of fibrogenesis, initially these cells were identified by light microscopy and electron microscopy based on morphological characteristics [21–24]. Epithelial-mesenchymal transition in renal fibrosis was first demonstrated by Strutz et al. [7] in a paper published almost more of a decade ago. Using fibroblast-specific protein (Fsp1) as a marker, these authors showed that tubular epithelial cells could express Fsp1, a calcium binding protein associated with the cytoskeleton, normally expressed in the fibroblasts, in an experimental animal model of nephropathy with tubular basement membrane antibodies. They postulated the presence of the epithelial-mesenchymal transition that could serve as a new way of generating fibroblasts in kidney fibrotic processes.

Subsequent studies in Lan’s group [25] provide evidence for the existence of morphological and phenotypic EMT in renal tissue remaining after 5/6 nephrectomy. Nadasdy Y et al. [6] reported that isolated cells or small groups of cells showing poorly organized epithelial markers could still be found in the interstitial renal biopsy samples from patients with end-stage kidney disease, according to the notion of epithelial-mesenchymal transition. Iwano M et al. [26] have provided convincing evidence showing that interstitial fibroblasts may be derived from renal tubular epithelium after injury cause obstruction. Epithelial cells showed marked morphological abnormalities, became disorganized and expressed fibroblast-transforming markers as Fsp1 and HSP47 [26].

Recent studies on biopsy specimens provide new arguments in EMT characteristics that seems to play an important role in progressive renal fibrosis, tubular epithelial cells that undergo phenotypic changes, as demonstrated by the expression of “de novo” myofibroblasts and cytokeratins loss [12].

In our study was observed a specific expression of epithelial markers as E-cadherin and cytokeratins intermediate filament loss associated the presence of vimentin as a mesenchymal marker. Moreover, in our study only vimentin expression was observed to be associated with creatinine level and renal filtration rate at the biopsy moment. Our finding is in agreement with previous studies that underline the specific vimentin expression in native kidney biopsies with fibrotic lesions [27, 28].

In our study, mesenchymal phenotype characteristics in tubular cells was associated with renal function reduction as well as with the interstitial fibrosis score independently of histological diagnosis confirming the hypothesis that tubulo-interstitial lesions can be important in chronic kidney progression [29–32]. Our study did not evaluate the epithelial to mesenchymal trans-differentiation process, we resumed at describing the relation observed in epithelial and mesenchymal markers expression.

Our study has some limitations. This is a retrospective study and the patients were included at different time points, it would be interesting to investigate in future study the presence of vimentin and the expression of epithelial markers in sequential protocol biopsies and to analyze whether they could be used as an earlier marker and could predict the development of tubular and glomerular proteinuria, rises in plasma creatinine and renal outcome.

**Conclusions**

This study demonstrated that introduction of vimentin as new marker in histopathological diagnosis of native kidney biopsy, in association with epithelial markers, can be important in tubulo-interstitial damage characterization. Both epithelial and mesenchymal characteristics can be specific markers in renal function evaluation, even in early stages of chronic kidney dysfunction.
Acknowledgments

This paper has been supported within the project entitled “Doctorate an Attractive Research Career”, contract number POSDRU/ID/88/1.5/S/52826 co-financed by European Social Fund through Sectoral Operational Programme for Human Resources Development 2007–2013.
References


Corresponding author
Ofelia Jercan, MD, PhD student, University of Medicine and Pharmacy of Craiova, 2–4 Petru Rareş Street, 200349 Craiova, Romania; e-mail: ofeliajercan@gmail.com

Received: June 16th, 2012    Accepted: November 9th, 2012