Int. J. Morphol.,
43(6):1964-1972, 2025.

Mast Cells and Capillary Rarefaction in Hypertensive Nephropathy - A Potential 'Double-Faced' Role?

Mastocitos y Rarefacción Capilar en la Nefropatía Hipertensiva: ¿Un Posible Doble Rol?

Stancho Stanchev[1]; Alexandar Iliev[1]; Nikola Stamenov[1]; Lyubomir Gaydarski[1]; Kristina Petrova[1]; Boycho Landzhov[1]; Vidin Kirkov[2]; Vesela Ivanova[3]; Tihomir Dikov[3]; Plamena Ermencheva[4] & Georgi Kotov[4,5]

STANCHEV, S.; ILIEV, A.; STAMENOV, N.; GAYDARSKI, L; PETROVA, K.; LANDZHOV, B.; KIRKOV, V.; IVANOVA, V.; DIKOV, T.; ERMENCHEVA, P. & KOTOV, G. Mast cells and capillary rarefaction in hypertensive nephropathy - a potential 'double-faced' role? *Int. J. Morphol., 43(6)*:1964-1972, 2025.

SUMMARY: Hypertension often leads to hypertensive nephropathy, characterized by nephrosclerosis and capillary rarefaction. Mast cells, tissue-resident immune cells, are implicated due to their perivascular location and release of bioactive substances, while vascular endothelial growth factor (VEGF) plays a complex role in renal health and angiogenesis. The aim of the present study was to analyze changes in mast cell number (MCN), VEGF expression and capillary density (CD) in two stages of hypertension-induced kidney injury and compare them to normotensive controls. Herein, we used spontaneously hypertensive rats (SHR) at 6 and 12 months of age and age-matched normotensive Wistar rats (WR). Kidney tissues were analyzed for mast cell tryptase, CD117 and VEGF using immunohistochemical expression and subsequent semiquantitative analysis for VEGF immunoreactivity and capillary density was quantified. Data were assessed statistically through the Mann-Whitney method. We reported a significantly higher MCN and lower CD in both 6- and 12-month-old SHR compared to controls. VEGF expression was highest in 6-month-old SHR and lowest in 12-month-old SHR. Our results suggest that the initial increase in MCN and elevated VEGF in early hypertension might be a compensatory angiogenic response to developing capillary rarefaction. However, in advanced stages, despite high MCN, depleted VEGF expression indicates the failure of this mechanism. Mast cells may contribute to capillary loss indirectly by promoting fibrosis and inflammation, which degrades the local microenvironment, overwhelming their potential pro-angiogenic capacity. These findings underscore the pivotal, albeit complex role of mast cells in the pathology of hypertensive nephropathy.

KEY WORDS: Mast cells; Vascular endothelial growth factor (VEGF); Kidney; Capillary rarefaction; Hypertension.

INTRODUCTION

Worldwide, hypertension remains a major factor for morbidity, targeting a variety of organs and systems and causing significant health and economic challenges (Hao *et al*., 2024). In regard to that, hypertensive nephropathy remains a serious challenge globally and it is one of the main factors for chronic kidney disease progression to end-stage renal disease (ESRD) (Costantino *et al*., 2021). Hypertension-induced renal injury leads to a continuous loss of nephrons and changes in the tubulointerstitium, development of nephrosclerosis and reduced capillary density with prominent vasoconstriction in both the cortex and the medulla of the kidney (Costantino *et al*., 2021).

Mast cells are tissue-resident immune cells, originating from multipotent progenitor myeloid cells (Stanchev *et al*., 2020). After their maturation, mast cells can be located in various organs, including kidney, often found in close proximity to vessels. This perivascular location positions them ideally to sense and react to hemodynamic changes, especially relevant under hypertensive conditions (Wang *et al*., 2024). While mast cell number (MCN) is relatively low under normal physiological conditions, it is known that under pathological conditions, including hypertensive nephropathy, it increases notably, highlighting their importance (Welker *et al*., 2008; Stanchev

[1]Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria.
[2]Department of Health Policy and Management, Faculty of Public Health 'Prof. Dr. Tzekomir Vodenicharov', Medical University of Sofia, Sofia, Bulgaria.
[3]Department of General and Clinical Pathology, Medical University of Sofia, Sofia, Bulgaria.
[4]Clinic of Rheumatology, University Hospital 'St. Ivan Rilski', Sofia, Bulgaria.
[5]Department of Rheumatology, Medical University of Sofia, Sofia, Bulgaria.
FUNDING. This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students - 2".

Received: 2025-07-18 Accepted: 2025-09-08

et al., 2020). The biological effect of mast cells is based upon the extensive spectrum of bioactive substances found inside their granules, including tryptase, chymase and renin which confer potent profibrotic role to mast cells (Veerappan *et al*., 2012). CD117, also known as KIT proto-oncogene receptor tyrosine kinase (c-Kit), is a type III transmembrane receptor expressed by resident renal progenitors, differentiated tubular epithelial cells, and infiltrating mast cells (Gomes *et al*., 2018). While it mediates reduced apoptosis and promotes tissue repair and functional recovery in resident renal cells, CD117 plays a pro-inflammatory and pro-fibrotic role in mast cells (Gomes *et al*., 2018).

