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HISTOMORPHOMETRIC ANALYSIS OF FIBROSIS IN THE RENAL INTERSTITIAL COMPARTMENT

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A b s t r a c t: The interstitium is the extravascular intertubular space of the renal parenchyma, which provides structural support to the functional renal units and is included at the same time in nearly all renal functions. Alterations to this renal compartment have been found in almost all glomerular diseases. During the last thirty years the studies of a few groups of investigators have shown that the degree of the renal dysfunction is strongly correlated with the changes in the tubulointerstitial compartment.

We made a morphometric study of a group of 10 renal biopsies, previously diagnosed as IgA nephropathy or membranoproliferative glomerulonephritis. For morphometric analysis we made colour extraction of the interstitial area on tissue sections stained with trichrom Masson using the LUCIA M–NIKON image analysing system with integrated software for statistical analysis of the data. We measured the surface of the marked fields and the results were expressed as a percentage of the total scanned area. The results were correlated with the serum creatinine at the time of biopsy.

We found fibrosis occupying more than 10% of the tubulointerstitial surface in all 10 patients. Six of them had a moderate level of fibrosis, occupying more that 20% of the tubulointerstitial space. The statistical analysis of these results showed a significant correlation between the degree of the interstitial expansion and the serum creatinine.

The results showing the correlation between these parameters will enable the quantitative histological analyses to be included in the process of the nephropathological diagnosis in order to evaluate the histological risk factors in glomerular diseases.

Key words: tubulointerstitium, fibrosis, serum creatinine, morphometry.

Introduction

The interstitium is the extravascular intertubular space of the renal parenchyma comprised of cellular elements and extracellular substances. From a morphological and functional point of view, the renal interstitium is divided into cortical and medullar interstitial compartments. The relative interstitial volume is different in various parts of the kidney and it increases going from the cortex to the medulla. According to the morphometric measurements done on experimental animals, the interstitial volume comprises 7–9% of the cortical compartment without the periarteriolar connective tissue and 30–40% of the inner medulla [1].

The interstitium with its elements structurally supports the functional renal units, but that is not its only function. It is involved in mediation and regulation of almost all exchange processes between the tubular and vascular elements of the renal parenchyma; it influences the glomerular filtration through the tubuloglomerular feedback mechanism; it influences the growth and differentiation of the parenchymal cells; it is involved in the production and regulation of the extracellular matrix; it influences the function of the peritubular microvasculature; the interstitial cells produce a variety of local and systemic hormones.

The pathological changes in the tubulointerstitial renal compartment responsible for the progression of kidney diseases with various ethiologies are called *tubulointerstitionephritides* (TIN). Histologically, these changes are presented with inflammatory infiltrate, oedema or fibrosis of the interstitium and tubular atrophy. On the basis of the existence of association between the pathological changes in the tubulointerstitial compartment and changes in other renal segments, the TIN is classified into *primary* (where pathological changes are concentrated in this renal compartment, without any previously existing disease in the other renal segments) and *secondary* (which is associated with diseases primary localised in the glomeruli, but also vascular, cystic, obstructive and other diseases).

Until the end of the sixties, the changes in the tubulointerstitial compartment of the kidney accompanying the glomerular diseases were thought to be

secondary manifestations without any clinical importance. During the last thirty years a few groups of investigators have focused their research on these changes, being able to show a strong correlation between the degree of the renal dysfunction and the changes in the tubulointerstitial compartment, as opposed to the weak correlation with the degree of the glomerular abnormalities [2, 3, 4].

Changes in this renal compartment have been found in almost all glomerular diseases – with or without the presence of glomerular inflammatory infiltrate (membranous nephropathy, membranoproliferative glomerulonephritis, focal-segmental glomerulosclerosis, IgA nephropathy, diffuse proliferative glomerulonephritis), with the exception of the minimal change nephropathy, as well as some systemic diseases with renal manifestations (lupus nephritis, diabetic nephropathy, etc.) [5, 6].

