CONSTANŢA "OVIDIUS'' UNIVERSITY
DOCTORAL SCHOOL
FACULTY OF MEDICINE
DISCIPLINE: PATHOLOGY

" CLINICAL AND MORPHOLOGICAL STUDY OF NEPHROPATHIES IN PATIENTS WITH DIABETES MELLITUS"

ABSTRACT

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LIST OF ABREVIATIONS

ABC - avidin biotin complex

AGE – advanced glycated end products

ANCA – anti-neutrophil cytoplasmic antibodies

ARN-m – messager ribonucleic acid

ASLO – antistreptolizin O

AT1 – angiotensin 1

AVC- cerebrovascular accident

BPOC – chronic obstructive bronchopneumopathy

CSMH – stromal cells of hematogenic marrow

CTGF- conjunctiv tissue growth factor

DAB - diaminobenzidin

DCCT- The Diabetes Control and Complications Trial

DZ – diabetes mellitus

EGF –epidermal growth factor

FGF-2- fibroblastic growth factor 2

FSP1 - fibroblastic specific protein

GSFS – focal and segmental glomerulosclerosis

HbA1c - A1c glycated haemoglobin

HDL – hihg density lipoproteins

HE – hematoxilin-eozin

HTA – arterial hypertension

HVS – left ventricular hypertrophy

IHC – immunohistochemistry

IDF- International Diabetes Federation

IGF-1 –insulin-like growth factor 1

Ig - immunoglobulins

IL – interleukin

IMA –acute myocardial infarction

IMC – body mass index

IPP2K - inositol pentakifosfat-2 kinaze

LEI – internal elastic lamina

KCNQ-1 - voltage-gated potassium channel single-nucleotid-1

KIM-1 – kidney injury molecule-1

LDL- low density lipoproteins

LES - systemic lupus erythematosus

MDRD – Modification of Diet in Renal Disease

NGAL - neutrophil gelatinase-associated lipocalin

PAS – periodic acid Schiff

PBS - phosphate buffered saline

PCNA – proliferating cell nuclear antigen

RFG - glomerular filtration rate

α-SMA- alpha smooth muscle actin

STNH –natrium-hydrogen transport system

TBC - tuberculosis

TEM - epithelial-mesenchymal transdifferentiation

TGF-beta – transforming growth factor beta

UKPDS – The United Kingdom Prospective Diabetes Study

VEGF- vascular endothelial growth factor

VSH – erythrocyte sedimentation rate

ZO-3 - zona occludens-3

KEY WORDS: diabetes mellitus, diabetic kidney disease, nephroangiosclerosis, diabetic nephropathy, renal infarction, nondiabetic nephropathies

INTRODUCTION

Diabetic kidney disease is one of the major chronic complications of diabetes mellitus. Patients with diabetes mellitus, especially those with type 2 diabetes, may develop non-diabetic kidney damage, favored by a number of specific diabetic conditions. The differential diagnosis of diabetic kidney disease is sometimes difficult to achieve requiring, besides clinical and laboratory data, pathological data obtained by performing renal biopsy or retrospectively at autopsy. The correlation between these data may lead to important conclusions for clinical practice.

This study aims to analyze the renal lesions occurring in diabetic patients and to evaluate whether there are correlations between them and clinical and laboratory data.

The study is retrospective and was performed on 127 patients with diabetes mellitus who died during hospitalization in Constanta County Emergency Hospital between 2006 and 2010 and whom necropsy were performed in the Pathology Department of the same institution. Pathological data were analyzed in relation to clinical and laboratory data collected from the clinical medical records of those patients aiming for 5 years prior to death.

The first part of the thesis analyzes the data from the literature and the second part is devoted to personal contributions. The thesis had a total content of 225 pages, of which general part - 64 pages and special part - 161 pages and is illustrated with 20 tables, 24 graphs and 54 original microscopic images.

MATERIAL AND METHOD

1. The investigation analzyed in the study

The study aimed to analyze some clinical parameters: gender, age, personal history, type and duration of diabetes mellitus, comorbidities, risk factors, type of antidiabetic therapy and associated diseases. The study tried to establish a correlation between laboratory data (blood glucose, glycated hemoglobin, blood lipids, urea, creatinine, urinary albumin) and pathological data.

The correlation between the urinary albumin excretion and kidney disease was demonstrated and described [1]. In the study group, laboratory tests performed for albuminuria screening were, in order of frequency: urinalysis, albumin / creatinine ratio in morning urine and urinary albumin excretion in urine nocturnal or in 24 hours urine.

Another analyzed aspect was the association between diabetes mellitus, obesity [2] and dyslipidaemia, additional risk factors for the emergence or worsening of kidney disease progression in patients with diabetes. Independent risk factors for development of diabetic nephropathy also include, according to some studies [3] male gender and smoking status. In this study were evaluated parameters most commonly enrolled in clinical medical records of patients with diabetes mellitus.

2. Histopathological material processing

From the pathological samples of the 127 patients in the study group, in order to obtain microscopic preparations, were collected suggestive fragments of renal parenchyma and of evident renal lesions. The collected fragments had a maximum area of 4-5 cm ^{2} and a thickness of 3-5 mm, for optimum processing, and then, subsequent operations, required for obtaining a long-lasting preparation have been carried out. Finally, slides were prepared for microscopic examination.

Microscopic examination

At the microscopic examination, we followed two important aspects:

1) The microscopic diagnosis based on standard morphological methods using conventional stains;

2) The microscopic diagnosis based on the immunomorphological methods using monoclonal antibodies.

1. Microscopic diagnosis based on standard morphological methods

This category include both conventional usual staining (Hemalaun Eosin and van Gieson) and special staining designed to highlight with high accuracy certain conjunctive tissue components (Masson's trichrome staining, orcein staining) and basal membrane integrity (PAS method) [4].

2) Microscopic diagnosis based on immunomorphological methods

Immunohistochemical methods enable to visualize tissue/cell antigens, having a higher fidelity than conventional staining in identify certain types of tumor and tissues.

Immunohistochemistry is based on antigen-antibody reaction using the monoclonal antibodies obtained by fusion of a normal plasmocyte that produce an idiotypic antibody and a plasmocyte immortalized from a plasmacytoma (hybridoma technique). The technique used was ABC (avidin-biotin complex) three-stage indirect [5, 6].

In addition with the immunohistochemical assays, immunofluorescence assays were performed to highlight the glomerular renal changes in diabetes mellitus. The view of histological preparations was performed using a Nikon Eclipse E600 microscope generation and capture microscopic images was performed by a computer program - Lucia Net, endowed with the capacity of dimensioning various renal structures.

3. Statistical analysis methods

In the present study, for collection, analysis and interpretation of the results we used a card type that included the investigated parameters. The collected data were entered into a computer database built in Microsoft Access 2010, which allowed us easy access to stored information and transfer of those data in the analysis and statistical interpretation program.

Graphs and statistical calculations were performed with the program MS Excel 2010 and MS Graph. For comparison we used STUDENT and CHI 2 tests. Statistical distribution of various parameters in relation to the group was pursued calculating the following indicators of central tendency and dispersion: mean, mode, median, standard error and standard deviation [7, 8].

In tests comparing the environments we used the following interpretation of the outcome p, the test:

$p < 0.05$ - significant dependence between the two factors

$p < 0.01$ - high significant dependence

$p < 0.001$ – very high significant dependence

$p > 0.05$ - not significant dependence between the two factors

To confirm the null hypothesis we set a $p > 0.05$. For a $p < 0.05$ (or $p < 0.01$) the null hypothesis is disproven.

The difficulties of our study were due to its retrospective nature, which allowed processing only the data existing in the clinical medical records of the patients. In some cases, insufficient data were entered on the patients' files and we cannot establish

correlations with all wanted parameters. On the other hand, by its retrospective and non interventional character, this study provides important data from current clinical practice and lead to useful conclusions to improve the therapeutic approach of patients with diabetes mellitus.

4. The study group characteristics

The study group consisted of 127 patients with diabetes mellitus who subsequently died during hospitalization in Constanta County Emergency Hospital between 2006 and 2010. Necropsies were performed in the Department of Pathology and clinical and laboratory data were collected from the clinical medical records of these patients.

Data regarding: gender, age, urban/rural area distribution, type and duration of diabetes mellitus, antidiabetic medication, causes of death and associated pathology were analyzed. Clinical and laboratory data were correlated with histopathological data.

Patients in the study group were aged between 33 and 88 years with a mean age of 66.72 years. No patient was under 20 years and between 20 and 40 years were only 4 patients. Most patients were between 60 and 80 years. This structure of the study group, with a high proportion of elderly patients, allows a better analysis of multiple risk factors.

The gender distribution in the study group was: 69 women (54.33 %) and 58 men (45.67%). Worldwide [9], the gender distribution of diabetes mellitus is relatively uniform, slightly higher in males (185 million cases in men versus 181 million cases in women, in 2011), but the study group consists predominantly in patients older than 65 years and life expectancy is higher among women than men.

Patients distribution relative to area of origin was as follows: 38 patients from rural areas (29.92 %) and 89 patients from urban areas (70.08 %).

EVALUATION OF THE STUDY GROUP RELATED TO CLINICAL ASPECTS

1. Distribution of the study group by the monitored parameters

The distribution of patients depending on the type of diabetes was as follows:

- Type 1 diabetes mellitus - 16 patients (12.6%)
- Type 2 diabetes mellitus - 108 patients (85.04%)

- Other types of diabetes - 3 patients (2.36 %)

Patient distribution related to time period from the diagnosis of diabetes mellitus was as follows: under 1 year - 17 patients (13.39 %), between 1 and 10 years - 38 patients (29.92 %), between 10 and 20 years - 53 patients (41.73 %) and over 20 years - 19 patients (14.96 %). 72 patients (56.69 %) in the study group had diabetes over 10 years old. The real duration of the disease progression is probably much higher, as suggested by the presence of chronic diabetic complications.

19 patients (14.96 %) had no antidiabetic therapy before admission, 54 patients (42.52 %) were receiving oral antidiabetic therapy, 25 patients (19.69%) combination therapy with insulin and oral agents and 29 patients (22.83 %) insulin therapy. During hospitalization, 108 patients received insulin treatment, while the rest remained on oral antidiabetic therapy.