Vascular endothelial growth factor-A (VEGF-A), often referred to in the literature and herein simply as VEGF, is the primary pro-angiogenic factor which plays a crucial role in both physiological and pathological conditions, hypertensive nephropathy where renal VEGF expression is significantly higher (Advani *et al*., 2007). As shown previously, this upregulation becomes depleted at later stages of hypertension-induced kidney injury (Stanchev *et al*., 2023). VEGF-A is crucial for the preservation of the fenestrated endothelium of glomerular capillaries and their repair following injury (Advani *et al*., 2007). The main sources of VEGF in the kidney include podocytes and epithelial cells of distal tubules, proximal tubules and collecting ducts (Advani *et al*., 2007). Additionally, VEGF release by mast cells has also been reported, suggesting an important interplay under hypertensive conditions (McHale *et al*., 2019). Furthermore, mast cells appear to converge to areas with higher levels of VEGF which creates a unique feedback where stronger VEGF production leads to higher mast cell counts, which release and further increase local VEGF levels (McHale *et al*., 2019).

Capillary rarefaction is one of the hallmarks of hypertensive nephropathy strongly associated with the development of interstitial fibrosis (Kida, 2020). Reduced capillary density in the context of the hypertensive kidney leads to hypoxia which further deteriorates kidney injury, reduces its regenerative capacity and promotes fibrosis (Afsar *et al*., 2018). It has been reported that capillary rarefaction can be detected before any fibrotic changes occur in the kidney implying potential prognostic value (Afsar *et al*., 2018). Also, capillary density can be used as a quantitative parameter in the assessment of developing and deteriorating fibrosis (Iliev *et al*., 2024).

The spontaneously hypertensive rat (SHR) is a well-established animal model for essential hypertension (Hultström, 2012). Furthermore, the pathological changes in the kidneys of these rats, which result from increased blood pressure, are comparable to those in humans, making this strain particularly suitable for this study (Hultström, 2012).

As outlined above, VEGF serves as a primary regulator of angiogenesis and is directly connected to mast cells, which produce it and react to it. The exact changes in MCN, VEGF expression and supposed capillary rarefaction over the course of development of hypertensive nephropathy, however, have not been fully elucidated. Therefore, the aim of the present study was to analyze changes in MCN, VEGF expression and capillary density in two stages of hypertension-induced kidney injury and compare them to normotensive Wistar rats (WR) as controls.

MATERIAL AND METHOD

Experimental animals. For the present study, we used two age groups of SHR - 6-month-old, representative of early established hypertension, and 12-month-old, representative of advanced hypertension (Hultström, 2012). As controls we used age-matched normotensive WR. The animals were chosen randomly from a large population of SHR and WR, respectively, available at the Laboratory of the Department of Anatomy, Histology and Embryology at the Medical University of Sofia, Bulgaria. Each group consisted of six male rats. The rats were housed in Macrolon cages at 22 ± 1 °C and 55 ± 15 % humidity with 12 h light/12 h dark cycle. All animals were allowed free access to food and tap water. We measured systolic and diastolic blood pressure using the tail-cuff method with a Model MK-2000ST device (Muromachi Kikai Co., Ltd., Tokyo, Japan). All animal experiments were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council on protecting animals used for scientific purposes and was approved by the Bulgarian Food Safety Agency (Approval Protocol No. 311 of 29 July 2021).

Tissue preparation. Rats were anesthetized with an intraperitoneal (i.p.) injection of 40 mg/kg Thiopental (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) in 0.05 M Tris-HCl buffer (pH 7.6). After opening the thorax, a transcardial perfusion was performed using 4 % (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The kidneys were then quickly excised, rinsed in oxygenated physiological saline, and fixed for a minimum of 24 h in 10 % neutral phosphate-buffered formalin. After removal of the renal capsule, they were bisected along their longitudinal axis as described previously (Stanchev *et al*., 2020). Following the described methodology, the kidney slides underwent standard processing for routine light microscopic examination as previously described (Stamenov *et al*., 2022).

Immunohistochemistry. We conducted an immuno histochemical study using the heat-induced epitope retrieval technique following the methodology described (Stamenov *et al*., 2022; Gaydarski *et al*., 2024). The following antibodies were used:

1. a mouse monoclonal anti-VEGF-A IgG antibody (Santa Cruz Biotechnology Catalog No. sc-7269, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) at a concentration of 1:250;
2. a mouse monoclonal anti-mast cell tryptase IgG antibody (Santa Cruz Biotechnology Catalog No. sc-59587, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) at concentration 1:100;
3. a rabbit polyclonal anti-c-kit IgG antibody (Dako Catalog No. A4502, Dako, Glostrup, Denmark) at concentration 1:400.

All other steps and procedures followed the standardized protocol described in a previous study by Stamenov *et al*. (2022) and Gaydarski *et al*. (2024).

Semiquantitative analysis. Semiquantitative analysis of the immunohistochemical expression of VEGF was conducted following the standardized procedure outlined in our previous study (Stanchev *et al*., 2023), and we adhered to the established protocol.

