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THE QUANTIFICATION OF GLOMERULAR ENDOTHELIAL AND MESANGIAL CELLS DURING THE HUMAN AGING

SUMMARY

Vital importance of the kidney function and the impossibility of its successful substitution, put on the need for the estimation of the morphologic and functional characteristics of the kidney during the aging process. Kidney's aging changes lead to the decrease of its function. So, it is very important, especially for the older individuals, to enable better differentiation of the pathologic from the age changes in the kidney's tissue.

Cadaveric tissue samples were used as material. Cadavers were classified into the different age groups according to the next scheme: I (20-29); II (30-39); III (40-49); IV (50-59); V (60-69) and VI (70 and older). After the routine histologic processing, slices were stereologicaly analyzed under the microscope with projection screen (Reichert Visopan) with 40 x lens magnification. M 42 test system was used. Hundred, by unbased method selected, glomeruli of each age group, were analyzed.

The average absolute number of the glomerular endothelial cells shows significant increase till the age of 50 (p<0.01), in regard to the age of the 20 to 29 years, while the number of the mesangial ceils during this period shows insignificant increase. After the age of 50 till the age of 70 years these parameters show insignificant increase. After the age of 70 years the number of glomerular endothelial cells significantly decrease in regard to the age of 40 to 49 years (p<0.01), while the number of mesangial cells shows significant decrease during this period in regard to the age of 20 to 29 years (p<0.05) and in regard to the age of 40 to 49 years (p<0.01).

Significant decrease of cell's number in the V and VI age group is the result of the glomerular loops decrease per area unit.

Key words: **mesangial cells, endothelial cells, glomerulus, aging**

INTRODUCTION

Numerous articles note involutional changes in kidneys during the aging process (1-3). Kidney's volume decrease is obvious after the age of 60 with further decrease tendency (1,2,4). Some authors claim that there is decrease of renal volume of 50% after the age of 50 and that this decrease is mostly the result of renal cortex reduction (3,5,6). Also, the increase of partially and completely sclerotized glomeruli is present in the older age groups, which leads to the decrease of the functionally active nephrons. In accordance with this data, the decrease of the kidney function is present in the older individuals (7,8). There are data in the present literature, that the remaining renal corpuscles increase their volume to preserve the normal renal function (8,9).

Mesangilal cells and matrix form the mesangium. It is separated from the capillary lumen by the endothelium and endothelial cells (10,11).

Mesangial cells, except their participation in structural support for the glomerular capillary loop, have contractile and phagocytic features, while the endothelial cells because of their negative surface electrical charge contribute to the selectivity of the glomerular capillary wall (12).

Today, very important problem is the dynamics of the glomerular capillary network mesangial, endothelial and epithelial cells number, as well as the amount of the mesangial matrix. There are data in the present literature, that the number of the mesangial and endothelial cells, as well as the amount of the mesangial matrix, increase in the glomeruli of the renal corpuscles (2,6,13 -15).

It is obvious, on the basis of the present literature data, that mesangial and endothelial cells are very important for the structure and function of the renal corpuscle. It is evident that numerical data, about the changes of previously cited cells during the aging process, are very poor. This fact pointed to the significance of their quantification analysis.

MATERIAL AND METHODS

The material was cadaveric kidneys, obtained from the autopsies at the Department for the forensic medicine, Medical faculty in Nis. Analyzed kidneys originated from the individuals which age ranged from the age of 20 to 70 years and their cause of death was accident. The kidneys were without the changes which were visible to the naked eye and without congenital anomalies. They were classified into the different age groups according to the following scheme: I (20-29); II (30-39); III (40-49); IV (50-59); V (60-69) and VI (70 and older).

The kidneys were cut with frontal section. Then, tissue sample was taken always on the same way, with dimensions 1cm x 1cm. Tissue was fixed in 10% formalin during the next 24 hours. After the fixation kidney tissue was processed by the routine histologic procedure and cut into the 5 jam thick which were stained with HE and PAS method. The total number of analyzed samples was 60, three samples from each age group.

Characteristic changes were additionally analyzed under the digital imaging system Olympus BX 50 with lens magnification 10 x and 20 x, eyepiece magnification 10 x and magnification 2 x in front of the camera.

Sterelogic analysis was performed under the light microscope with projection screen (Reichert Visopan) with 40 x lens magnification. M_{42} multipurpose test system was used. The distance between two adjacent test points (d) was defined by the test system calibration with object micrometer (1:100) at the 40 x lens magnification. Then the test system area (A,) and the area of the test system which belongs to one test point (a) were calculated. Characteristics of the used test system were P_t =42, At = 0.058mm^2, a =0.014 mm^2.