The distribution according causes of death was as follows: cardiovascular diseases - 78 patients (61.42 %), infectious causes - 22 patients (17.32 %), end stage renal disease - 10 patients (7.87 %), acute diabetes complications - 4 patients (3.15 %), malignancies-3 patients (2.37%), other causes - 10 patients (7.87%). The main cause of death in the study group was represented, as expected, of cardiovascular diseases (Chart 8).

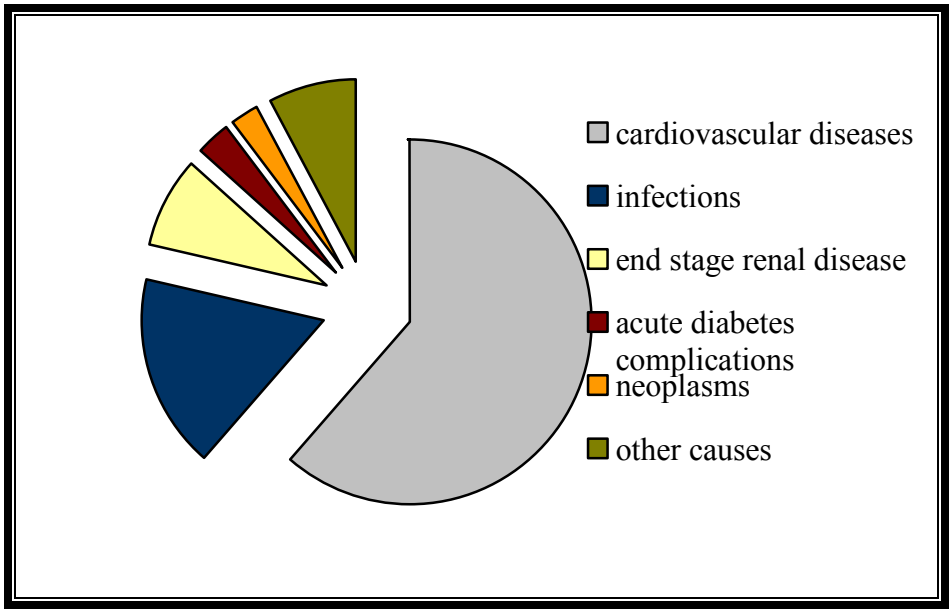

Chart 8 – Distribution according causes of death

2. Clinical characteristics in the study group

In most cases, diabetes-related pathology was multiple, especially in age groups over 65 years. The most common comorbidities were hypertension (105 patients, representing 82.7 %) and coronary ischemic heart disease (78 patients, representing 61.4%). Arterial hypertension induces renal vascular lesions that are difficult to be differentiated from those due to advanced age [10]. The characteristic histopathological renal lesions correlates with the presence of arterial hypertension are represented by nephroangiosclerosis.

Out of the study group, 103 patients (81.10 %) were overweight or obese. Of these, 34 (26.77 %) were overweight, 54 (42.52 %) had first degree obesity, 11 (8.66%) second degree obesity and 4 (3.15 %) third degree obesity.

3. Laboratory characteristics in the study group

Given that of the 127 patients in the study group, 105 (82.7%) were hypertensive and in 78 patients (61.4%) the cause of death was a cardiovascular event, was determined the frequency in which investigations were performed to qualitative and quantitative albuminuria measurement.

A total of 17 subjects had diabetes mellitus duration below one year. Of the 110 patients with diabetes mellitus duration more than one year, the assessment of albuminuria, in the range of five years followed, was performed in 101 patients (91.8 %), achieving a rate of 0.6 determinations / patient / year , respectively one investigation every 1.7 years. In the case of patients diagnosed with diabetic renal disease, microalbuminuria measurements were performed with a higher frequency, that means 1.4 measurements/ patient / year and one measurement every 0.7 years.

Determination of serum creatinine, which allows measurement of glomerular filtration rate based on MDRD formula, was performed with a frequency of 0.47 determinations / patient / year, respectively one investigation every 2.1 years. In patients diagnosed with diabetic renal disease, were made 1.2 investigations / patient / year, respectively one determination of creatinine every 0.8 years. Uric acid measurement was performed with lower frequency, which not allowed establishing correlations.

Glucose dosing frequency was difficult to assess because a significant percentage of patients self monitoring their blood sugar at home. HbA1c measurement was

performed with a frequency of 0.83 determinations/ patient/ year, respectively one measurement every 1.2 years.

Most patients had dyslipidemia (113 of 127 patients- 88.98 %). The association between diabetes mellitus, overweight, dyslipidemia and arterial hypertension explain the large share of cardiovascular causes of death in the study group. For an important number of patients, data on HDL- cholesterol level cannot be obtained, the literature data [11] indicating a correlation between low HDL-cholesterol and diabetic nephropathy.

EVALUATION OF THE STUDY GROUP RELATED TO ANATOMOPATHOLOGICAL FEATURES

1. Renal histopathological changes in the study group

Microscopic study of the cases allowed highlighting the varied pathological changes in all renal structures, classified as follows:

Glomerular lesions – various degrees of alterative, proliferative and/or exudative glomerulitis.

Tubular lesions: tubular atrophy,"endocrine" tubules, tubular hypertrophy, tubular dilatation, ischemic tubular changes, renal tubular cylinders.

Arterial lesions: subendothelial fibrosis of interlobar, arcuate and interlobular arteries, peritubular, interstitial fibrosis or hyperemia of capillaries and vasa recta.

Interstitial lesions - the common feature of all cases was the interstitial fibrosis and inflammation - was noted to be dominant chronic lymphocytes- monocytes- plasmacytes inflammation with diffuse or nodular form aggregates, sometimes with germinal center formation , similar to lymphoid follicles.

2. Pathological changes associated with diabetes mellitus and arterial hypertension

Specific pathological renal lesions in diabetes mellitus were first described by Kimmelstiel and Wilson in 1936 (diabetic glomerulosclerosis Kimmestiel -Wilson) [12]. From a morphological point of view, are several types of classical lesions highlighted by optical microscopy in diabetic nephropathy [13]: diffuse lesions, nodular lesions, exudative lesions, vascular and tubulointerstitial lesions. In the evolution occur nodular sclerosis and diffuse sclerosis changes.

In the study group, were revealed diverse renal lesions. Characteristic were the nodular lesions, sometimes combined with arteriolar capillary microaneurysms, exudative or hyaline lesions. Histopathological lesions ranged from diffuse forms of mesangial sclerosis to decrease in the mesangial matrix and uniform thickening of the capillary wall.

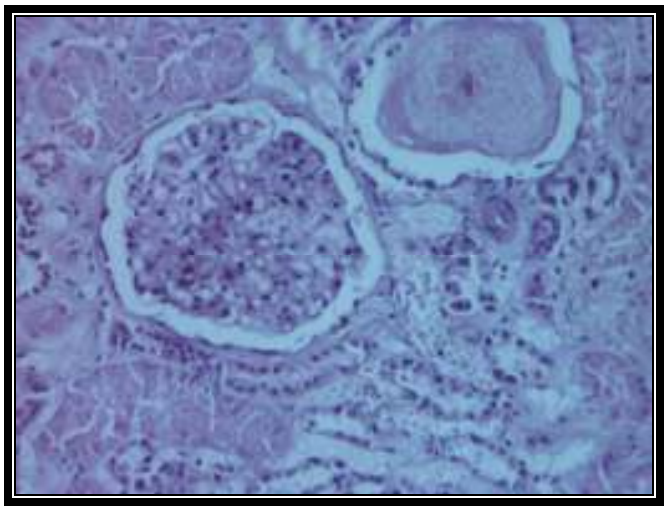

Figure 1. Glomerulosclerosis. Hyalinized glomerulus and fat load glomerulus. Periglomerular urinary tubules with granular vacuolar dystrophy phenomenon (HE stain, × 100) (case 7105/2007)

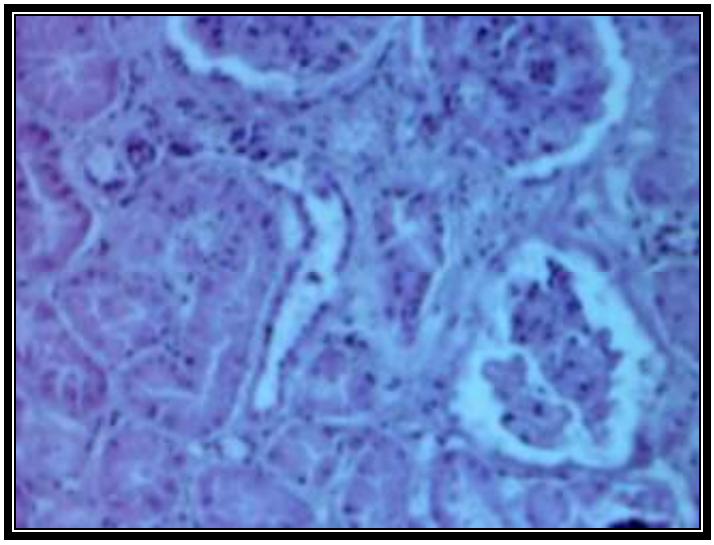

Figure 2. Renal glomerulus with fibrosis, sclerotic atrophy and ischemic glomerular lesions. The surrounding urinary tubules were cloudy intumescent (granular vacuolar dystrophy) (HE staining, x100) (case 9123/2008)

Hyaline exudative lesions occur more frequently in later stages of the disease and with various sites [14]. In the study group, arteriolar hyalinosis lesions were common,

especially given the frequent association between arterial hypertension, dyslipidemia and advanced age. PAS-positive arteriolar hyalinosis interested both afferent and efferent arterioles, sometimes causing muscle cell replacement in arteriolar walls. At the arteriolar vessels, in some cases was found a pronounced degree of hyaline intimal and media thickening, with endothelial cell proliferation, even in the absence of significant glomerular lesions.