Morphometric analysis of MCN and CD. In accordance with the previously standardized protocol, we evaluated MCN using tryptase-stained and CD117-stained slides (Stanchev *et al*., 2020). Haematoxylin and eosin-stained slides from each animal were used to evaluate the CD following the previously described protocol (Iliev *et al*., 2023).

Statistical analysis. The obtained quantitative data were analyzed using the SPSS software package (IBM Corp., Armonk, NY). Data distribution was not normal, as assessed through the Kolmogorov-Smirnov test. The Mann-Whitney nonparametric test was then used to test for statistically significant differences between MCN in 6-month-old SHR and age-matched controls; MCN in 12-month-old SHR and age-matched controls; CD in 6-month-old SHR and age-matched controls and CD in 12-month-old SHR and age-matched controls. In all statistical tests, we employed a standard level of significance a (p-value = 0.05). Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Blood pressure measurement. The mean values of the systolic and diastolic pressure of all examined animals are presented in Table I.

Changes in MCN and CD in SHR *vs*. WR. In both hypertensive and normotensive models, mast cells were found on slides stained for tryptase and CD117 as either single cells or groups of cells. They were observed as oval or irregularly shaped cells with intensively stained cytoplasmic granules in the periglomerular and peritubular interstitial tissue of the renal cortex (Figs. 1 and 2). A statistically significant difference in MCN per high-power field was reported between 6-month-old SHR and their respective age-matched normotensive controls, regardless of the staining method used for visualization (tryptase or CD117) (Table II; Fig. 3). A similar tendency was noted in 12-month-old hypertensive animals when compared to age-matched WR (Table III; Fig. 3). Interestingly, in 12-month-old SHR, all randomly examined high-power fields contained at least one mast cell and as many as three per field.

Table I. Mean systolic and mean diastolic blood pressure (mmHg) of 6- and 12-month-old spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar rats (WR).

Age group	Mean systolic blood pressure (mmHg) ± SD	Mean diastolic blood pressure (mmHg) ± SD
6-month-old SHR	180.1 ± 2.5	111.2 ± 4.2
6-month-old WR	111 ± 3.3	75.9 ± 3.6
12-month-old SHR	199.2 ± 2.3	124.1 ± 3.8
12-month-old WR	125 ± 4.5	84.6 ± 3.3

SD – standard deviation.

Table II. Descriptive statistics for the parameters capillary density (CD) per high-power field on hematoxylin and eosin-stained slides and mast cells number (MCN) per high-power field on slides immunohistochemically stained for tryptase and CD117 in the renal cortex of 6-month-old spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar rats (WR).

Parameter	N	6-month-old SHR					6-month-old WR					p-value
		Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	
CD	600	18.2	18	5.2	9	27	23.9	25	5.0	23	33	p<0.001
MCN tryptase	600	0.72	1	0.8	0	2	0.34	0	0.5	0	1	p<0.001
MCN CD117	600	0.69	0	0.8	0	2	0.28	0	0.5	0	1	p<0.001

N – number of high-power fields; SD – standard deviation.