Average glomerular volume was estimated for the 100 randomly selected renal corpuscles by Weibel and Gomes method. Both mesangial and endothelial cells were counted simultaneously and their average number per test system area unit was estimated (16). The absolute number of endothelial and mesangial cells per one glomerulus was calculated from their average number and glomerular average volume.

Obtained quantitative data were showed in tables, charts and statistically processed. Average value *(X)* of estimated parameters, its standard deviation (SD) and standard error (SE) were calculated. The statistical significance was tested by Student t - test for small samples.

RESULTS

Histological analysis

Figure 1 shows renal tissue in the first age group, which is stained by PAS method. Bowman's capsule lamina, capillary basal lamina, mesangial cells and mesangial matrix are PAS positive structures, so they are more clearly visible in regard to the other glomerular structures. Glomerular capillary network is composed of clearly visible capillary loops and nuclei of endothelial cells (with more irregular shape), which are located next to the mesangium, distant from the urinary space. These nuclei are located next to the capillary basal lamina. Mesangial matrix with numerous central, dark and small mesangial cells nuclei is located in the space between capillary loops.

Figure 2 shows renal tissue of the IV age group, stained with HE method. Three renal corpuscles with central position are obvious. Glomerular capillary network is multiplied, mesangial matrix is more distinct and the proliferation of the mesangial and endothelial cells nuclei is evident.

Figure 3 shows the same section of renal tissue as figure 2, but in this case it is stained by PAS method. The proliferation of the mesangial and endothelial cells is evident. Glomerular capillary are enlarged and between them is enlarged amount of mesangial matrix. Basal lamina of the glomerular capillary is distinct positive structures.

Figure 4 shows renal tissue of the VI age group which is stained by HE method. There are two completely preserved renal corpuscles with multiplied capillary network and reduced mesangial matrix. Renal corpuscle in the center of the figure is smaller with completely reduced glomerular capillary network, which is replaced with connective tissue.

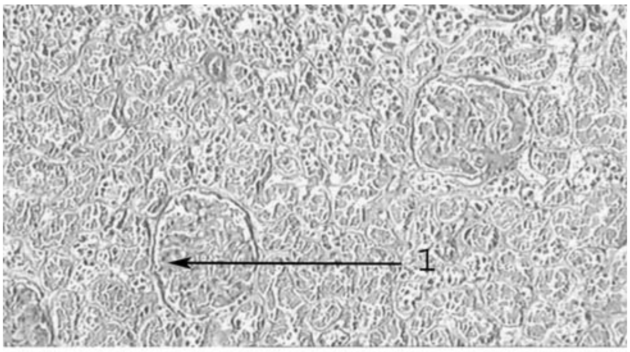

Figure 1. Kidney's tissue of the I age group; PAS; 1- normal renal corpuscle; microscope magnification 10 x 10 and in front of the camera 2x

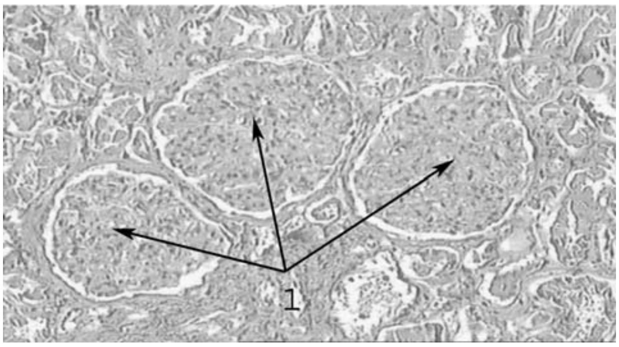

Figure 2. Kidney's tissue of the IV age group; HE; 1- Mesangial and endothelial nuclear proliferation; microscope proliferation 20 x 10 and in front of the camera 2x

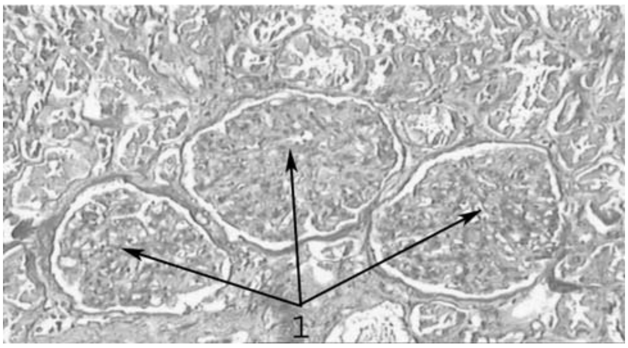

Figure 3. Kidney's tissue of the IV age group; PAS; 1- Mesangial and endothelial nuclear proliferation; microscope proliferation 20 x 10 and in front of the camera 2x