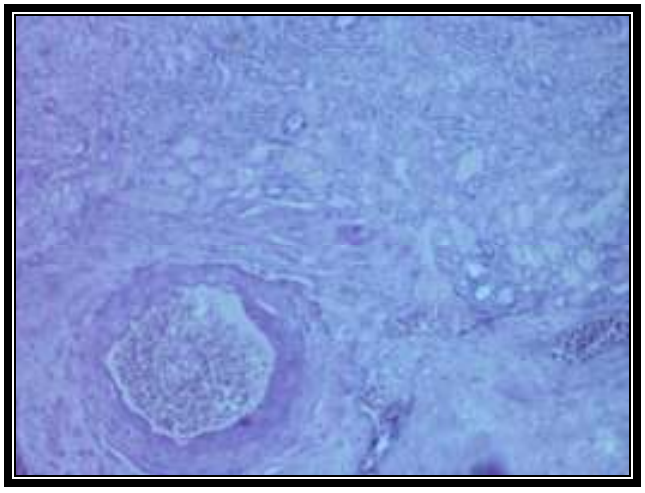

Figure 4. Hyalinized renal vessel. (hematoxylin-eozin, × 40) (case 1599/2008)

During aggravation, the glomerular lesions are associated with tubulointerstitial changes. Tubular pathological aspects encountered were diverse from normal tubules to atrophic tubules or atubular glomeruli. Tubular epithelial cells were infiltrated with glycogen and may show in the usual stains, the appearance of vacuolar dystrophy.

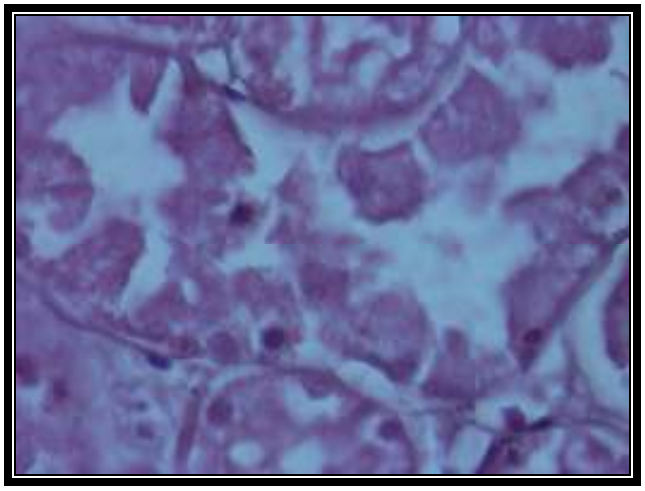

Figure 7. Urinary tubes with clear and turbid intumescent of intraluminal epithelium. (hematoxylin-eozin, x 400) (case 7327/2010).

The glomeruli had sclerosis and fibrosis with adjacent tubules atrophy, decreased endothelial cell size and lower tubular lumen. Tubular basement membrane can be thickened based on the degree of tubular atrophy.

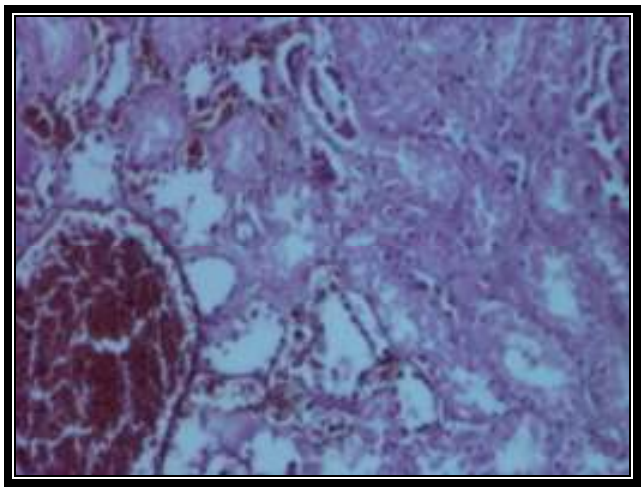

Figure 8. Granular vacuolar dystrophy aspects associated with glomerular sclerosis and renal arterial hyperemia (hematoxylin eosin, × 100) (case 2157/2009)

Peritubular blood vessels suffer hyaline arteriosclerosis and arteriolosclerosis phenomena, with varying degrees of thickening of vascular intima, a frequent early manifestation of diabetic kidney disease, especially when associated with hypertension and advanced age [15].

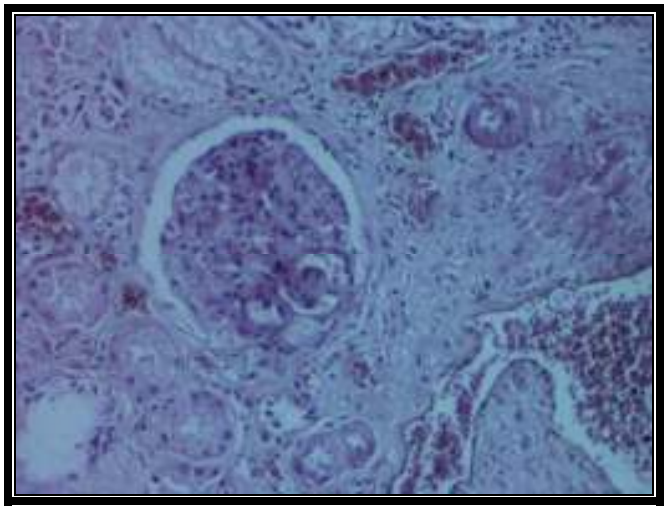

Figure 11. Association of tubuloglomerular renal lesions in diabetes mellitus: glomerular stasis, intraglomerular hyaline degeneration, urinary tubules with granular vacuolar dystrophy, hyalinosis and hyperemia of peritubular vessels (HE, x 100) (1235/2009)

3. Chronic renal stasis.

Chronic renal stasis occurs in chronic renal glomerular hypertension, associated with persistent and uncontrolled hyperglycemia and arterial hypertension. The kidney is enlarged and easy to be decapsulated, in newly diagnosed diabetic patients [1] the renal capsule being tense. Microscopy shows glomerular lesions. Renal glomerulus appears increased, with glomerular vessels dilated and filled with erythrocytes.

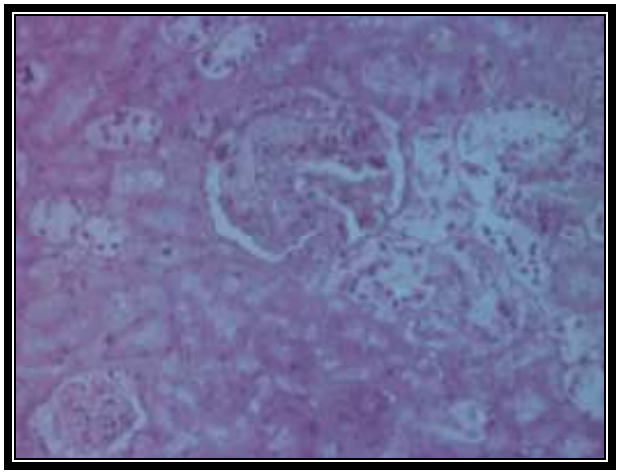

Figure 12. Chronic renal stasis. Dilated renal glomerulus, with reduced subcapsular space. Periglomerular urinary tubules are flattened. (H-E, x 100) (case 7105/2007)

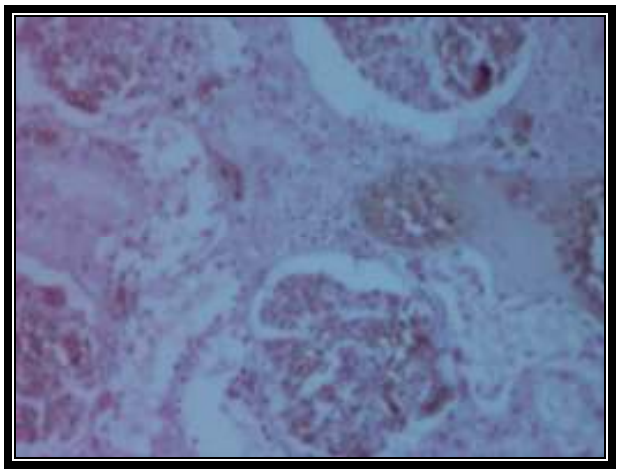

Figure 14. Chronic renal stasis (increased glomeruli, dilated capillary, intraglomerular fibrosis phenomena, extravasated erythrocytes and hemosiderin pigment) (HE, x 100) (case 5154/2008)

Peritubular arterial and arteriolar vessels are dilated, the hyperemia phenomena being present at renal parenchymal level. The characteristic aspect is represented by increased glomeruli with dilated vessels and intraglomerular fibrosis phenomena. Chronic

renal stasis can coexist with chronic renal glomerulosclerosis, nephroangiosclerosis, hyaline endothelial or mesangial deposits and glomerular basement membrane thickening [16].

4. Renal infarction

In the study group, 9 patients showed characteristic features of renal infarction. Macroscopic, renal capsule overlying is whitish, adherent to the kidney area. On section, the lesion is triangular, with the apex to the hilum and base to cortical.

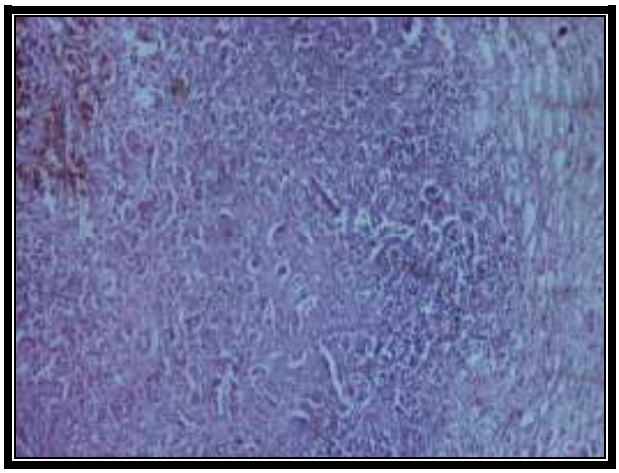

Figure 15. Renal infarction. Area of ischemic necrosis with debris uriniferi tubules, atrophied glomeruli, perinecrotic leukocyte inflammatory infiltrate (HE x 40) (case 7357/2006)

Microscopy highlights eosinophilic material composed of cellular, glomerular and tubular debris, while in the periphery is observed leukocyte inflammatory infiltrate.

