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EXPRESSION OF COLLAGEN TYPES I AND III IN HUMAN KIDNEY DURING THE SECOND AND THIRD TRIMESTERS OF DEVELOPMENT

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Methanephros or definitive kidney begins its development on the 5th week of gestation from two mesodermal sources: the ureteric bud and the metanephric blastema. Collagen tipes I and III are the proteins from the fibrilar group and it is found in conestive tissues. Collagen molecules in kidney are present in the extracellular matrix. The aim of this study was to determine the volume density of type I and III collagen in second and third trimester of gestation in human kidney. For the material we used 12 human kidneys from embryos and fetuses. Kidneys were fixed in 10% buffered formaline solution, and taken to paraffin molds by using standard procedure. 5μm slices are made, and stained with hematoxilin-eosin methode and imunnohystochemic methode by using rabbit policlonal anti collagen tipe I and III antibody. For the quantification of type I and III collagen in different developing stages of the kidney, we determined its volume density. Type I and III collagen are present in connective tissue of the kidney in second and third trimester of development. There is no statistically significant difference between the study groups in amount of type I and III collagens. Type I and III collagens are are present in kidney connective tissue and basement membranes during fetal development. The volume density of collagen tipe I and III is constant during second and third trimester.

Keywords: kidney; metanephros; collagen

Introduction

The extracellular matrix (ECM) is a dynamic, highly organized network of proteins and polysaccharides that provides structural support and plays a pivotal role in tissue morphogenesis, homeostasis and repair (1,2). One of the essential components of the ECM in mammals is collagen, of which type I collagen (COL1) constitutes approximately 90% of the body's total collagen content (2,3). As a fibrillar collagen, COL1 consists of two α1 and one α2 chains that intertwine into a right-handed triple helix and is stabilized by post-translational modifications such as hydroxylation of proline and lysine, glycosylation and interchain crosslinking. These post-translational modifications give to COL1 its biomechanical strength and stability, therefore providing the tensile properties of connective tissues COL1 fibers (1,2,4,5).

During kidney development, nephron formation begins at the 5th week of gestation and continues through the 36th week, resulting in approximately one million nephrons in each kidney (6,7). When kidney development is at its peak, around the time of birth, nephron progenitor cells are rapidly dividing, the ureteric bud is branching out and assembling the glomeruli and tubules (8). The extracellular matrix and its collagen component play a crucial role in directing the progression of these developmental events by acting as a framework and a repository of signals that control cell adhesion, movement, and differentiation (1,9).

The cells responsible for the collagen type I secretion in the developing kidney include interstitial fibroblasts, mesenchymal stem cells, and, under specific conditions, bone marrow-derived cells and renal tubular epithelial cells (10,11). The levels of collagen I are influenced by a number of growth factors and signal pathways including TGF-beta, Wnt/beta-catenin, and bone morphogenetic proteins. These factors integrate external messages to control the production and reshaping of the ECM (6,12).

The aim of the paper was to determine the volume density of collagen type I in the cortex and medulla of the human kidney during the 2nd and 3rd trimesters of development in order to examine its contribution to the developing kidney’s ECM.

Material and methods

The material consisted of eight human fetal kidneys, of both genders, obtained legally and in accordance with ethical principles. Samples were collected postmortem following premature deliveries, as well as after spontaneous and artificial abortions. The criterion for sample selection

was the absence of macroscopic damage, pathological changes, and autolytic alterations of kidney tissue. Fetal age was determined using medical documentation and by measuring crown–rump length. The study was conducted at the Center of Pathology and Pathological Anatomy of the Clinical Center in Niš and at the Department of Histology and Embryology, Faculty of Medicine, University of Niš. All examined samples were classified according to trimesters and gestational weeks (Table 1).

Table 1. Number of samples in the study, classified by trimester and gestational week.

