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Expression of collagen type IV in human kidney during prenatal development

Ekspresija kolagena tipa IV u ljudskom bubregu tokom prenatalnog razvoja

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Abstract

Background/Aim. Type IV collagen belongs to the group of nonfibrillar collagens and is an important component of the basement membranes, where it accounts for approximately 50% of its structural elements. The aim of the study was to describe the expression and distribution of collagen type IV in the embryonic and fetal metanephric kidney and to determine the volume density of collagen type IV in kidney tissue in each trimester of development. **Methods.** The material consisted of 19 human embryos/fetuses, in the gestational age from 8th to 37th week. Kidney tissue specimens were routinely processed to paraffin molds, stained immunohistochemically using polyclonal anti-collagen IV antibody and counterstained with Mayer hematoxylin and eosin. Stained slides were examined using a light microscope, and images of the selected areas under different lens magnification were captured with a digital camera. Volume density of collagen type IV was determined using ImageJ 1.48v and a plugin of the software, which inserted a grid system with 336 points. For the data comparison, the One-Way Analysis of Variance (ANOVA) was used. **Results.** Strong collagen IV immunopositivity was seen in all specimens, with a distribution in the basement membranes of urinary bud, parietal leaf of Bowman's capsule, glomerular basement membrane, basement membrane of interstitial blood vessels, and basement membranes of nephron tubules and collecting ducts. No statistically significant difference in the volume density of type IV collagen was found among the different trimesters of the embryonic and fetal development. **Conclusion.** The synthesis and secretion of collagen type IV simultaneously follow the development of nephron structures, collecting system and blood vessels. The volume density of collagen type IV remains constant throughout all the trimesters of metanephric kidney development, indicating that it plays a crucial role in the normal development of nephron and collecting system structures, as well as in maintaining the normal kidney function.

Key words:
growth and development; histological techniques; collagen type iv; fetus; immunohistochemistry; kidney.

Apstrakt

Uvod/Cilj. Kolagen tipa IV pripada grupi nefibrilarnih kolagena i predstavlja značajnu komponentu bazalnih membrana u kojima čini oko 50% u odnosu na sve strukturne elemente. Cilj rada bio je da se opiše ekspresija i distribucija kolagena tipa IV u embrionalnom i fetusnom bubregu i da se odredi volumenska gustina kolagena tipa IV u svim trimestrima razvoja. **Metode.** Materijal je činilo 19 humanih embriona/fetusa, gestacione starosti od 8. do 37. nedelje. Uzorci tkiva bubrega su rutinski obrađeni do parafinskih kalupa, bojeni imunohistohemijski upotrebom poliklonalnog anti-kolagen IV antitela i kontrastirani Mayer-ovim hematoksilinom i eozinom. Nakon bojenja, uzorci su analizirani na svetlosnom mikroskopu i upotrebom digitalne kamere, na različitim uveličanjima, napravljena je fotodokumentacija. Volumenska gustina kolagena tipa IV određivana je u programu ImageJ 1.48v uz upotrebu „plagina" kojim je na digitalne slike postavljana mrežica od 336 tačaka. Za statističku analizu dobijenih podataka korišćen je *One-Way Analysis of Variance* (ANOVA) test. **Rezultati.** Jaka imunopozitivnost kolagena tipa IV bila je prisutna na svim ispitivanim uzorcima, sa distribucijom u bazalnim membranama ureterskog pupoljka, parijetalnog lista Baumanove kapsule, glomerularnoj bazalnoj membrani i bazalnim membranama tubula nefrona i sabirnih kanalića. Nije pronađena statistički značajna razlika u volumenskoj gustini kolagena tipa IV između različitih trimestara embrionalnog i fetalnog razvoja. **Zaključak.**

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Sinteza i sekrecija kolagena tipa IV dešava se istovremeno sa razvojem struktura nefrona, sabirnog sistema i krvnih sudova. Volumenska gustina kolagena tipa IV ostaje konstantna u svim trimestrima razvoja metanefričnog bubrega, što ukazuje na to da kolagen tipa IV ima značajnu ulogu u normalnom razvoju struktura nefrona i sabirnog sistema, kao i u održavanju normalne funkcije bubrega.

Ključne reči:
rast i razvoj; histološke tehnike; kolagen, tip iv; fetus; imunohistohemija; bubreg.