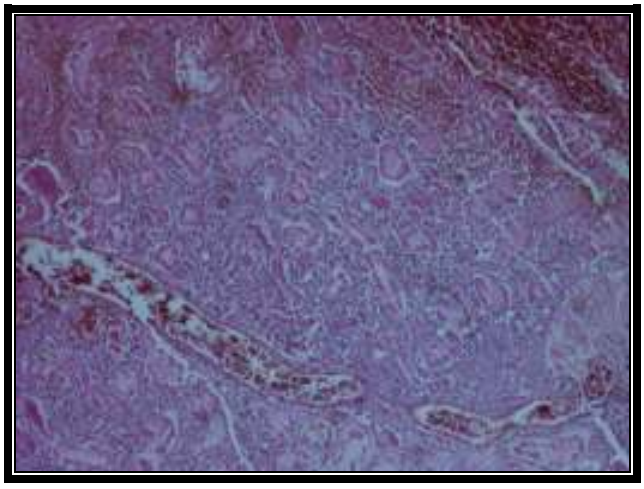

Figure 17. The hyperemic area surround the ischaemic necrosis area. The blood vessels are dilated to allow leukocyte infiltration around necrotic area (hematoxylin-eosin × 40) (case 6793/2008)

5. Selective presentation of some studied cases.

Case 1. Renal cortical with marked reduction of glomeruli and various lesions varying from the capillary loops collapse to the proliferative changes. Proximal convoluted tubules are crowded, with granular vacuolar dystrophy lesions. In renal medulla, the urinary tubules appear dilated with epithelial atrophy. Arcuate and interlobular arteries have marked narrowing of the lumen, to complete obliteration.

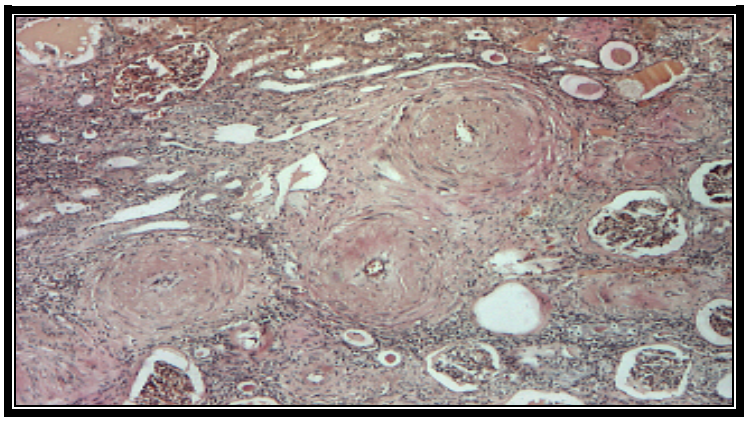

Fig.20 Arcuate arteries, with reduced lumen by proliferation of hyalinized smooth muscle fibers (van Gieson x 40) (case 9137/2006)

Case 3. Marked reduction in glomerular number, the remaining showing marked hyaline fibrosis that interest both capsule and capillary loop. Massive convoluted tubule multiplication with various aspects.

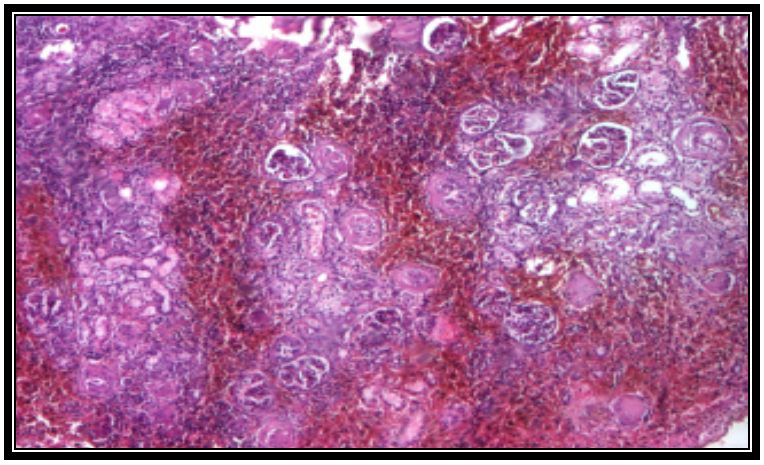

Fig.23. Glomeruli with marked subcapsular fibrosis, nephrocytic ballooning (HE x 40) (case 5579/2009)

Interstitial appears diffuse and sometimes nodular polymorphous inflammatory infiltrate in the hyperplastic collagen tissue, with marked proliferation of fibroblasts and neocapillary, generating aspect of granulation tissue. Interlobular, arcuate and

interlobular arteries had distorting arteritis lesions, marked thickening of the endothelium, with narrowing of the lumen, internal elastic lamina (LEI) endoreduplication.

Case 8. Marked reduction of glomerulotubular structures; length persistence of a small number of dilated convoluted tubules with nephrocytes atrophy; the renal glomeruli are absent.

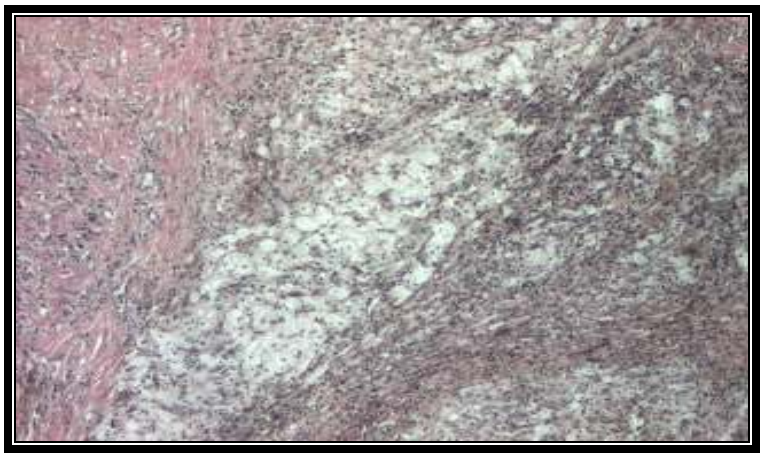

Fig.28. Granulomatous inflammation with foamy macrophages (van Gieson x 40) (case 3117/2009)

The interstitium shows intensive collagen and fibroblast proliferation, that surrounding extensive cellular areas with predominant foamy cytoplasm macrophages, accompanied by a neocapillary proliferation and associated with areas of parenchymal necrosis and acute purulent inflammation. The rare arcuate arteries have collapsed or dilated lumen.

Case 10. Glomeruli are numerically reduced to extinction; the remaining exhibit phenomena of obsolescence. Tubules were atrophic, with extensive extinction areas.

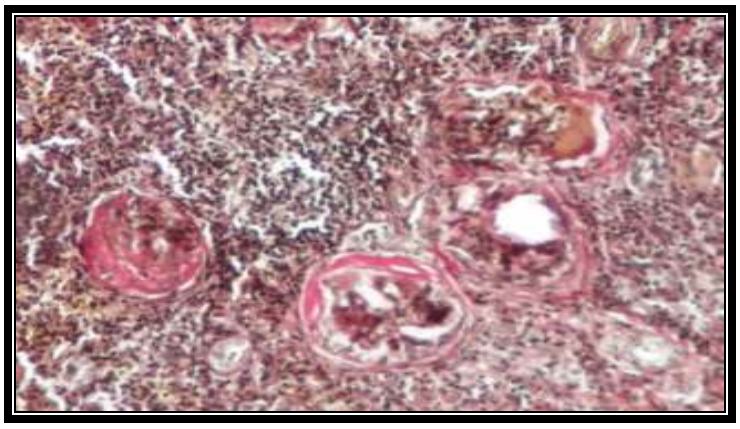

Fig.31. Glomeruli with partial or total obsolescence (van Gieson x 100) (case 3651/2010).

Diffuse interstitial fibroblastocollagen proliferation, abundant diffuse plasma cell infiltrate with lymphocytes forming focal lymphoid aggregates, edema and hyperemia. Interlobular arteries shows asymmetric narrowing of the lumen, subendothelial collagenization and edema, myocytic hyperplasia, internal elastic lamina strong corrugated, globular interlobular and arcuate arteries, without lumen, muscle tunica being edematous and hypertrophic.

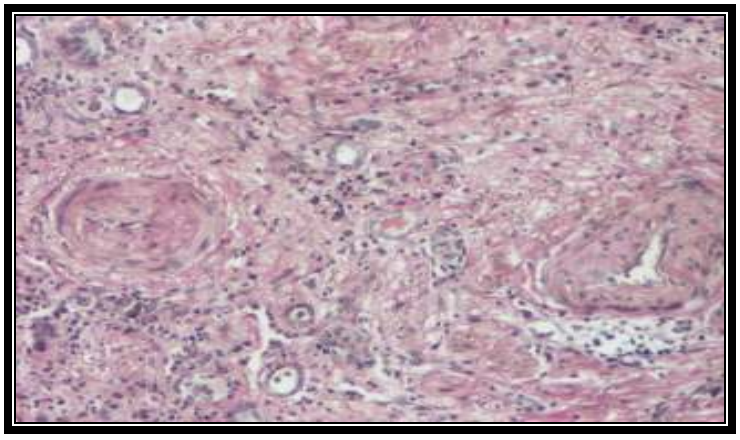

Fig.32. Arcuate arteries located in the cortex, with reduced lumen (van Gieson x 100) (case 3651/2010)

Case 12. Glomeruli with predominant obsolescents lesions. Convoluted tubules had atrophy and agglomeration areas, due to intense interstitial fibrosis. Tubular epithelium had vacuolated granular dystrophy and cystic dilatation. Interstitial, occurs collagen proliferation and marked inflammatory infiltrate.

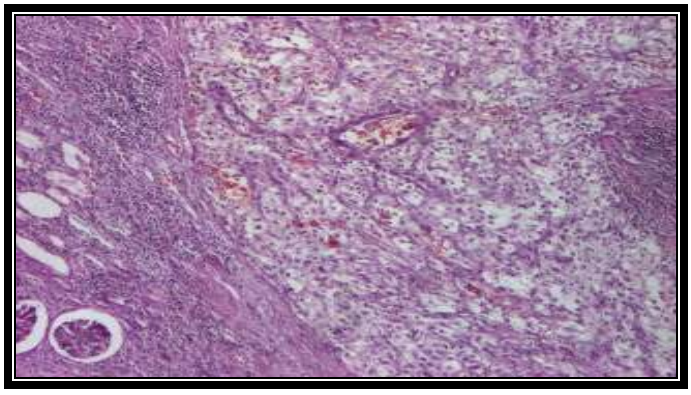

Fig.34. Proliferation of malignant clear cytoplasm cells (hex 40) (case 4371/2008).