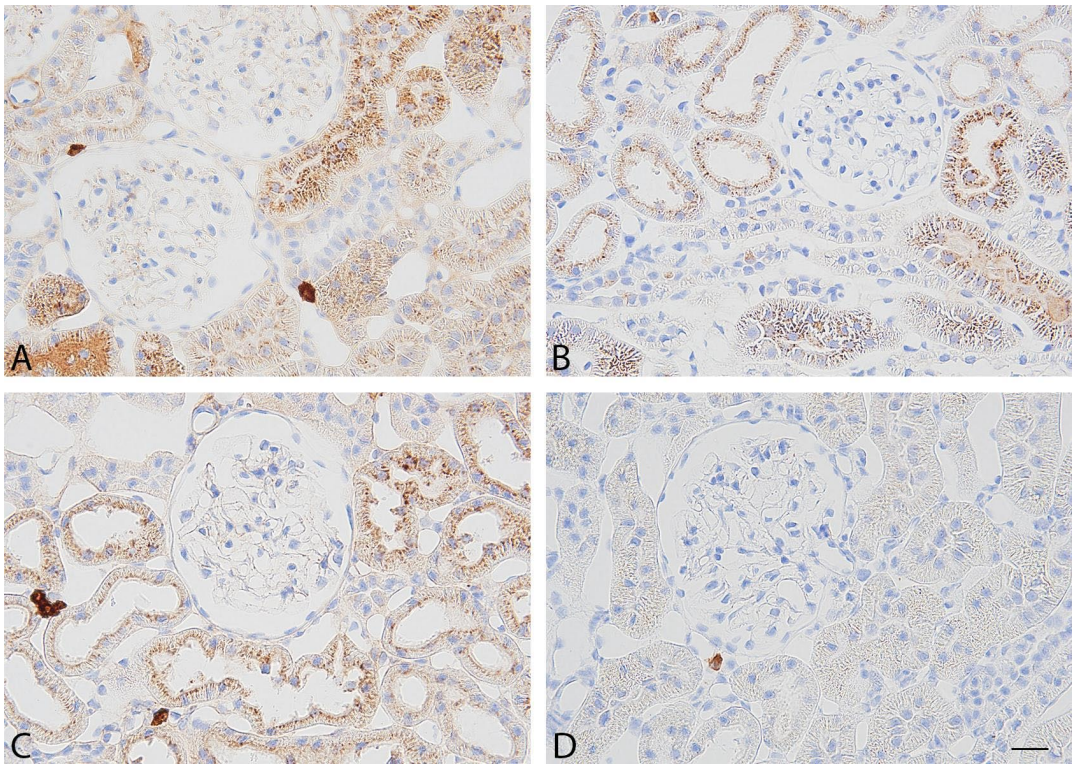

Fig. 1. Mast cells in the kidney of rats stained immunohistochemically with anti-tryptase antibody. A) renal cortex (RC) of 6-month-old spontaneously hypertensive rat (SHR). B) RC of 6-month-old Wistar rat (WR). C) RC of 12-month-old SHR. D) RC of 12-month-old WR. Scale bars in all panels – 20 µm.

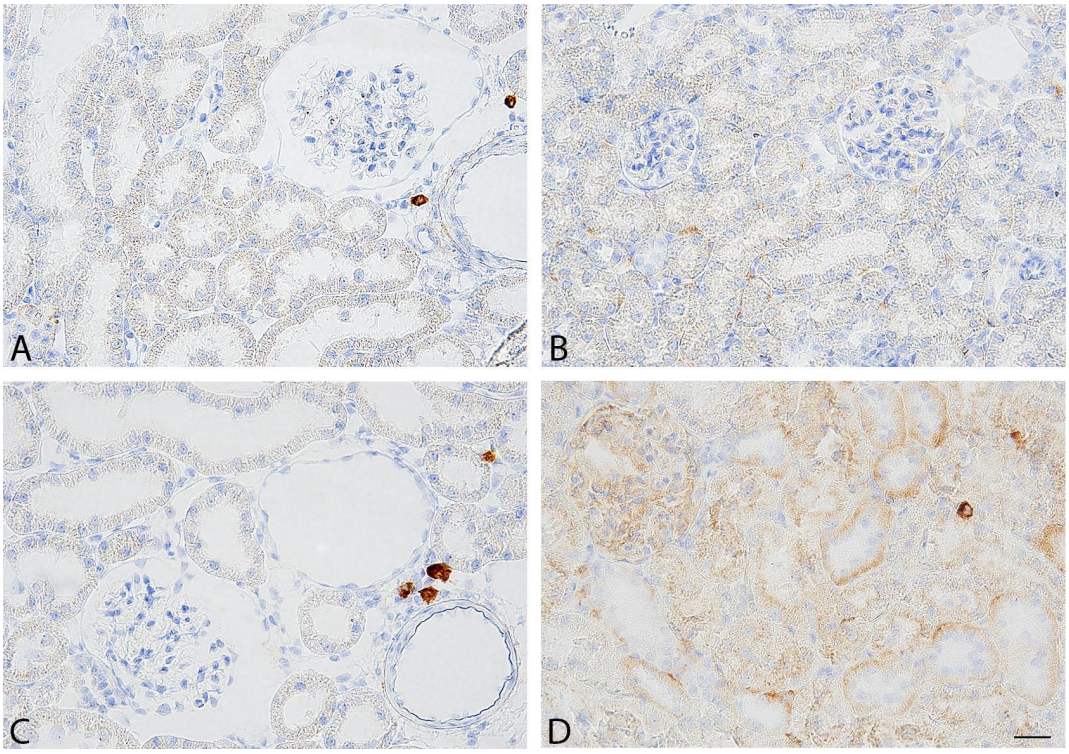

Fig. 2. Mast cells in the kidney of rats stained immunohistochemically with anti-c-Kit antibody. A) renal cortex (RC) of 6-month-old spontaneously hypertensive rat (SHR). B) RC of 6-month-old Wistar rat (WR). C) RC of 12-month-old SHR. D) RC of 12-month-old WR. Scale bars in all panels – 20 µm.

Table III. Descriptive statistics for the parameters capillary density (CD) per high-power field on hematoxylin and eosin-stained slides and mast cells number (MCN) per high-power field on slides immunohistochemically stained for tryptase and CD117 in the renal cortex of 12-month-old spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar rats (WR).

Parameter	N	12-month-old SHR					12-month-old WR					p-value
		Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	
CD	600	8.6	8.5	3.0	10	14	16.6	16	2.5	16	28	p<0.001
MCN tryptase	600	1.85	2	1.0	1	3	0.52	0	0.7	0	2	p<0.001
MCN CD117	600	1.79	2	1.0	0	3	0.54	0	0.8	0	2	p<0.001

N – number of high-power fields; SD – standard deviation.

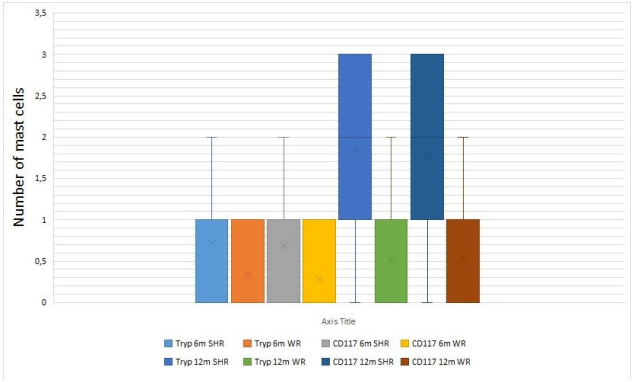

Fig. 3. Box and whisker plots illustrating the comparative statistical analysis for mast cell number (MCN) in renal cortex between 6- and 12-month-old spontaneously hypertensive rats (SHR) and age-matched Wistar rats (WR) using slides stained with anti-tryptase antibody (Tryp) and anti-c-Kit antibody (CD117).