Period of development		Week of gestation	№ of kidneys	Σ№
Fetus	Second trimester	17	1	4
		18	2	
		20	1	
	Third trimester	24	1	4
		25	1	
		36	1	
		37	1	

Immunohistochemistry. The tissue sections were rehydrated using a descending series of alcohols (100%, 96%) and distilled water. The slides were then submitted to heat-induced antigen retrieval for 30 minutes at 95-98°C. The slides were subsequently treated with 3% hydrogen peroxide to eliminate endogenous peroxidase activity. The rabbit polyclonal antibody against collagen type I (Abcam, ab34710, 1:100) was applied overnight at 4°C. EnVisionFLEX High pH system (manufacturer: Agilent, catalogue number: K8000/8002) was used to visualise the antibody in tissue sections. Between steps, the tissue was rinsed with TWEEN buffer. The slides were counterstained with Mayer's hematoxylin and mounted with cover slips by using Canada balsam. For analysis, three sections from each kidney were used.

Morphometric analysis. Photodocumentation was prepared from microscopic slides using an Olympus BX50 light microscope (Olympus, Japan) equipped with a Leica DFC295 digital camera (Leica Microsystems, Germany) at the Department of Histology and Embryology, Faculty of

Medicine, University of Niš. Microphotographs were taken at magnifications ranging from ×40 to ×400 and saved as TIFF (Tagged Image File Format) files. For this study, the collagen I volume density was calculated on images taken at x400 magnification.

To quantify collagen type I in different trimesters of kidney development, its volume density was determined. Volume density represents the percentage proportion of collagen type I (examined phase) relative to the examined compartment of kidney tissue. In kidneys from the second and third trimester of development, the volume density was determined separately for kidney tissue compartments: (1) cortex and (2) medulla.

For the determination of volume density, the program ImageJ v.1.48v (Wayne Rasband, National Institute of Health, USA) was used. An 80-point test system was superimposed on the image, and the number of points falling on the examined phase of the kidney tissue compartment (Vf) was counted. Volume density (V_V) was calculated according to the formula: $V_V=Vf/Vt$, where Vt represented the total number of points of the test system. The obtained results were multiplied by 100 and expressed as percentages. In each group, 60 view fields were analyzed (30 for cortex and 30 for medulla).

Statistical analysis. Statistical analysis was performed using SigmaStat 3.5 (Systat Software Inc., USA, 2006). Normality of distribution was tested with the Kolmogorov–Smirnov test. For statistical analysis, the ANOVA (one-way analysis of variance) was used.

Results

In all examined samples, the cortex and medulla were clearly outlined and showed morphological features similar to those seen in the adult kidney. Glomeruli and Bowman's capsules were distinctly visible as parts of the renal corpuscles. Podocytes throughout the second and third trimesters exhibit a cuboidal shape. Proximal and distal tubules are found in the cortex and are lined by simple cuboidal epithelium; however, some less mature tubules lack a clearly visible lumen (Figure 1). The medulla contains collecting ducts lined with simple cuboidal epithelium, Henle's loop lined with simple squamous epithelium, and in the renal papilla region, large papillary ducts lined with urothelium were observed (Figure 2).

Collagen I and III were expressed in the kidney interstitium, glomerular basement membrane, and the basement membranes of tubules, collecting ducts, loops of Henle, and papillary ducts. Tubular and ductal epithelial cells, as well as endothelial cells, podocytes, mesangial cells, cells of the outer

sheath of Bowman's capsule, and endothelial cells were consistently immunonegative for collagen types I and III.

The results of our study indicate that there is no statistically significant difference in the volume density of collagen types I and III in the cortex and medulla of the developing kidney during the second and third trimesters. The results are shown in Graph 1.