Material and methods

Material

The study was done at the Institute of Pathology, Faculty of Medicine in Skopje, in collaboration with the Department of Nephrology, Clinical Centre, Skopje, and the Macedonian Academy of Arts and Sciences. The study included morphometrical analyses of 10 renal biopsies, diagnosed previously with standard histological and immunofluorescent analyses as IgA nephropathy (IgAN) or membranoproliferative glomerulonephritis (MPGN). For the study we used biopsies analysed in the period between 2000 and 2004. The final analyses included only biopsies from those patients for whom laboratory data for the estimation of the renal function at the time of biopsy were available.

Methods

We used sections from biopsies previously being treated with standard histological technique. The sections with a thickness of 4–5 μ were stained with trichrom Masson, a histochemical staining that highlights the collagenous tissue (Fig. 1).

For the morphometrical analyses, in order to evaluate the extent of the fibrosis, we used the LUCIA M–NIKON image analysing system with integrated software for statistical analysis of the gathered data.

Using the image analysing system, we made a colour extraction of the cortical interstitial compartment, avoiding the perivascular and the subcapsular space (Fig. 2). The measurements were made on 10 high-power fields (x400) on each biopsy cylinder. We repeated the measurements ten times for each visual field in order to avoid possible errors due to subjectivity in the colour

extraction. The surface of the marked fields was measured and the results were expressed as a percentage of the total scanned area. After that, we calculated the mean values with standard deviation, minimal and maximal values with the integrated software. Finally, we calculated the mean values for the extent of the collagenous tissue in the entire renal biopsy cylinder.

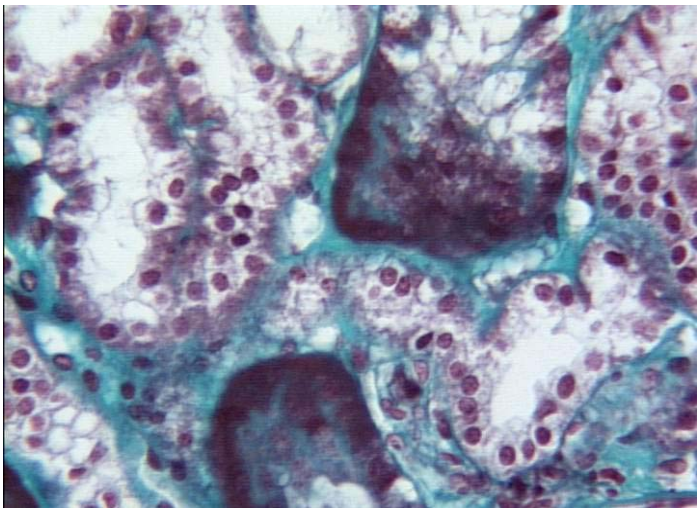


Figure 1 – *Trichrom Masson, x 400*

Слика 1 – *Trichrom Masson, x 400*

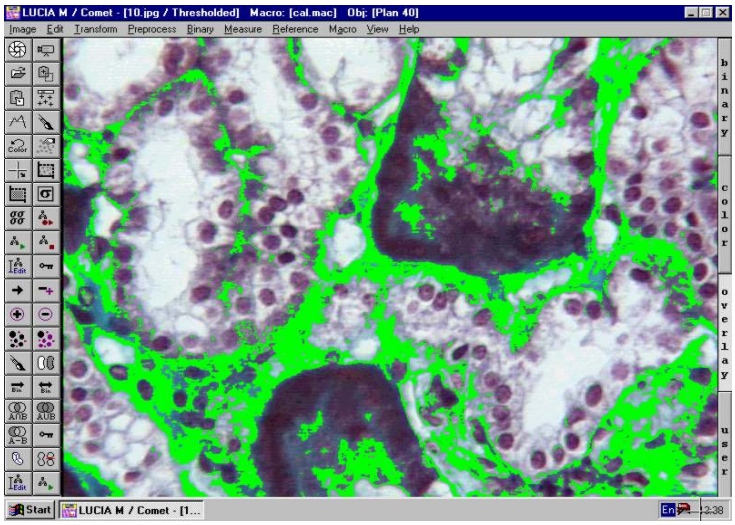


Figure 2 – *Colour extraction of the collagenous tissue*

Слика 2 – *Колор екстракција на кологено ткиво*

The results were statistically correlated with the serum creatinine levels at the time of the biopsy using the STATISTICA commercial statistical software for Windows.