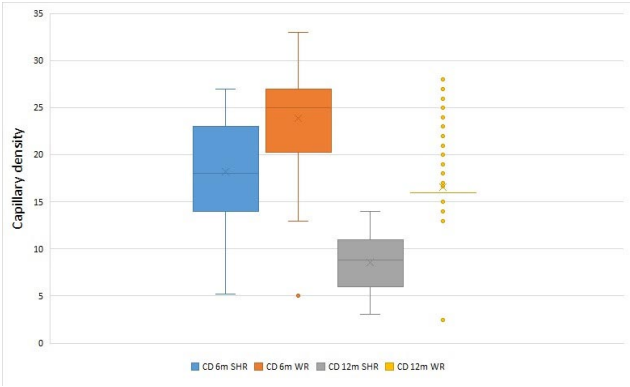

Fig. 4. Box and whisker plots illustrating the comparative statistical analysis for capillary density (CD) in renal cortex between 6- and 12-month-old spontaneously hypertensive rats (SHR) and age-matched Wistar rats (WR) using slides stained with hematoxylin and eosin.

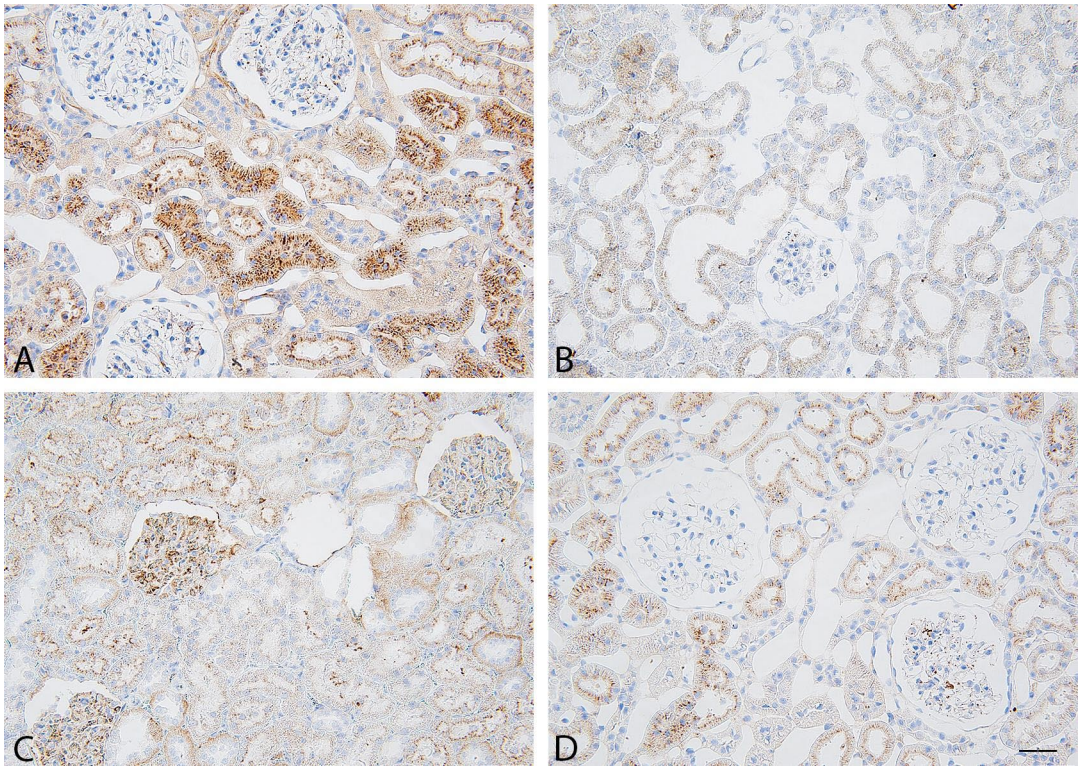

Fig. 5. Immunohistochemical staining for vascular endothelial growth factor (VEGF) in the kidney of rats. A) renal cortex (RC) of 6-month-old spontaneously hypertensive rat (SHR). B) RC of 6-month-old Wistar rat (WR). C) RC of 12-month-old SHR. D) RC of 12-month-old WR. Scale bars in all panels – 20 mm.

Table IV. Semi-quantitative analysis of the intensity of immunohistochemical staining for vascular endothelial growth factor (VEGF) in the renal cortex in 6- and 12-month-old spontaneously hypertensive rats (SHR) and age matched normotensive Wistar rats (WR), used as control group experimental animals.