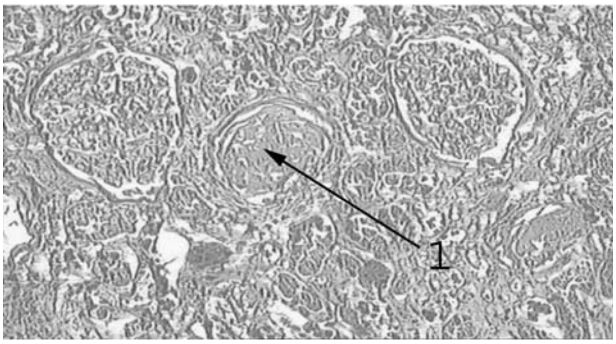

Figure 4. Kidney's tissue oftheVl age group; HE; 1 - sclerotized renal corpuscle; microscope proliferation 20 x 10 and in front of the camera 2x

Morphometric analysis

Table 1 and figure 5 show total average number of mesangial cell per one glomerulus. This number increases insignificantly from the I age group in which it amounts 727 to the III age group in which it amounts 820. Then, this parameter shows decrease. It amounts 394 in the VI age group and this is significant decrease in regard to the I age group ($p<0.05$) and in regard to the III age group ($p<0.01$), too.

Table 1. The average absolute number of mesangial cells per one glomerulus during the process of aging

AGE GROUPE (years)	N	Nm X SD
I (20-29)	3	*111* ± 126
II (30-39)	3	737 ± 136
ffl (40-49)	3	820 ± 276
IV (50-59)	3	764 ± 144
V (60-69)	3	520 ± 172
VI (=70)	3	394 ± 88[ab]

a - p < 0.05 in regard to the 1 age group
b - p < 0.01 in regard to the III age group

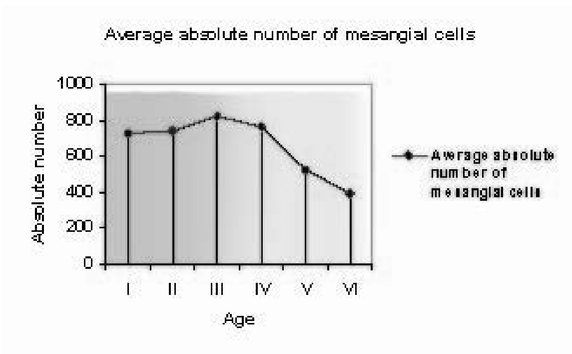

Figure 5. The average absolute number of mesangial cells per one glomerulus during the process of aging

Table 2 and figure 6 show the change of glomerular endothelial cells average absolute number per one glomerulus. The number of endothelial cells significantly increases ($p < 0.01$) in the III age group in which it amounts 553 in regard to the I age group in which it amounts 283. In the VI age group this number amounts 264, which presents significant decrease in regard to the III age group ($p<0.01$).

Table 2. The average absolute number of endothelial cells per one glomerulus during the process of aging

AGE GROUP (years)	N	Ne
		X SD
I (20-29)	3	283 + 38
II (30-39)	3	269 ± 45
III (40-49)	3	553 ± 182 [a]
IV (50-59)	3	443 ± 67
V (60-69)	3	348 ± 87
VI (> 70)	3	264 ± 64 [b]

a - $p < 0.05$ in regard to the I age group
b - $p < 0.01$ in regard to the III age group

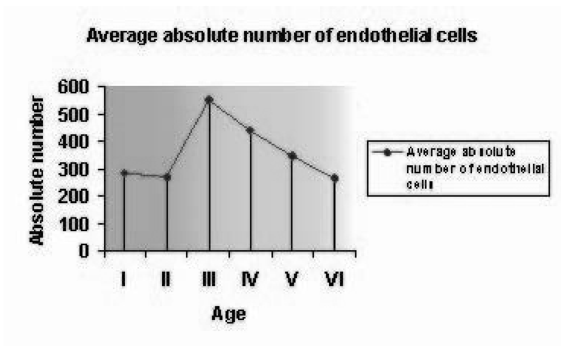

Figure 6. The average absolute number of endothelial cells per one glomerulus during the process of aging

DISCUSSION

Structural and functional glomerular changes, which influence on kidney function, occurr during the process of aging. The number of glomerular and tubular cells, as well as the glomerular loops per space unit, decreases during the aging process. However, the size of the Malpighian corpuscle, glomerular loops, glomerular and tubular cells nuclei increases during the aging process (2,6,17).