Introduction

The human kidney is a complex organ consisting of functional units – nephrons, connected to a highly branched collecting duct system. Nephrogenesis, the process of kidney formation, ends around the 36th week of development and comprises a plethora of intertwined processes, such as epithelial-mesenchymal interactions, epithelial branching, cell migration, differentiation, and cell division, as well as cell-extracellular matrix interactions [1–3]. The unique feature of kidney development is the mesenchymal-epithelial transition that occurs during the formation of the nephron and the differentiation of highly specialized structures, such as the glomerulus [1, 3].

The definitive mammalian kidney development is a complex process that occurs through the formation of three excretory structures from intermediate mesoderm: pronephros, mesonephros, and metanephros, of which the first two are temporary and involute, while the metanephros will give rise to the definitive kidney [4]. The pronephros and mesonephros formations are necessary for the development of the metanephric kidney, and the interruption in the development of these two precursor excretory structures will lead to renal agenesis [5]. The metanephric kidney occurs during the 5th week of development and is a result of the interaction of the nephric duct and metanephric mesenchyme, both of which originate from the intermediate mesoderm [6]. The intermediate mesoderm is a narrow strip of mesoderm located between the somite and lateral plate mesoderm [7]. Its ventral part will give rise to the nephric duct, while the posterior part of the intermediary mesoderm, referred to as the nephrogenic cord, will become condensed near the hindlimb buds, thus giving rise to metanephric mesenchyme [6]. The nephric duct is a tubular structure covered with simple cuboidal epithelium, directed toward the cloaca of the embryo with whom it connects. It is shown that glial cell-line-derived neurotrophic factor (GDNF), a protein secreted by the metanephric mesenchyme cells, binds to the Ret receptors on the epithelial cells of the distal part of the nephric duct and initiates the formation of the ureteric bud [8]. The ureteric bud plays a crucial role in the formation of metanephros, and its branching in the metanephric mesenchyme will give rise to the collecting system of the kidney [9]. The metanephric mesenchyme contains multipotent self-renewing Six2+ progenitors that will give rise to the main body of the nephron, as well as self-renewing Foxd1+ progenitor cells that will give rise to the stroma of the interstitium, mesangium, and pericytes in kidney [10].

Type IV collagen belongs to the group of nonfibrillar collagens and is an important component of the basement membranes (BM), where it accounts for approximately 50% of its structural elements [11, 12]. In BM, collagen type IV forms a polygonal network that, along with other molecular components of the BM, has a supporting and barrier function [11]. Moreover, it has a role in supporting tissue integrity, cell survival, cell signaling, morphogenesis, and tissue regeneration [13]. The type IV collagen molecule is a heterotrimer (protomer) consisting of three alpha chains that have a similar primary structure [11, 14]. To date, six genes encoding alpha chains, denoted COL4A1-COL4A6, were identified [15]. Alpha chains are interconnected into a three-helix structure of type IV collagen molecules. Three molecular isoforms of type IV collagen are described: $\alpha1_2\alpha2$, $\alpha3\alpha4\alpha5$ and $\alpha5_2\alpha6$ [16]. The $\alpha1_2\alpha2$ collagen IV isoform is found in all basement membranes, the $\alpha3\alpha4\alpha5$ isoform is found in the kidney, lung, and at the neuromuscular junction, while the $\alpha5_2\alpha6$ isoform is present in smooth muscle and at the neuromuscular junction [16, 17].

The aim of the study was to describe the expression and distribution of collagen type IV in the embryonic and fetal metanephric kidney and to determine the volume density (Vv) of collagen type IV in kidney tissue in each trimester of development.

Methods

Material

The material consisted of 19 human embryos/fetuses, in the gestational age from 8th to 37th week, obtained following all legal and ethical guidelines. The material was obtained after spontaneous or artificial miscarriages and premature births due to prenatal deaths. There was no macroscopic damage or any pathological/autolytic changes in the specimens, and both sexes were represented in the sample. Gestation week was determined using medical history, as well as by measuring the crown-rump length. The study was performed at the Department of Histology and Embryology, Faculty of Medicine, University of Niš. All examined samples were allocated into three groups based on the trimester of development (Table 1).

Tissue preparation

Kidney tissue specimens were isolated and fixated in 10% buffered formalin and routinely processed to paraffin blocks. A 5 μm thick tissue section was cut on Leica RM2255 microtome (Leica Micro-Systems, Rueil-Malmaison, France) and stained with hematoxylin and eosin for histological examination.