VEGF	SHR	WR
6-month-old animals	High-positive (3+) – 37 % Positive (2+) – 39% Low-positive (1+) – 22 % Negative (0) – 2%	High-positive (3+) – 0 % Positive (2+) – 44% Low-positive (1+) – 38 % Negative (0) – 18 %
12-month-old animals	High-positive (3+) – 0 % Positive (2+) – 12 % Low-positive (1+) – 50 % Negative (0) – 38 %	High-positive (3+) – 0 % Positive (2+) – 20 % Low-positive (1+) – 53 % Negative (0) – 27 %

CD was highest in the group of 6-month-old WR, where the maximum reported number of capillaries per high-power field was 33, with a mean of 23.9 (Table II; Fig. 4). This CD was higher than the one observed in age-matched SHR and the difference was statistically significant. Furthermore, the mean CD of 12-month-old hypertensive models was the lowest recorded in all four age groups at 8.6 capillaries per high-power field and was significantly lower than the value reported in age-matched WR (Table III; Fig. 4).

Changes in VEGF expression in SHR *vs.* WR. VEGF immunoreactivity in the renal cortex was observed in the cytoplasm of cells lining the walls of proximal and distal tubules, as well as mesangial cells in the glomerular bodies (Fig. 5). The semi-quantitative assessment of staining intensity is reported in Table IV. In general, VEGF expression was highest in 6-month-old SHR where three-quarters of the examined fields were either high-positive or positive. In contrast, in age-matched WR, more than 80 % of the fields were read as either positive or low-positive but no fields were read as high-positive. This trend, however, was reversed in 12-month-old models, where no high-positive fields were reported in SHR and WR alike. In both groups, half the fields showed low-positive staining intensity, however, in SHR, 38 % of fields were read as negative, which was down to 27 % in the group of 12-month-old normotensive animals, suggesting that VEGF expression was lowest in SHR with advanced hypertensive kidney injury.

DISCUSSION

The present study aimed at investigating the changes in MCN in the context of well-studied capillary rarefaction in experimental models of two stages of hypertensive kidney injury (established vs advanced). We reported a statistically significant increase in MCN coupled with a statistically significant decrease in CD in both groups of SHRas opposed to age-matched normotensive WR. In addition, VEGF immunoreactivity was examined as a major driver of angiogenesis and we noted higher expression in the early stage of hypertensive kidney injury as opposed to age-matched normotensive controls - a difference, which was not reported in advanced hypertensive kidney injury when compared to the respective age group of WR.

A consistent finding across studies of both human hypertensive nephropathy and various experimental animal models of kidney disease is a notable increase in MCN within the renal tissue (Welker *et al*., 2008). In a study examining kidney tissue from middle-aged individuals with primary essential hypertension, the renal mast cell count was found to be approximately fivefold higher than in age-matched normotensive controls, indicating a significant accumulation of these cells in the hypertensive renal microenvironment (Welker *et al*., 2008). Furthermore, the extent of mast cell infiltration often shows a positive correlation with the severity of tubulointerstitial injury and the overall rate of renal disease progression, suggesting that the presence of these cells is linked to the extent of kidney damage (Santos *et al*., 2020). SCF observed in human hypertensive kidneys provides a potential mechanistic basis for this increased mast cell presence (Welker *et al*., 2008). SCF is a critical cytokine for mast cell growth, differentiation, survival, and migration, acting through its receptor c-Kit (CD117), which is expressed on mast cells (Wang *et al*., 2024). While an array of cells, including fibroblasts, endothelial cells and tumor cells may release SCF, it has been reported that mast cells themselves may produce it and therefore engage in local autocrine regulation (Bradding & Pejler, 2018). The marked increase in SCF expression within the hypertensive renal tissue suggests an active recruitment and/or local proliferation process, rather than merely a passive accumulation of these cells. This implies a sustained signaling environment within the diseased kidney that actively promotes and supports an expanded mast cell population, highlighting the SCF/c-Kit axis as a potential therapeutic target for limiting mast cell numbers in hypertensive nephropathy.

Mast cells have emerged as critical players in the intricate process of renal fibrogenesis (Veerappan *et al*., 2012). Their contribution is mediated by a number of secreted products that can influence virtually every step of the fibrotic cascade, from initiating inflammation to activating fibroblasts and promoting collagen deposition in the extracellular matrix (ECM). Tryptase is the most abundant enzyme stored in mast cell granules and is a key mediator of their pro-fibrotic effects (Wang *et al*., 2024). Upon release, tryptase can exert multiple actions that favor ECM accumulation. It has been shown to stimulate the proliferation of fibroblasts through FGF-2, increase their synthesis of collagen and other ECM proteins, and promote their differentiation into myofibroblasts, the principal effector cells in fibrosis (Stanchev *et al*., 2020; Wang *et al*., 2024). Tryptase can also enhance the production of interleukin-6 (IL-6) by fibroblasts, a cytokine with pro-inflammatory and potentially pro-fibrotic properties, and can directly activate latent transforming growth factor beta (TGF-b) to its biologically active form (Santos *et al*., 2020). Chymase, another prominent mast cell protease, exhibits a complex and sometimes paradoxical role in tissue remodeling and fibrosis. It converts angiotensin I to angiotensin II, thereby promoting its pro-fibrotic effects and can also directly activate latent TGF-b, further amplifying the fibrotic cascade (Hao *et al*., 2024). On the other hand, chymase can degrade certain ECM components, such as fibronectin, and activate matrix metalloproteinases (MMPs) (Wang *et al*., 2024). This suggests that chymase might, under specific circumstances, contribute to matrix remodeling in a way that limits excessive scar formation. This 'double-edged sword' nature means that the net effect of chymase on renal fibrosis likely depends on the balance of its various substrates in the local microenvironment, the stage of the disease, and the presence of other regulatory factors (Wang *et al*., 2024).