Polygonal tumor cells with clear cytoplasm and round / angular nuclei, were highlighted centrally located and arranged as islands separate of fine connective septa,

accompanied by large areas of necrosis, hemorrhage and neo-vasa hyperplasia with thin walls. Arcuate and interlobular arteries had marked narrowing lumen.

Case 15. Afferent arterioles had hyperemia and extensive fibrinoid necrosis in the wall, degenerative changes in the vascular wall structures (nuclear pyknosis / cariorexis) and inflammatory phenomena. Vasa recta and peritubular capillaries appears hyperemics. The interstitium shows proliferation of fibrillar texture connective tissue, in contents of which are noted diffuse or nodular aggregates of lymphomonocytic inflammation and hemorrhage areas.

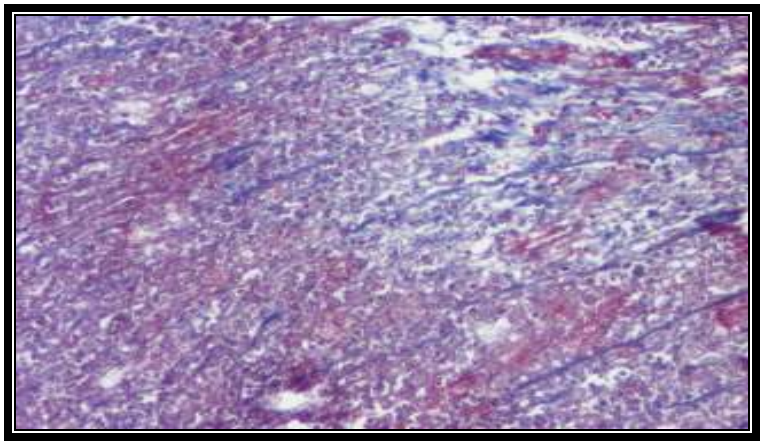

Fig.39. Interstitium with fibrillar texture areas and chronic inflammation (Masson x 40).Chronic pyelonephritis (case 6621/2007).

6. Immunohistochemical and immunofluorescence issues.

Rigorous microscopic preparations research highlights that the main histopathological feature is interstitial fibrosis. The tissue element that is responsible for the initiation and progression of fibrosis is the fibroblast, whose presence in the renal interstitium is controversial [17, 18]. Myofibroblasts are also involved in the kidney fibrogenethics events. It derived from fibroblast differentiation, migration of smooth muscle cells of the vessel wall, changing of tubular cells [18, 19].

Observation, confirmed by this study, that the tubulointerstitial fibrosis is predominantly peritubular suggests that tubular epithelial cells have an important pathogenic role. Some cells become apoptotic, contributing to tubular atrophy, and others begin to express cytokines, promoters for process of "epithelial- mesenchymal transition" or "transdifferentiation" (TEM) .

In order to assess TEM mechanism, a minimal panel of monoclonal antibodies was realized (Table 6).

Table No. 6. Monoclonal antibodies used for highlighting TEM

TYPE OF MARKER	**SPECIFIC MARKER**	**TYPE OF REACTION**	**PRODUCT SOURCE**
Cell proliferating markers	**PCNA** (proliferating cell nuclear antigen)	Nuclear reaction	DAKO Monoclonal Mouse antiProliferating Cell Nuclear Antigen, clone PC 10, isotype IgG2a, kappa
Epithelial markers	**cytokeratin** intermediate filament (fundamental marker of epithelial differentiation)	Intracytoplasmic reaction	DAKO Monoclonal Mouse anti-Human Cytokeratin, clone MNF 116, isotype IgG1, kappa
Mesenchymal markers	**vimentin** (component of the cytoskeleton)	Intracytoplasmic reaction	DAKO Monoclonal Mouse anti-Vimentin, clone Vim 3B4, isotype IgG2a, kappa
	type I collagen	Collagen fiber reaction	Chemicon International Mouse anti-Collagen type I Monoclonal Antibody MAB 3391
Muscular markers	**α-SMA** (microfilament composed of globular monomers)	Intracytoplasmic reaction	DAKO Monoclonal Mouse anti-Human Actin, clone 1A4, isotype IgG2a, kappa
Fibroblastic markers	**FSP1 – S100A4** (calcium-dependent heterotrimeric protein complex)	Intracytoplasmic and nuclear reaction	DAKO Rabbit anti-Human S100 A4

We have found varying degrees of positivity in tubular cells and fibroblasts for PCNA, showing a significant increase in their proliferative capacity and a decrease to absence of expression of epithelial markers in tubular epithelial cells.

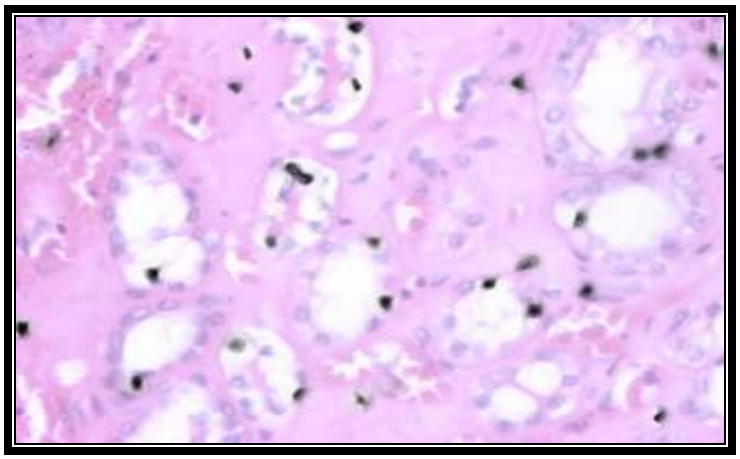

Fig. 41. PCNA ++ in tubular cells and interstitial fibroblasts (x100) (caz 5579/2009).

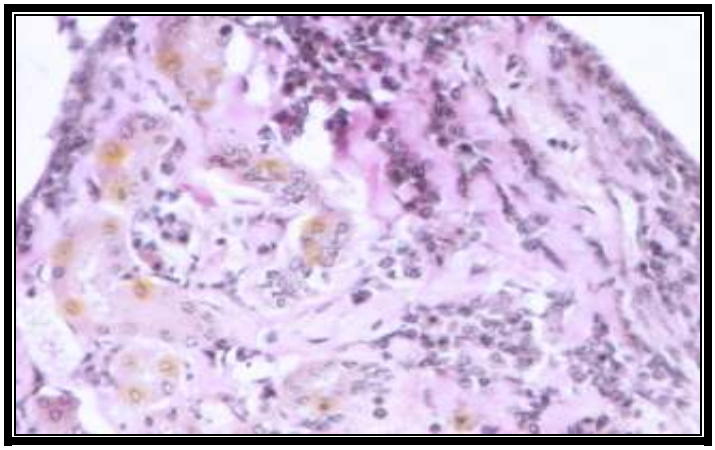

Fig.43. Low + reaction to cytokeratin in tubular epithelial cells (x 200) (case 2413/2009).

FSP1-S100A4 expression was present in all fibroblasts, latent or active (myofibroblasts). In the studied cases, which revealed fibrosis associated with persistent inflammation, polyclonal anti-FSP1 serum gave positive reactions in fibroblasts from interstitial collagen deposition areas and in tubular epithelium adjacent to inflammatory foci, composed mainly of lymphocytes, confirming the hypothesis that, in most cases, fibroblasts derived from local conversion of the epithelium by TEM. There have been detected tubular and interstitial cells expressing vimentin, but positive reaction to the monoclonal antibody did not have constant distribution. Number of tubular cells with mesenchymal phenotypic characters was directly proportional to the degree of interstitial fibrosis.

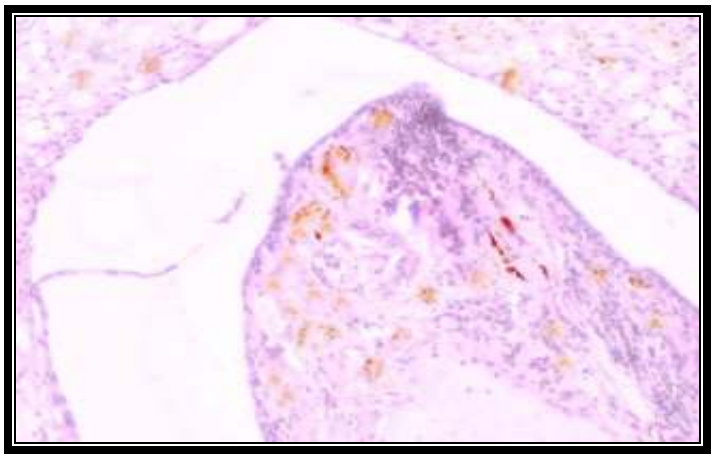

Fig. 45. Positive reaction to vimentin in tubular cells and fibroblasts, directly proportional over the degree of fibrosis (x 100) (case 4761/2010).

In addition, tubular and interstitial cells were positive for type I collagen, fiber type characteristic for mature fibrous tissue involved in tissue repair, whose expression was observed in direct proportion to the degree of fibrosis.

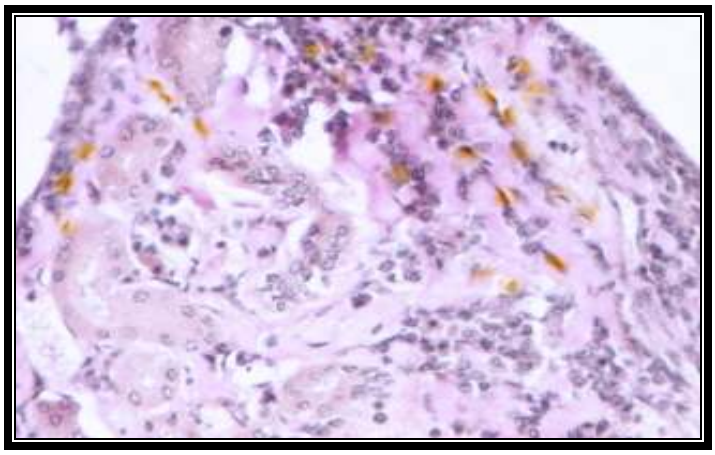

Fig. 46. Positive reaction to collagen I (x 200) (caz 6487/2008).