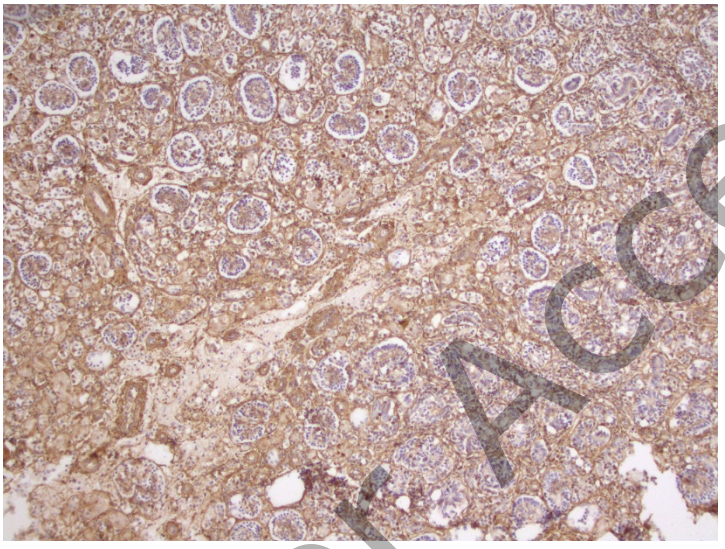

Figure 1. Immunoreactivity of collagen type I and III in the cortex of the fetal kidney during the 25th week of gestation x100.

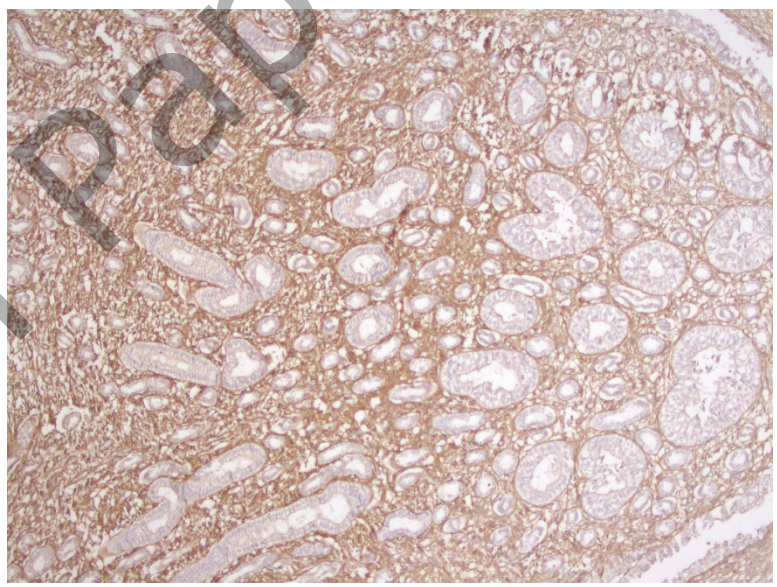

Figure 2. Immunoreactivity of collagen type I and III in the medulla of the fetal kidney during the 17th week of gestation x100.

The results of our study indicate that there is no statistically significant difference in the volume density of collagen types I and III in the cortex and medulla of the developing kidney during the second and third trimesters. The results are shown in Table 2.

Table 2. Volume density of collagen type I and III in the cortex and medulla of the fetal kidney in second and third trimesters of development. The values are given in percentages (%)

Period of development	V_V **(%) in the second trimester** $\bar{X}$**±SD**	V_V **(%) in the third trimester** $\bar{X}$±SD
Cortex	58,89±11,36	50,93±16,91
Medulla	48,13±8,26	48,75±8,84

V_V - volume density, $\bar{X}$ - mean value, SD – standard deviation

Discussion

In the present study we examined the volume density of collagen type I and type III in the cortex and medulla in the human kidney during the second and third trimesters of development. The results of our study showed that there is not a statistically significant difference in the percentage distribution of these collagen types between the examined kidney compartments. These results imply that the synthesis and secretion of collagen type I and III during the second and third trimesters of development remain stable and constant during the examined period of development.