Mast cells are also known to possess a repertoire of mediators that can influence vascular growth and remodeling. Studies primarily in cardiac tissue have shown that mast cells can produce and release VEGF-A, a key angiogenic factor, and VEGF-C, the principal lymphangiogenic mediator (Poto *et al*., 2024). In the hypertensive myocardium, cardiac mast cells have been implicated in releasing VEGF and FGF-2, both of which promote the process of angiogenesis (Kotov *et al*., 2020; Poto *et al*., 2024). However, it is crucial to note that much of the evidence comes from non-renal tissues or from contexts other than hypertensive kidney disease (Corrêa Rassele *et al*., 2025). The promotion of VEGF-A synthesis in the kidney of SHR appears to have a renoprotective effect owing to a reduced inflammatory cell infiltration in the tubulointerstitium, preservation of the normal morphology of the glomerular filtration barrier and maintenance of renal microvasculature integrity (Dimke *et al*., 2015). Nevertheless, direct evidence demonstrating that renal mast cells are a quantitatively significant source of VEGF or other key angiogenic factors specifically within the microenvironment of the hypertensive kidney in animal models is not strongly presented in the available literature. Although mast cells possess the enzymatic machinery to produce VEGF, their specific quantitative contribution to the overall VEGF milieu within the hypertensive kidney, relative to other well-established renal sources of VEGF (such as podocytes, tubular epithelial cells, or even infiltrating macrophages), is not well elucidated in the current literature. It is plausible that mast cells contribute to renal VEGF levels indirectly. For example, pro-inflammatory mediators released from activated mast cells (e.g. tumor necrosis factor-alpha or IL-6) could stimulate resident renal cells or other infiltrating immune cells to upregulate their own VEGF production. Conversely, a chronic inflammatory environment heavily influenced by mast cells might also dysregulate normal VEGF signaling pathways (Hao *et al*., 2024). The lack of strong, direct evidence from hypertensive kidney models points to a potential knowledge gap: either the role of mast cell-derived VEGF in this specific renal context is minor, highly dependent on specific circumstances not yet identified, or it remains an under-investigated area.

In the context of the present study, the higher MCN in 6-month-old SHR compared to age-matched normotensive WR, coupled with a stronger VEGF expression, may indicate that in early hypertension-induced kidney injury, mast cells may promote angiogenesis as a compensatory mechanism for imminent capillary rarefaction with the onset of more pronounced interstitial fibrosis. This mechanism, however, appeared to be depleted at the stage of advanced hypertensive damage (12-month-old SHR), where VEGF expression was lowest despite the higher MCN. It is plausible that other molecular cascades, including the apelin/apelin receptor pathway and neuronal nitric oxide synthase (nNOS)-derived production of nitric oxide (NO), may act as compensatory mechanisms at that point with the ultimate goal of restoring VEGF production (Gaydarski *et al*., 2024). On the other hand, considering the 'double-edged sword' nature of mast cells in the hypertensive kidney, one could hypothesize that the increase in MCN promotes further local inflammation and fibrosis which ultimately depletes other major sources of VEGF such as podocytes and tubular epithelial cells. This theory would be in line with our earlier findings of positive correlations between MCN and markers of kidney injury, such as the glomerular sclerosis index and tubulointerstitial damage index (Stanchev *et al*., 2020).

A common pathological feature of progressive hypertensive nephropathy is capillary rarefaction - a

reduction in the density of peritubular and sometimes glomerular capillaries (Gaydarski *et al*., 2025). Several indirect mechanisms could link mast cell activity to capillary rarefaction. Firstly, mast cell-driven fibrosis can lead to the compression and eventual obliteration of capillaries as the interstitial space becomes overloaded with ECM. Secondly, mast cell-derived mediators, particularly angiotensin II generated via mast cell renin and chymase can cause sustained vasoconstriction of renal arterioles and potentially peritubular capillaries (Veerappan *et al*., 2012). Chronic hypoperfusion resulting from such vasoconstriction can lead to endothelial cell injury, apoptosis, and eventual capillary dropout. Thirdly, the chronic inflammatory state perpetuated by mast cells can lead to endothelial dysfunction, impairing the ability of endothelial cells to survive, proliferate, and form stable capillaries. Thus, despite their capacity to produce some angiogenic factors, the dominant pro-inflammatory and pro-fibrotic actions of mast cells in the hypertensive kidney might create a microenvironment that is hostile to endothelial cell survival and normal angiogenesis, thereby contributing indirectly to capillary rarefaction. This loss of microvasculature is detrimental, as it exacerbates renal hypoxia, promotes inflammation, and contributes significantly to the progression of interstitial fibrosis and loss of renal function (Liu, 2011). Furthermore, peritubular capillary rarefaction contributes to the development of hypertensive nephrosclerosis and shows a positive correlation with the severity of tubulointerstitial injury (Kida *et al*., 2020). These findings suggest a potential 'vicious cycle' in which mast cells may very well play a 'double-faced role' as either an 'unsung hero' with compensatory renoprotective impact through promotion of angiogenesis or the 'chief villain' orchestrating the progression of kidney damage through capillary rarefaction and promotion of renal fibrosis.