Identification of α-SMA, a marker of myofibroblasts, has been observed in the microscopic field, but has not been observed in all the fibroblasts FSP1 +, suggesting that smooth muscle actin is not a fibroblast specific marker.

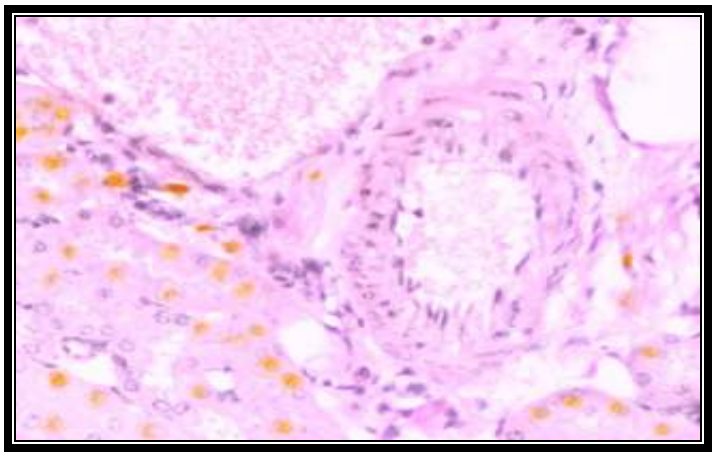

Fig. 47. + + Reaction at FSP1 in the interstitial fibroblasts and in the tubular cells (x 200) (case 3269/2010).

It was found the presence of hypertrophied epithelial cells that expressed both cytokeratin and α-SMA, which indicates that they were caught in an intermediate stage of the process of TEM. The qualitative analysis used the following rating scale: 0 – absence of α-SMA + cells from microscopic field, + - a single α-SMA + cell per microscopic field, + + - groups of α-SMA + cells in a microscopic field, + + + - groups of α-SMA + cells in several microscopic fields. There was a significant correlation between the number of tubular α-SMA + cells, accumulation of α-SMA + myofibroblasts and severity of tubulointerstitial fibrosis, knowing that fibrogenetic activity of myofibroblast is more intense than fibroblasts (Muller, 1992) [20].

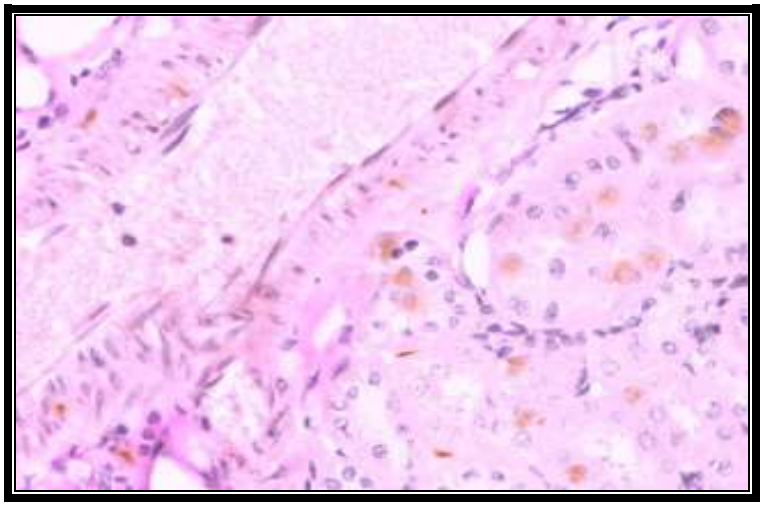

Figure 50 + Reaction to α-SMA in hypertrophied epithelial cells (x 200) (case 7463/2010).

Correlating all those data, results the idea that FSP1 - S100A4 is the most sensitive marker for the fibroblast of epithelial origin, being an important early indicator of epithelial-mesenchymal transition. The distribution of α -SMA in tubular and interstitial areas confirme the TEM appearance of myofibroblasts and the reaction α -SMA + in the arterial wall and interstitium raises the possibility of vascular myocyte transformation in myofibroblast.

The results of the immunohistochemical methods suggest that, by transition to mesenchymal phenotype, the tubular cells can produce, via myofibroblasts and fibroblasts, extracellular matrix proteins, directly intervening in the process of interstitial fibrosis. .

The immunofluorescence complete the classic morphological diagnosis and the immunohistochemical methods, by exploiting the characteristics of the antibodies used, that act, specifically on the complementarity principle, with some amino acid sequences of cellular protein structure, sequences known as epitopes. Antibodies used are grouped in isotypes - dimers to immunoglobulin A and pentamers to immunoglobulin M [21].

Our study detected a positive immunofluorescence for some types of glomerulopathies and glomerulonephritis associated with diabetes mellitus in the context of renal damage, detecting a positive complement fraction C3 in membranoproliferative glomerulopathies and in IgA glomerulonephritis Immunofluorescence also detected segmental glomerulosclerosis - positivity by applying IgG and lupus nephropathy - immunofluorescence positivity of C1 complement fraction.

ANATOMOCLINICAL CORRELATIONS IN THE STUDY GROUP

1. Anatomoclinical correlations in patients with diabetic kidney disease

The main objective of the study group was to identify diabetic nephropathy lesions and their correlation with clinical and paraclinical parameters.

Renal lesions characteristic for diabetic nephropahy were found in a total of 39 patients out of 127, representing a rate of 30.71 %. Of these patients, 11 had type 1 diabetes (9 with diabetes duration greater than 20 years, 1 patient had a duration of 16

years and 1 patient had inaugural diabetic coma). 28 patients had type 2 diabetes mellitus.

The patients were divided into two groups:

- Group A - with diabetic nephropathy
- Group B - without diabetic nephropathy

There were analyzed the two groups with respect to a series of clinical and paraclinical parameters. The gender distribution in the two groups is shown in Table 7.

Group	Men	Percent of men(%)	Women	Percent of women(%)	Total
Group A	13	33,33	26	66,67	39
Group B	45	51,14	43	48,86	88

Table 7– Distribution according gender in the two groups

In both groups predominated patients from urban areas, as is the entire group of patients studied.

The number of patients presenting histopathological lesions of diabetic nephropathy increased with age, the highest number of cases being in the age group between 70 and 79 years, the age group that included most patients (53 of total). In the age group over 80 years, the number of cases of diabetic nephropathy is lower, but this can be interpreted in the light of the small number of patients (9 in total).

Distribution of diabetic nephropathy was analyzed related to duration of diabetes (Table 8) and patients from the 2 groups were divided into 2 subgroups- with diabetes duration less than 10 years and more than 10 years.

Duration of diabetes mellitus	Number of patients group A	Percent of patients group A	Number of patients group B	Percent of patients group B
Under 10 ani	7	17,95%	45	51,14%
Over 10 ani	32	82,05%	43	48,86%

Table 8. Distribution of diabetes nephropathy according diabetes duration

An important issue studied was the correlation between diabetic renal disease and some clinical and paraclinical parameters. In type 1 diabetes, arterial hypertension is often a consequence of diabetic renal disease. In patients with type 2 diabetes mellitus, hypertension usually precedes the appearance of diabetes and diabetic renal disease.

In the studied patients, was took into account the average systolic and diastolic blood pressure recorded in the clinical medical records of those patients over a period of 5 years prior to death. The presence of diabetic nephropathy lesions correlated with systolic blood pressure values above 160 mmHg (p = 0.009) (Table 9)

Systolic blood pressure	Group A	Group B
Mean	158,97	149,15
Standard Error	3,08	1,91
Median	164	152
Mode	165	145
Standard Deviation	19,25	17,93
Kurtosis	0,75	-0,18
Skewness	-0,90	-0,57
Range	77	82
Minimum	110	110
Maximum	187	192
Count	39	88
Confidence Level (95.0%)	6,24	3,80
p	0,009	

Table 9 – Correlation between systolic blood pressure and diabetic nephropathy

Statistical analysis in the study group demonstrated that the presence of diabetic nephropathy correlated with diastolic blood pressure, with the most important correlation with diastolic blood pressure greater than 95 mmHg (p = 0.0007).

Diastolic blood pressure	Group A	Group B
Mean	90,85	83,75
Standard Error	1,70	1,05
Median	95	84
Mode	100	70
Standard Deviation	10,62	9,84
Kurtosis	-0,67	-1,05
Skewness	-0,64	0,20
Range	35	35
Minimum	70	70
Maximum	105	105
Count	39	88
Confidence level (95.0%)	3,44	2,08
p	0,0007	

Table 10- Correlation between diastolic blood pressure and diabetic nephropathy

It can be concluded that blood pressure values above 160/95 mmHg are a risk factor for the emergence or worsening of diabetic nephropathy lesions.

Persistent hyperglycemia and fluctuations in blood glucose and glycated hemoglobin levels are important in the development of diabetic renal disease [22, 23], pathogenic mechanisms being different. We analyzed whether these correlations are applied in the study group. Was chosen as the reference the average fasting glucose entered the clinical medical records of the patients in the past five years prior to death.

The patients were divided into 4 groups (table 11):

- With average fasting blood glucose greater than 100 mg /dl,
- With average fasting blood glucose between 100-129 mg /dl,
- With average fasting blood glucose between 130-159 mg/dl,
- With average fasting blood glucose over 160 mg / dl

Glycaemia	Groupe A	Percent groupe A	Groupe B	Percent groupe B
< 100 mg/dl	0	0,00 %	0	0,00 %
100 - 129 mg/dl	3	7,69 %	14	15,91 %
130 - 159 mg/dl	7	17,95 %	19	21,59 %
> 160 mg/dl	29	74,36 %	55	62,50 %

Table 11– Patients distribution according average of glycaemia

In patients with diabetic renal disease (group A), the presence of characteristic histological lesions correlated with the mean fasting glucose levels and the most important correlation was with the average blood glucose over 160 mg/dl (p = 0.001) (Table 12). Due to the low number of cases, data are not statistically significant.