Table 1

The number of samples, allocated to different groups based on the trimesters of the kidney development

Development period	Gestation week	Samples (n)	Total (n)
Embryo			
first trimester			
	8	2	6
	9	1	
	10	1	
	11	2	
second trimester			
	17	1	6
	19	2	
	20	1	
	21	2	
Fetus			
third trimester			
	29	1	7
	32	1	
	34	1	
	35	1	
	36	2	
	37	1	

Immunohistochemistry

Tissue sections were deparaffinized in xylene, rinsed in alcohols with descending concentrations (100%, 96%, 75%), and rehydrated with distilled water. Heat-induced antigen retrieval with citrate buffer (0.01M, pH 6) was performed for 30 minutes. The endogenous peroxidase was blocked with 3% H_2O_2 for 15 min at room temperature. The kidney tissue was then exposed to the anti-collagen IV antibody (Rabbit polyclonal, Abcam, USA, ab6586, 1:250) overnight at 4 ℃. The secondary antibody was applied for 45 min, and the tissue specimens were then stained with diaminobenzidine (DAB) and counterstained with Mayer hematoxylin. Secondary antibody and DAB were used from EnVisionFLEX, HighpH visualization system (Agilent, Denmark GV80011-2). The rinses between the steps were performed with phosphate buffer (0.1 M, pH 7.4). Stained slides were examined using a light microscope Olympus BX50 (Olympus, Japan), and images of the selected areas, under different lens magnification, were captured in TIFF format with a digital camera Leica DFC295 (Leica Microsystems, Germany).

Morphometric and statistical analysis

The Vv is a relative variable, which shows how much overall space is occupied by the observed space in volume units [18]. The Vv of collagen type IV was determined by using ImageJ v. 1.48v (Wayne Rasband, National Institute of Health, USA) and a plugin of the software which inserted a grid system with 336 points (Vt). The number of points overlapping the collagen IV positive structures (Vf) within the kidney tissue was counted. The Vv was determined using the following formula: $Vv = Vf/ Vt$ [18]. The obtained results were multiplied by 100 and presented in percentages. For each trimester, the Vv of collagen type IV was determined for the entire kidney tissue of the examined sample. In the kidneys obtained from fetuses belonging to the third trimester, the Vv of collagen type IV was determined in specific regions of the tissue: (1) cortex (renal corpuscles, proximal and distal tubules, Ferrein's pyramides (medullary rays) and blood vessels); (2) medulla (collecting ducts, loops of Henle, and blood vessels). The distribution of data was tested using the Kolmogorov-Smirnov test. For data comparison, the One-Way Analysis of Variance (ANOVA) was used.

Results

Collagen type IV was expressed in kidney tissue specimens in all the trimesters. In the histological sections of the kidney tissue during the 8th week of development, there were visible renal corpuscles and proximal and distal tubule (Henle's loops were not present in our tissue sections), along with the components of the ductal system and interstitial blood vessels. During the late first trimester (11th and 12th week), the number of nephrons was increasing, and all parts of the nephrons and ductal system were clearly visible. The collagen type IV was clearly and strongly expressed in the basement membranes of urinary bud, parietal leaf of Bowman's capsule, glomerular basement membrane (GBM), basement membrane of interstitial blood vessels, tubules, and collecting ducts (Figure 1a).