There were several limitations to the present study. First, we did not establish a direct causal link between the increase in MCN and the reported parallel decrease in CD. Therefore, these two events may not be directly related; however, as implied above, mast cells are able to indirectly influence capillary rarefaction through promotion of fibrosis and alteration of VEGF synthesis. Second, as outlined above, mast cells are not the only source of VEGF in the kidney and therefore changes in VEGF expression may not be solely attributed to mast cell function. Third, VEGF immunoreactivity was assessed semi-quantitatively and despite the use of dedicated software is subject to bias. Fourth, our observations on hypertensive kidney injury were confined to the renal cortex and should not be extrapolated to the renal medulla. Last but not least, the species-specific unipapillar structure of the rat kidney does not allow for a clear extrapolation of the present results to the multipapillar human kidney.

CONCLUSIONS

The present study reported a statistically significant higher MCN in SHR compared to age-matched normotensive controls which mirrored the observed decrease in CD. Changes in VEGF expression were somewhat less straightforward and suggest that in early hypertension-induced kidney injury, its elevated levels aim to compensate for capillary rarefaction. Based on earlier findings, it can be suggested that mast cells contribute to the loss of capillaries through several pathogenic mechanisms but as shown in other tissue, may also ultimately be a source of VEGF, thus promoting angiogenesis and exhibiting renoprotective functions. These results highlight that mast cells are emerging as pivotal players in the complex pathobiology of hypertensive kidney disease, primarily by fueling inflammation and driving renal fibrosis. While their direct impact on renal VEGF and capillary dynamics requires more focused investigation, their established pro-fibrotic roles make them compelling therapeutic targets. Addressing the identified knowledge gaps through rigorous future research will be essential to fully harness the potential of modulating mast cell activity for the prevention and treatment of hypertensive nephropathy.

ACKNOWLEDGMENTS. This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students - 2".

STANCHEV, S.; ILIEV, A.; STAMENOV, N.; GAYDARSKI, L.; PETROVA, K.; LANDZHOV, B.; KIRKOV, V.; IVANOVA, V.; DIKOV, T.; ERMENCHEVA, P. & KOTOV, G. Mastocitos y rarefacción capilar en la nefropatía hipertensiva: ¿un posible papel doble? *Int. Morphol., 43(6)*:1964-1972, 2025.

RESUMEN: La hipertensión a menudo conduce a la nefropatía hipertensiva, caracterizada por nefroesclerosis y rarefacción capilar. Los mastocitos, células inmunitarias residentes en los tejidos, están implicados debido a su ubicación perivascular y la liberación de sustancias bioactivas, mientras que el factor de crecimiento endotelial vascular (VEGF) desempeña un papel complejo en la salud renal y la angiogénesis. El objetivo del presente estudio fue analizar los cambios en el número de mastocitos (MCN), la expresión de VEGF y la densidad capilar (CD) en dos etapas de la lesión renal inducida por hipertensión y compararlos con controles normotensos. En este estudio utilizamos ratas espontáneamente hipertensas (SHR) a los 6 y 12 meses de edad y ratas Wistar normotensas (WR) de la misma edad. Los tejidos renales se analizaron para triptasa de mastocitos, CD117 y VEGF mediante expresión inmunohistoquímica y análisis semicuantitativo posterior para inmunorreactividad de VEGF y se cuantificó la densidad capilar. Los datos se evaluaron estadísticamente a través del método de Mann-Whitney. Informamos un MCN significativamente mayor y una CD menor en SHR de 6 y 12 meses

de edad en comparación con los controles. La expresión de VEGF fue más alta en SHR de 6 meses de edad y más baja en SHR de 12 meses de edad. Nuestros resultados sugieren que el aumento inicial de MCN y la elevación de VEGF en la hipertensión temprana podría ser una respuesta angiogénica compensatoria al desarrollo de rarefacción capilar. Sin embargo, en etapas avanzadas, a pesar de la elevada concentración de MCN, la disminución de la expresión de VEGF indica la falla de este mecanismo. Los mastocitos pueden contribuir indirectamente a la pérdida capilar al promover la fibrosis y la inflamación, lo que degrada el microambiente local y sobrepasa su potencial capacidad proangiogénica. Estos hallazgos subrayan el papel fundamental, aunque complejo, de los mastocitos en la patología de la nefropatía hipertensiva.

PALABRAS CLAVE: Mastocitos; Factor de crecimiento endotelial vascular (VEGF); Riñón; Rarefacción capilar; Hipertensión.

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Corresponding author:
Georgi Kotov
Clinic of Rheumatology
University Hospital 'St. Ivan Rilski'
Department of Rheumatology
Medical University of Sofia
13 Urvich St.
1612 Sofia
BULGARIA

E-mail: gn_kotov@abv.bg