Results

The ten patients whose biopsies were analysed were between 17 and 64 years of age (mean 40.2), all of them being male.

The clinical and demographic data of the patients, as well as the results from the histopathological and morphometrical analysis, are summarized in Table 1.

Table 1 – Табела 1

Clinical and demographic data of the patients and histological and morphometrical findings of their biopsies

Клинички и демографски податоци за пациентите и хистолошки и морфометриски наоди од нивните биопсии

Patient	Sex	Age	Histopathological diagnosis	Serum creatinin μmol/L	Interstitial fibrosis (%)
1	M	45	IgAN	134	22.2
2	M	42	IgAN	139	23.6
3	M	20	IgAN	91	14.7
4	M	17	IgAN	52	17.3
5	M	29	MPGN	91	23.4
6	M	64	IgAN	189	28.9
7	M	46	IgAN	171	24.3
8	M	56	IgAN	74	19.7
9	M	49	MPGN	134	32
10	M	34	IgAN	110	23

Histopathologically, 8 of the patients had IgAN and 2 patients had MPGN.

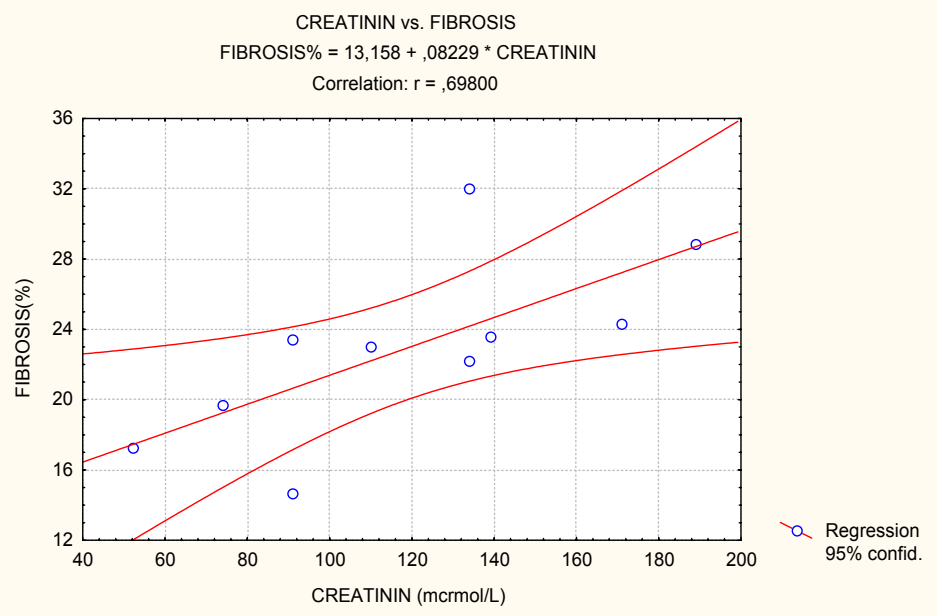
Clinically, the values of the serum creatinine were between 52 and 189 μmol/L (normal values 45–109 μmol/L).

The morphometrical analyses showed an extent of interstitial fibrosis between 13 and 32%. Four of the biopsies presented a low extent of fibrosis

(10–20%) and 6 biopsies had interstitial fibrosis between 20 and 40%, meaning that most of the patients had a moderate extent of interstitial fibrosis in their renal biopsies.

The statistical analysis of the data showed a strong correlation (Pearson Product-moment correlation r = 0,7, p < 0,05) between the serum creatinine level at the time of the biopsy and the extent of the interstitial fibrosis (see Graph 1).

Graph 1 – Графикон 1



Correlation between the serum creatinine level at the time of the biopsy and the extent of the interstitial fibrosis

Корелација меѓу нивото на креатининот во серумот во времето на изведување на бубрежната биопсија и степенот на интерстицијална фиброза

Discussion

All progressive renal diseases finally result in a process of destructive fibrosis in the tubulointerstitial compartment [7, 8]. This is a result of various complicated interactions among the cells of the inflammatory infiltrate, the

damaged tubular epithelial cells and the resident interstitial fibroblasts, under the influence of the numerous mediators that modulate the process of the final collagen production [9].