Glycaemia	Group A	Group B
Mean	212,36	176,23
Standard Error	9,51	4,81
Median	211	168
Mode	158	163
Standard Deviation	59,37	45,09
Kurtosis	-1,16	-0,51
Skewness	0,15	0,56
Range	201	172
Minimum	123	107
Maximum	324	279
Count	39	88
Confidence Level (95.0%)	19,24	9,55
p	0,001	

Table nr.12 – Correlation between diabetic renal disease and mean fasting glycaemia

We analyzed the presence of diabetic renal disease lesions in relation to the average total cholesterol in the last 5 years prior to death.

The distribution of patients in both groups (with diabetic nephropathy - group A and without diabetic nephropathy - group B) based on mean value of cholesterol in serum are presented in Table 13. In the group A and in the group B, the largest number of patients had shown mean values of serum cholesterol above 200 mg / dl.

Total cholesterol (mg/dl)	Number of patients group A	Percent of patients group A	Number of patients group B	Percent of patients group B
< 200	6	15,38%	24	27,27%
200 - 250	18	46,15%	45	51,14%
> 250	15	38,46%	19	21,59%

Table 13– Distribution of patients according to the mean cholesterol

In the group of patients with average cholesterol levels over 250 mg / dl, most were those in group A, with diabetic nephropathy. In the study group was established a correlation between the mean serum cholesterol and the presence of diabetic kidney disease ($p = 0.0324$), but due to the small number of cases, data may not be statistically significant.

Pathological data were analyzed and compared with the mean values of serum triglycerides in the two groups (with and without diabetic nephropathy) (table 15)

Triglycerides (mg/dl)	Number of patients group A	Percent of patients group A	Number of patients group B	Percent of patients group B
< 150	5	12,82 %	11	12,50 %
150 - 200	8	20,51 %	39	44,32 %
> 200	26	66,67 %	38	43,18 %

Table 15 – The distribution of patients according the average serum triglycerides

The presence of diabetic nephropathy lesions correlated with the average serum triglycerides (p=0.0484), especially with triglycerides levels above 200 mg/dl.

Given the evidence from statistical analysis can build a picture of the risk factors for developing diabetic renal disease, represented in our study by: diabetes duration more than 10 years, blood pressure values higher than 160/95 mmHg, fasting plasma glucose values above 160 mg/dl, total serum cholesterol values above 250 mg/dl and triglycerides levels above 200 mg/dl. Age may be an additional risk factor, possibly explained by longer the development of diabetes and by associated comorbidities.

2. Anatomoclinical correlations in patients with nephroangiosclerosis

Of the study group, nephroangiosclerosis lesions were found on a number of 100 patients representing 78.74% of total. There was a significant correlation between nephroangiosclerosis lesions and patients age (p = 0.0000001) (Table 17).

Patients age	With nephroangiosclerosis	Without nephroangiosclerosis
Mean	69,18	57,59
Standard Error	0,87	2,53
Median	71	59
Mode	71	44
Standard Deviation	8,69	13,16
Sample Variance	75,56	173,10
Kurtosis	0,59	-1,02
Skewness	-0,64	-0,09
range	50	46
Minimum	38	33
Maximum	88	79
Count	100	27
Confidence Level	1,72	5,20
	p	0,0000001

Table 17 - Correlation between the presence of nephroangiosclerosis and patients age

The data are consistent with the literature [24, 24, 26], showing a high prevalence of nephroangiosclerosis lesions in elderly patients. It is difficult to say whether those lesions are a consequence of age or associated pathology [27].

Following statistical analysis, positive correlations were established between the presence of nephroangiosclerosis and duration of diabetes (p = 0.0834) in patients with diabetes older than 5 years (Table 18).

Diabetes mellitus duration	With nephroangiosclerosis	Without nephroangiosclerosis
Mean	11,89	9,70
Standard Error	0,70	1,59
Median	12	8
Mode	1	1
Standard deviation	6,96	8,26
Sample Variance	48,42	68,22
Kurtosis	0,40	-1,56
Skewness	0,59	0,28
Range	33	23
Minimum	1	1
Maximum	34	24
Count	100	27
Confidence Level	1,38	3,27
	p	0,0834

Table 18 - Correlation of nephroangiosclerosis presence and duration of diabetes.

Nephroangiosclerosis lesions are considered to be secondary to high blood pressure [28]. In the study group, of the 100 patients with nephroangiosclerosis, 86 (86%) had a medical history of hypertension. Nephroangiosclerosis lesions correlated with

blood pressure values and the most important correlation was with the systolic blood pressure values between 140 and 159 mmHg (Chart No. 19).

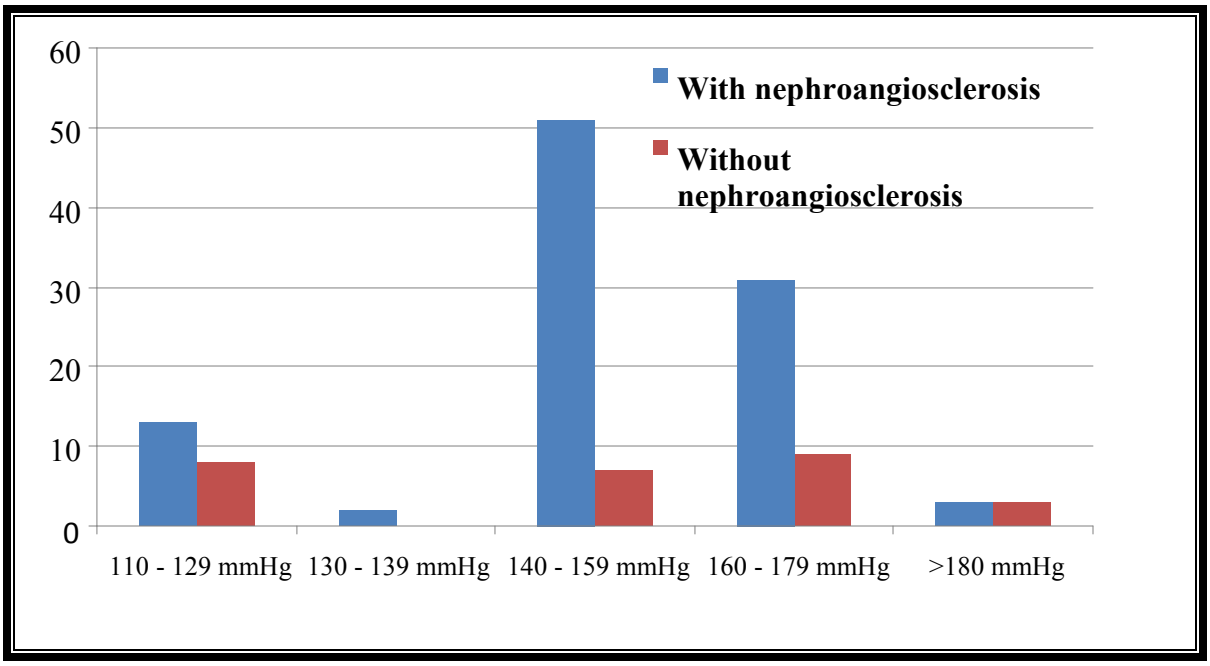

Chart No. 19 - Nephroangiosclerosis correlation with arterial hypertension

This study try to establish a correlation between the presence of renal lesions and metabolic status of the patients, the presence of nephroangiosclerosis being correlated ($p=0.006205$) with mean fasting glucose values for the 5 years prior to death. The most important correlation was with the average value glucose more than 160 mg/dl (table 19).

In the study group patients, there was a correlation between the presence of hypertriglyceridemia and nephroangiosclerosis lesions at triglycerides levels higher than 160 mg/dl, but the data were not statistically significant.

Smoker or nonsmoker status could not be indicated in a significant percentage of patients. Nephroangiosclerosis lesions were not correlated with gender, rural or urban area of origin, body mass index or total cholesterol value.

Glycaemia	With nephroangiosclerosis	Without nephroangiosclerosis
Mean	181,32	209,56
Standard Errror	4,84	11,79
Median	169	214
Mode	163	214
Standard Deviation	48,36	61,28
Sample Variance	2338,72	3754,72
Kurtosis	0,00	-1,16
Skewness	0,78	-0,17
range	204	217
Minimum	107	107
Maximum	311	324
Count	100	27
Confidence Level	9,60	24,24
	p	0,006205

Table 19 - Correlation between nephroangiosclerosis and average fasting glycaemia

Correlations established between nephroangiosclerosis lesions and patients age, duration of diabetes, systolic blood pressure and mean fasting glucose levels indicates that the etiology is multifactorial for this pleading also data from the literature [29, 30]. Regardless of etiology, evolution is to the end stage renal disease, the superposition of several risk factors precipitating this development [31, 32, 33].

3. Anatomoclinical correlations in patients with acute renal infarction

The association of diabetes with advanced age, arterial hypertension, various cardiovascular disorders (especially permanent atrial fibrillation and valvular heart) [34], obesity and dyslipidemia [35, 36, 37] increase the risk of renal vessels atheromatosis and renal infarction.

In the study group, renal infarction was revealed in 9 patients of the total of 127, representing a rate of 7.1 % (chart no.22).