Although, we didn't in this research estimated -proportional relation between mesangial and endothelial cells, Wehner showed in his study that the portion of the mesangial and endothelial cells significantly increases during the aging and it ranged from 6.2% of glomerular volume in the middle age to 10.4% in the older age (18).

The results of our research (table 1 and 2) are in accordance to the results of the other authors (4,15,17). Cited authors claimed that the size of the renal corpuscles is significantly larger in older individuals (the age 40 to 50 years) and that this is the result of the mesangial matrix increase and expansion due to collagen fibers proliferation. But, the renal corpuscle size increase is not followed the increase of glomerular size.

In our research, we established the increase of the mesangial and endothelial cells number per one glomerulus till the age of 50. After the age of 50 this number started to decrease significantly. We also established that mesangial cells proliferation cause the increase of the glomerular capillary network volume and consequently renal corpuscle volume, which is in accordance with present literature data (13-15). Sorensen, in his research, paid attention to glomerular constituents and their mutual relationships (19). He investigated the relationship between mesangial cells and mesangial matrix, especially. The results of his research pointed to the conclusion that the mesangial cells proportionally compose the largest part of glomerulus. Kostjukov concluded during his electron microscopic research that the main renal corpuscle aging changes are thickening, shrinkage and reduplication of the glomerular capillary basal lamina, the changes of lamina densa and the increase of the mesangial and endothelial cells number (4). Mesangial matrix changes its protein characteristics and proliferates, which causes the increase the size of the whole glomerulus. All these changes influence on the age dependent glomerulosclerosis occurrence (8,9,15). Hattori et al. associate the origin of glomerulosclerosis and other glomerular lesions with cellular changes, especially with phenotypic changes of the mesangial cells which are considered as the initiators of the glomerular sclerosis (20).

Statistically significant decrease of cell's number which we were detected in the V and VI age group is the result of the decrease of nephrons number, the number of glomerular loops per area unit and general atrophy of all kidney structures, which was noted by other authors (2, 6,14,21).

CONCLUSION

The total average number of mesangial cells per one glomerulus increases insignificantly till the III age group, then It decreases significantly in the VI age group in regard to the I and the III age group.

The total average number of endothelial cells per one glomerulus increases significantly till the III age group in regard to th;e I age group, then it shows statistically significant gradual decrease till the VI age group in regard to the I age group.

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KVANTIFIKACUA MEZANGIJALNIH I ENDOTELNIH ĆELIJA U GLOMERULU U TOKU PROCESA STARENJA KOD ČOVEKA

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SAŽETAK

Vitalni značaj bubrežne funkcije i nemogućnost njene uspešne supstitucije nameću potrebu izučavanja morfoloskih i funkcionalnih karakteristika bubrega u toku starenja. Starosne promene u bubregu smanjuju njegovu funkciju pa je zato značajno, naročito kod osoba starije životne dobi, omogućiti bolju diferencijaciju šta je patološko, a šta pripada procesu starenja.

Kao materijal u istraživanju korišćeni su isečci kadaveričnih bubrega osoba Starosne dobi od 20 do iznad 70 godina, koji su svrstani u različite uzrasne grupe prema šemi: I (20-29); II (30-39); HI (40-49); IV (50-59); V (60-69) i VI (preko 70 godina). Nakon klasične histoloske obrade preseci su analizirani stereološki na projekcionom mikroskopu sa ekranom (Reichert Visopan) na povećanju objektiva 40 puta sa testnim sistemom M42. Analizirano je 100 slučajno odabranih glomerula iz svake Starosne grupe.

Prosečan apsolutni broj endotelnih ćelija u glomerulu progresivno se povećava do 50. godine sa statistički značajnom razlikom ($p<0.01$) u odnosu na period od 20. do 29. godine, dok se broj mezangijalnih ćelija u ovom periodu povećava bez statističke značajnosti. U periodu do 70. godine dolazi do postepenog smanjenja ispitivanih parametara, da bi nakon ovog perioda pad broja endotelnih ćelija bio statisticki značajan u odnosu na period od 40. do 49. godine ($p<0.01$), a pad broja mezangijalnih ćelija statisticki značajan u odnosu na period od 20. do 29. godine ($p<0.05$) i od 40. do 49. godine ($p<0.01$).

Pad u broju ćelija koji je statisticki značajan u V i VI starosnoj grupi posledica je smanjenja broja glomerularnih petlji po jedinici površine.

Ključne reči: **mezangijalne ćelije, endotelne ćelije, glomerul, starenje**