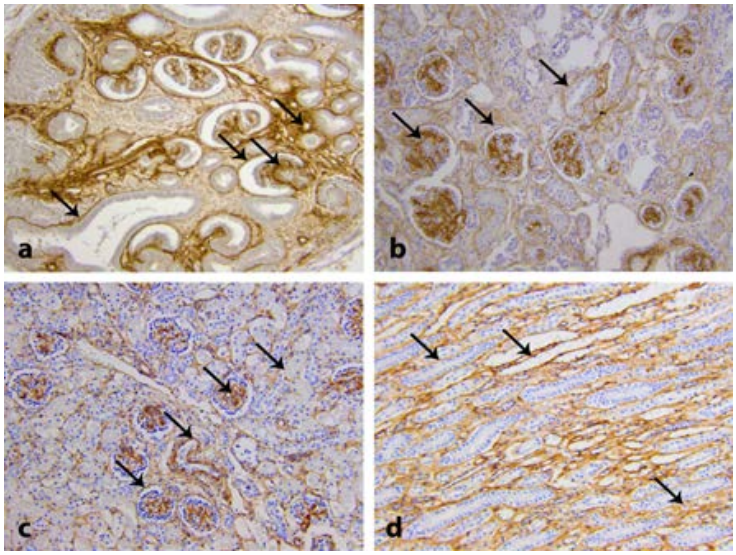

Fig. 1 – Expression of collagen type IV in the kidney in the 8th (a), 21st (b), 32nd (c) and 36th (d) week of development: a) The strong positivity is seen in basement membranes of the ureteric bud, parietal leaf of Bowman's capsule, glomerular basement membrane, and basement membranes of interstitial capillaries, ×200; b) Immunopositivity in the renal cortex is seen in the glomerular basement membrane, parietal leaf of Bowman's capsule, basement membrane of the distal tubule, ×200; c) Immunopositivity in the renal cortex is seen in the parietal leaf of the Bowman's capsule, glomerular basement membrane, basement membranes of interstitial blood vessels, and proximal renal tubule, ×200; d) Immunopositivity in the renal medulla is present in the basement membranes of collecting ducts, blood vessels, and Henle's loops, ×200.

Table 2

Volume density (Vv) of collagen type IV in the kidney, presented by the trimesters of the kidney development

Period of development	Vv (%), mean ± SD
1st trimester	15.97 ± 9.58
2nd trimester	14.34 ± 5.89
3rd trimester	16.25 ± 4.02

SD – standard deviation.

A clear difference between renal cortex and medulla was present in the kidney specimens of the second trimester. The growth of the fetal kidney was followed by an increase in the number of nephrons and extensive branching of the ductal system. During the second and third trimester of development, the collagen IV expression and distribution had the same pattern as in the late first trimester, i.e., in basement membranes belonging to the renal corpuscle and tubules of the nephron, collecting ducts, and blood vessels (Figures 1b, 1c, and 1d).

The volume densities of type IV collagen in the kidney, presented by trimesters of development, are shown in Table 2. No statistically significant difference in the volume density of type IV collagen was found between the different trimesters of the kidney development. Volume densities of type IV collagen in the elements of the renal cortex and medulla in the third trimester of development are shown in Figures 2 and 3.

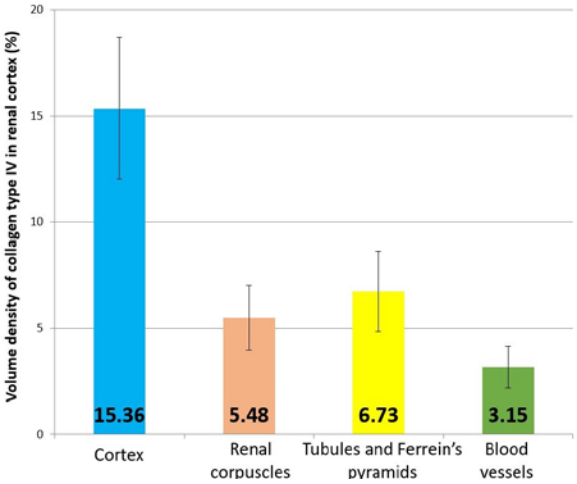

Fig. 2 – Volume density of collagen type IV in the renal cortex during the third trimester of the kidney development.

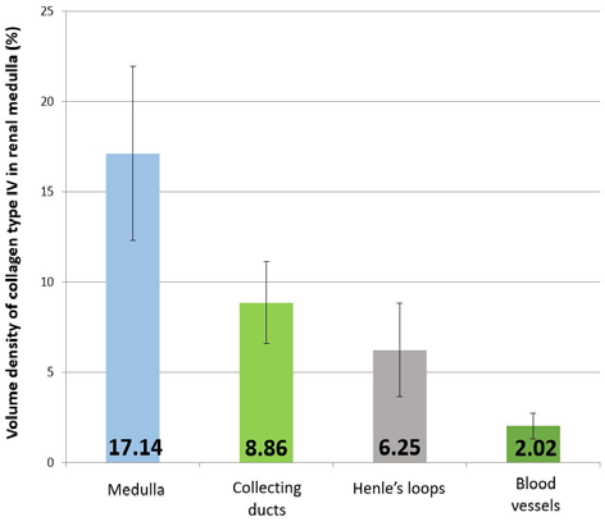

Fig. 3 – Volume density of collagen type IV in the renal medulla during the third trimester of the kidney development.