The extracellular matrix plays an essential role in the proper development of organs. Studies on human organoid models of epithelial branching show that extracellular matrix composition influences branching patterns and epithelial proliferation (13,14). These findings suggest a role for the extracellular matrix in coordinated, time-specific development during organogenesis. In the interstitial spaces of the kidney, fibres made of collagen types I and III form a scaffold that supports renal tubules and vasculature. This allows the nephrons to maintain their structural integrity and positional stability, facilitating efficient reabsorption and secretion. The balance between collagen synthesis and degradation underpins tissue remodelling and repair without compromising function (10,15).

Collagen type I is primarily found within the interstitial ECM of the developing kidney, where it is produced mainly by fibroblasts and interstitial mesenchymal cells (1,3). Its expression is closely

regulated both spatially and temporally, reflecting the changing requirements for tissue remodelling during nephrogenesis (3,16). In contrast, collagen type IV is the main component of basement membranes, including those of the glomerulus and renal tubules, and exhibits a relatively consistent level of expression throughout fetal kidney development (17,18). The difference between fibrillar collagens (types I and III) and network-forming collagens (type IV) accounts for their complementary roles in providing tensile strength and filtration barrier properties, respectively (2,8).

Studies examining the components of the extracellular matrix in developing kidneys showed that laminin, collagen IV, and fibronectin appear in a coordinated pattern during nephron development. Furthermore, it was shown that they act as regulators of kidney development and might serve as determinants of the final nephron number (15,19,20). Also, it was reported that the development of the glomerular basement membrane in the renal corpuscle is a gradual, coordinated process, and that the extracellular matrix participates in its regulation (21).

Understanding the distribution patterns of various components of the healthy renal extracellular matrix during development is crucial for comprehending the pathogenesis of certain congenital renal malformations. Disorders such as renal dysplasia, obstructive nephropathy, and congenital anomalies of the kidney and urinary tract are often linked to abnormal collagen deposition (22).

In conclusion, the current study shows that the volume density of collagen types I and III does not vary significantly between the cortex and medulla of the human kidney during the second and third trimesters. This consistency likely indicates coordinated ECM development across renal regions and enhances our understanding of normal renal growth.

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EKSPRESIJA KOLAGENA TIPA I I III U HUMANOM BUBREGU TOKOM DRUGOG I TREĆEG TRIMESTRA RAZVIĆA

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Metanefros ili definitivni bubreg počinje da se razvija u 5. nedelji iz dva mezodermalna izvora: ureterskog pupoljka i metanefrosnog blastema. Kolageni tipa I i III spadaju u grupu fibrilarnih kolagena i zastupljeni su u ekstracelularnom matriksu bubrega u razviću. Cilj rada bio je da se odredi volumenska gustina kolagena tipa I i III u kori i meduli humanog fetusnog bubrega u drugom i trećem trimestru razvića. Materijal je činilo 8 humanih fetusnih bubrega (4 u drugom i 4 u trećem trimestru). Bubrezi su fiksirani u 10% puferisanom formalinu i dovedeni do parafinskih kalupa rutinskom metodom. Pravljeni su preseci debljine 5µm i bojeni imunohistohemijski uz korišćenje zečijeg poliklonalnog antitela na kolagen tip I i III.U cilju kvantifikacije kolagena tipa I i III u drugom i trećem trimestru razvića bubrega određivana je njihova volumenska gustina. Kolageni tipa I i III se eksprimiraju u intersticijumu kore i medule bubrega, kao i u bazalnim membranama u drugom i trećem trimestru razvića. Između ispitivanih grupa nije pronađena statistički značajna razlika u volumenskoj gustini kolagena tipa I i III između ispitivanih grupa. Kolageni tipa I i III su široko rasprostranjeni tokom embrio-fetalnog razvića u ekstracelularnom matriksu i bazalnim membranama bubrega. Njihova volumenska gustina se ne menja tokom drugog i trećeg trimestra.

Ključne reči: bubreg; metanefros; kolagen