The histomorphometrical analyses performed in many studies showed a strong correlation between the intensity of the interstitial inflammatory infiltrate and the fibrosis on the one, and the glomerular filtration rate (GFR) and the serum creatinine level on the other hand [4, 10, 11]. The results of many investigations show that these parameters are useful in the estimation of renal survival, not overlooking the degree of the glomerular changes [7, 12].

The fact that the values of serum creatinine, as one of the parameters for estimating renal function, are increased in those patients with glomerular diseases that also manifest marked interstitial changes, can be explained by the expansion of the interstitial space with consequent reduction of the number and area of the postglomerular blood vessels, thus reducing the glomerular blood flow. Besides that, the expansion of the cortical interstitium on the one, and the vasoactive effects of numerous mediators produced by the inflammatory cells and the damaged tubular epithelial cells on the other hand, result in deranged functional and nutritive vascularization of the tubular epithelium. These mechanisms reduce its resorptive and secretory function. The reduced resorption of sodium followed by an increase of its concentration in the distal segments of the tubular system activates the tubuloglomerular feedback and reduces the glomerular blood flow and GFR [7].

Our investigation confirmed the other researchers' findings of the existence of fibrosis in the renal cortical interstitium in patients with glomerular diseases, as well as its correlation with the values of serum creatinine as an indicator of the renal function. The biopsies from all ten patients included in our series showed interstitial fibrosis greater than 10% which, according to the literature data based on stereological measurements, is considered to be the upper limit for the normally present collagenous tissue in the interstitium. Besides that, in 6 of them the extent of the fibrosis was of moderate grade, above 20%. The statistical analysis of the results showed a strong positive correlation ($r = 0,7$) between the degree of the interstitial expansion and the serum creatinine level.

Conclusion

The above-stated findings lead to the conclusion that the employment of quantitative microscopic analyses (which are simplified with the development

of computer technology) in the process of the nephropathological diagnosis could be useful in the evaluation of the histological risk factors in glomerular diseases. The correlation between the clinical parameters for estimation of the renal function and the results of the histomorphological evaluation of the changes in the tubulointerstitial compartment give valid parameters for the prognosis of the progressive renal dysfunction.

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Резиме

ХИСТОМОРФОМЕТРИСКА АНАЛИЗА НА ФИБРОЗАТА ВО ИНТЕРСТИЦИЈАЛНИОТ БУБРЕЖЕН КОМПАРТМАН

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Интерстициумот е екстраваскуларниот интертубуларен простор на бубрежниот паренхим кој со своите градбени елементи обезбедува структурна поддршка на функционалните бубрежни единици, но истовремено учествува во речиси сите бубрежни функции. Промени во овој бубрежен компартман се најдени скоро кај сите видови гломеруларни заболувања. Во текот на последните триесетина години испитувањата на неколку групи истражувачи покажале дека степенот на бубрежната дисфункција силно позитивно корелира со промените во тубулоинтерстицијалниот компартман.

Направивме морфометриска студија на 10 бубрежни биопсии кај кои со претходна хистолошка анализа е дијагностицирана IgA нефропатија или мембранопролиферативен гломерулонефритис. За морфометриска анализа на пресеците боени со trichrom Masson е користен системот за анализа на сликата LUCIA M-NIKON со интегриран софтвер за статистичка обработка на добиените податоци. Мерена е површината на обележените полиња, а резултатите се изразени како процент од вкупната анализирана површина. Направена е корелација со вредностите на креатининот во серумот во време на изведувањето на бубрежната биопсија.

Кај сите 10 пациенти е најдена фиброза која зафаќа повеќе од 10% од тубулоинтерстицијалната површина. Кај 6 од нив фиброзата е од умерен степен и надминува 20%. Статистичката анализа на добиените резултати покажа значителна позитивна корелација меѓу степенот на интерстицијалната експанзија и концентрацијата на креатининот во серумот.

Добиените резултати за постоење на корелација меѓу овие параметри, отвораат простор за вклучување на квантитативните хистолошки анализи во нефропатолошката дијагностичка процедура со цел проценка на хистолошките фактори на ризик кај гломеруларните заболувања.

Клучни зборови: тубулоинтерстициум, фиброза, серумски креатинин, морфометрија