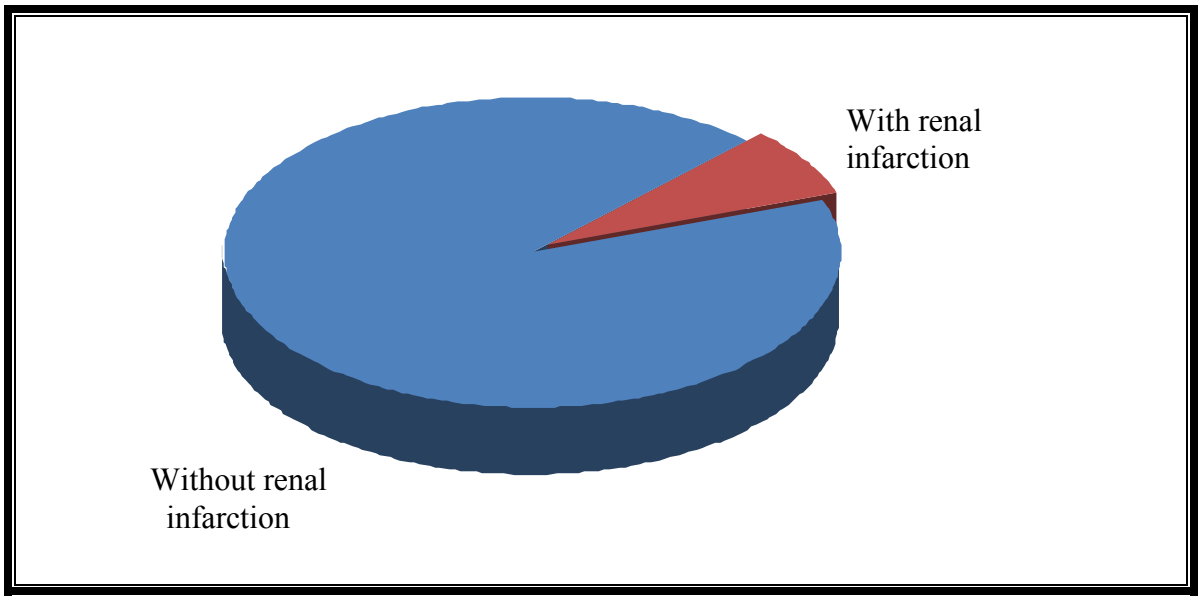

Chart no.22 – Renal infarction distribution in the study group

Analyzing the data, it was determined a correlation between the age of patients in the study group and the presence of renal infarction (p=0.041341339). The number of cases increased with patient age and with a mean systolic blood pressure greater than 140 mmHg.

In most cases, renal infarction was not diagnosed before death, because small renal infarctions often had nonspecific symptoms [38]. 5 patients had associated favoring clinical conditions (permanent atrial fibrillation and valvular heart disease). The small number of cases, however, not allowed obtain data statistically significant.

No correlation could be established between the presence of renal infarction and gender, urban areas, type of diabetes, duration of diabetes, mean serum cholesterol, triglycerides and glucose levels.

4. General discussions

The progression and prognosis of renal impairment in diabetes mellitus is different depending on the aetiology. In the study group, patients' final outcome was unfavorable, to death. However, only in 10 patients death was caused by end stage renal disease and the most commonly cause of death was cardiovascular disease.

The presence of diabetic nephropathy lesions correlated with age, diabetes duration greater than 10 years and glycemic control, although the small number of cases did not permit obtaining data statistically significant. The association between diabetes mellitus and hypertension increased the risk of proteinuria and accelerate the decline in renal function as shown by the literature [39].

Most patients in the study group were associated with multiple cardiovascular risk factors (age, dyslipidemia, hypertension), as proven by the large renal vascular lesions, isolated or confounding with other types of kidney damage. The most common identified renal lesion was nephroangiosclerosis and was correlated with patient age, duration of diabetes, systolic blood pressure value and the mean fasting glucose.

Renal infarction was present in 7.08% patients studied, percentage explained by the accumulation of risk factors in diabetic patients included in the study group and to the fact that diagnosis is made retrospectively, at necropsy, given that small renal infarcts symptoms atypical.

Nondiabetic kidney disease have a lower progression rate than the natural progression of diabetic renal disease, but a nondiabetic nephropathy is superimposed on diabetic nephropathy accelerates the decline in renal function.

In the study group were found two cases of membranoproliferative glomerulonephritis, which is slowly progressive to chronic renal failure. Kidney involvement in systemic lupus erythematosus usually has a poor prognosis, progressing to chronic renal failure. In the study group, there was only one case of lupus glomerulonephritis. In this case diabetes had a short duration and was secondary to cortisone therapy. Necropsy did not reveal other types of renal lesions superimposed to lupus nephritis and the death was due to the underlying disease that progressed to end stage renal disease.

The original elements of the study results from the retrospective analysis of a group of patients with diabetes from the point of view of clinical and laboratory data obtained from current clinical practice and in terms of the elements of renal histopathology. A clinical profile of risk for the development of renal damage was obtained.

A feature of the patients in the study group was the increased number of patients aged over 65. This age group is often associated with multiple comorbidities (hypertension, dyslipidemia) or cognitive decline [40] and the evolution of various nephropathies to end stage renal disease is influenced by the interrelationship between these factors [41]. Those patients required multidisciplinary approach [42]. Distribution by age, with higher percentage of patients over 65 years has provided data on the association of multiple comorbidities and their effect on renal damage.

Distribution by causes of death, with the presence on the first place of cardiovascular diseases is consistent with the literature, but the presence on the second place of infectious causes showed the precarious infectious defense in diabetic patients and the importance of preventing infectious processes in these patients, with a good metabolic control and prevention of characteristic diabetic foot lesions.

Microscopic study of renal lesions showed that the main histopathological characteristic is renal interstitial fibrosis with predominantly peritubular distribution.

Immunhistochemical study revealed in cases of fibrosis associated with of persistent inflammation a marked reduction of the expression of epithelial markers, such as cytokeratin, with increased expression of fibroblast markers (FSP1), proving the hypothesis that fibroblasts derived from local conversion of epithelium through epithelial-mesenchymal transition (TEM). Most faithful marker to indicate early TEM process was FSP1 - S100A4.

However, there have been varying degrees of positivity for markers of cell proliferation as a DNA polymerase accessory protein (PCNA) in the tubular cells and fibroblasts, indicating a significant increase in their proliferative ability. Identification of myofibroblasts marker α -SMA was not achieved in all FSP1 + fibroblasts, suggesting that smooth muscle actin is a not a specific marker of fibroblast. On the other hand, there

was a correlation between the number of α -SMA + tubular cells and the severity of interstitial fibrosis.

The presence of a large number of patients with renal lesions characteristic for the association between diabetes mellitus and hypertension, has created a picture of the risk of renal impairment in a patient with diabetes mellitus. Kidney damage is related to duration of diabetes greater than 10 years, the average values of blood pressure greater than 160/95 mmHg, mean fasting blood glucose above 160 mg/dl, mean total cholesterol over 250 mg/dl and triglycerides above 160 mg/dl.

The highlighting of renal pathological characteristics in patients with diabetes and the establishment of correlations with commonly clinical and laboratory allowed conclusions that may have practical applications in diabetologists practice.

FINAL CONCLUSIONS

1. Diabetes is a disease whose prevalence has increased significantly in recent years, increasing the number of patients who may develop chronic complications.
2. The differential diagnosis of diabetic renal disease is sometimes difficult, requiring in addition to clinical and laboratory data, pathological data obtained prospectively by performing renal biopsy puncture or retrospectively at necropsy.
3. The original elements of the study results from the retrospective analysis of a group of patients with diabetes from the point of view of clinical and laboratory data in terms of current medical practice and in terms of the elements of renal histopathology.
4. The analyzed group, consisting of 127 patients, has a preponderance of females and a higher proportion of patients aged over 65 years with multiple risk factors, which has provided us with data about their effect on kidney injury.
5. Distribution of the causes of death, with the presence on first place of cardiovascular diseases (61.4 % of total) is consistent with the literature, but the presence on the second place of infectious causes shows the precarious infective defense in diabetic patients and the importance of preventing infectious processes in these patients.
6. The most common renal lesion was nephroangiosclerosis (100 of 127 patients, representing 78.74 %). The presence of nephroangiosclerosis correlated with patients' age, diabetes duration, mean fasting glucose over 160 mg/dl and systolic blood pressure greater than 140 mmHg.
7. Typical lesions for diabetic renal disease have met a number of 39 of the total 127 patients (30.71 %) and correlates with the duration of diabetes of more than 10 years, blood pressure values higher than 160/95 mmHg, fasting plasma glucose values above 160 mg/dl, serum total cholesterol over 250 mg/dl and triglyceride levels over 200 mg/dl.

8. Renal lesions characteristic for renal infarction has been shown in a number of 9 patients (7.08 % of total). The occurrence of renal infarction correlated with age and systolic blood pressure, but due to low number of cases the data were not statistically significant.
9. In a small number of patients in the study group were found specific damages for other renal diseases (stage I and stage II membranoproliferative glomerulonephritis, lupus nephritis, segmental glomerulosclerosis, renal clear cell carcinoma, chronic pyelonephritis, renal papillary necrosis).
10. In our study was detected a positive immunofluorescence for some types of glomerulonephritis and glomerulopathies, detecting the positivity complement fraction C3 in membranoproliferative glomerulopathies, C1 fraction in lupus nephropathy and positivity by applying IgG in segmental glomerulosclerosis
11. Microscopic study of renal lesions showed histopathological that dominant was renal interstitial fibrosis with predominant peritubular disposition, a process in which TGF-β (transforming growth factor) occupies a central position by stimulating protein synthesis in interstitial fibroblast and by facilitating epithelial-mesenchymal transition (TEM), inducing the expression of specific proteins of fibroblasts (FSP1) in epithelial cells from this process.
12. Imunhistochimic study revealed in cases with fibrosis associated with persistent inflammation decreased expression of epithelial markers such as cytokeratin, with increased expression of fibroblast markers as FSP1, most faithful marker to indicate the early TEM early being FSP1 - S100A4. In these cases, polyclonal anti-FSP1 serum gave positive reactions in fibroblasts from collagen deposition areas of and in tubular epithelium adjacent to inflammatory foci.
13. We have found varying degrees of positivity for markers of cell proliferation as DNA polymerase accessory protein PCNA in the tubular cells and fibroblasts, indicating a significant increase in their proliferative ability.
14. Identification of myofibroblasts marker α -SMA was not achieved in all FSP1 + fibroblasts, suggesting that smooth muscle actin is not a specific marker of fibroblast, but there was a correlation between the number of α -SMA + tubular cells and interstitial fibrosis severity.

15. The results of the immunohistochemical methods suggest that, in transition to mesenchymal phenotype, the tubular cells can produce, via myofibroblasts and fibroblasts, extracellular matrix proteins, intervening directly in the process of interstitial fibrosis.
16. Highlighting renal pathological characteristics in patients with diabetes and establishing correlations with commonly clinical and laboratory data allowed conclusions that may have practical applications in medical practice.
17. In patients with diabetes, especially in those with advanced age, glycemic control, blood pressure and renal function should be carefully monitored to reduce cardiovascular mortality.

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