Discussion

Our results show that the expression of collagen type IV occurs early in kidney development and is limited to basement membranes of the ureteric bud, different parts of nephrons, collecting ducts, and blood vessels. These findings are in accordance with the previous reports that the formation of ureteric bud in the early stages of metanephros development is simultaneously followed by the assembly of basement membrane containing collagen type IV on the basal part of its cells [19–21]. During the further development, the formation of nephrons and collecting ducts, as well as blood vessels in the kidney, are followed by the deposition of basement membranes containing, among the other molecules, collagen type IV [22, 23]. Basement membranes have an important role in the modeling of parts of the nephron and collecting tubules of the kidney during development, and maintaining the normal tissue structure [23–25]. The results of type IV collagen testing on mouse kidney tissue show that developmental changes in different segments of the nephron and the collecting system are closely related to the expression of this type of collagen. From the aspect of nephrogenesis, type IV collagen is the most important protein of the basement membrane since its expression occurs immediately at the beginning of the formation of renal structures [26]. The collagen type IV is not only important for structural support of developing nephrons and collecting system, but it also has an important role in maintaining their function, i.e., the glomerular filtration, as well as the tubular reabsorption [27, 28].

Studies with developing human kidneys showed that the $\alpha1_2\alpha2$ isoform of type IV collagen is first synthesized and secreted in the GBM, but at the late capillary stage, there occurs a substitution of this collagen IV isoform with $\alpha3\alpha4\alpha5$ [29, 30]. The experiments with murine developing kidneys show that collagen $\alpha3\alpha4\alpha5$ appears in discontinuous, nonlinear patterns in parts of laminin α5-positive GBM that does not contain either isoform of collagen IV [31]. The $\alpha1_2\alpha2$ isoform of type IV collagen in the renal corpuscle is secreted by endothelial cells, podocytes, and mesangial cells, while the $\alpha3\alpha4\alpha5$ isoform is secreted exclusively by the podocytes of the visceral leaf of the Bauman capsule [32]. It is believed that these isoform transitions play key roles in the establishment of the glomerular filtration barrier, as well as in the maintenance of endothelial cells and podocytes inside the glomerulus [33]. Concerning the other parts of the nephron, the α3 through α6 chains of type IV collagen are abundant in the distal tubular basement membrane (TBM) in humans, while α1 and α2 chains are found ubiquitously in TBM. In the basement membrane of the parietal leaf of Bowman's capsule, the major collagen IV chains are α1, α2, α5, and α6 [34].

Although there are several studies dealing with the temporal and spatial expression of different isoforms of collagen type IV in developing kidneys, there are virtually no data concerning the quantification of collagen type IV in prenatal human kidneys. Jalali et al. [35] used a semiquantitative approach to determine the amount of collagen type IV in a murine model of kidney development. Their results indicate that the first traces of collagen type IV were observed during the E13 and that its amount gradually increased until the E18, to finally reach its maximum around day 5 postnatally. The earliest specimen used in our research was at the 8th week of gestation, and strong collagen IV positivity was already seen around all the tubular structures in the developing kidney. The quantified Vv of collagen type IV showed no significant statistical difference compared to the later stages of development. Moreover, the Vv is a relative variable and does not reflect the absolute amount of collagen type IV in developing kidneys, but rather its volume presence expressed in percentages within the organ and compared to all the other structural kidney components.

The genetic disorders of collagen IV synthesis especially affect the kidney due to the dependence of its functions on the stability and normal morphology of the BM. Glomerular filtration and tubular filtration are highly specialized kidney features, and the failure of the kidney to perform these functions may lead to end-stage renal disease with life-threatening consequences. The two major syndromes occurring as a result of mutation of genes for collagen type IV are Alport's and Goodpasture's syndromes [36]. Alport's syndrome occurs as a result of mutations in any of the three genes encoding components of the $\alpha3\alpha4\alpha5$ collagen type IV network (COL4A3, COL4A4, and COL4A5). Most mutations prevent assembly and/or secretion of $\alpha3\alpha4\alpha5$ heterotrimers of collagen type IV such that all 3 proteins are absent from the GBM [37]. Clinically, it manifests with persistent hematuria, sensorineural hearing loss, and ocular abnormalities [38]. Goodpasture syndrome is an autoimmune disease caused by the development of autoantibodies against the GBM that leads to kidney failure [39].

Conclusion

The collagen type IV is an important part of basement membranes in the kidney, whose synthesis and secretion simultaneously follows the development of nephron structures, collecting system and blood vessels. The volume density of collagen type IV remains constant throughout all the trimesters of metanephric kidney development, indicating that it plays a crucial role in the normal development of nephron and collecting system structures, as well as in maintaining the normal kidney function.

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Conflict of interest

The authors declare no conflict of interest.

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