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Source / Izvornik: **Pathology, Research and Practice, 2007, 203, 647 - 652**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1016/j.prp.2007.06.005

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:282415

Tomić, K., Mladinov, D., Batelja-Vuletić, L., Spajić, B., Mijić, A., Tomas, D., Belicza, M., Krušlin, B. (2007) *Morphometric analysis of renal arteries in patients with renal cell carcinoma.* Pathology - Research and Practice, 203 (9). pp. 647-652.

http://www.elsevier.com/locate/issn/0344-0338

http://www.sciencedirect.com/science/journal/03440338

http://dx.doi.org/10.1016/j.prp.2007.06.005

http://medlib.mef.hr/295

MORPHOMETRIC ANALYSIS OF RENAL ARTERIES IN PATIENTS WITH RENAL CELL CARCINOMA

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Running title: Morphometry of renal arteries

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The study was accomplished at Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia.

Presented in part at the 20th European Congress of Pathology, Paris, France, September 3-8, 2005.

Keywords: renal artery, renal cell carcinoma, tunica media, atherosclerosis, fibromuscular dysplasia

SUMMARY

The aim of the study was to analyze morphometric parameters of renal arteries (longest diameter and tunica media thickness) in patients with renal cell carcinoma (RCC), their relationship to tumor necrosis, and compare them with morphometric parameters recorded in a control group.

We analyzed archival cases of RCC diagnosed in the year 2003 that also contained routinely sampled specimens of distal segments of renal artery. Control group consisted of specimens from both renal arteries obtained from 16 patients at routine autopsy during the 2004-2005 period. Autopsy as well as further histological analysis did not disclose any malignant disease in the control group. Morphometric analysis of diameter and thickness of the renal artery tunica media was performed by use of Issa 3.1 software (Vamstek 2002, Zagreb, Croatia).

Comparison of tunica media thickness showed that renal arteries from RCC cases were significantly thicker when compared with distal parts of renal arteries in the control group ($p=0.0002$). Although renal artery samples from cases with necrotic tumor areas were thicker than those without tumor necrosis, the difference was not statistically significant.

It is concluded that significantly thicker tunica media characterized renal arteries in the group of patients with RCC when compared with the control group.

INTRODUCTION:

Renal cell carcinoma (RCC) accounts for 1%-3% of all human cancers and over 90% of malignant renal tumors in adults. It is 2-3 times more common in men, and the average age at diagnosis is 55-60 years [3, 15]. Tobacco smoking, obesity and hypertension are considered as the most important risk factors [1, 3]. Although hypertension is a well-known risk factor in patients with RCC, it can also represent a paraneoplastic symptom of the tumor itself [7].

The major prognostic factors for the outcome of RCC patients are tumor size, histological type, nuclear grade, stage of disease, and metastatic dissemination [2]. Recently, the presence and extent of tumor necrosis are also considered as a predictor of more aggressive tumor behavior and poorer prognosis [6, 17]. The majority of authors suggest that decreased tumor microvessel density leads to chronic hypoxia of the tumor tissue, which then causes coagulative necrosis [10, 17]. However, some authors suggest that pathologic changes in renal arteries could also be responsible for tumor necrosis in RCC [11, 20].

Different lesions, most frequently atherosclerosis, and less commonly fibromuscular dysplasia (FMD) or some other conditions such as Takayasu arteritis, radiation injury and congenital malformations [15], may affect the main renal artery. Atherosclerosis is a generalized progressive arterial disease associated with localized arterial occlusions that accounts for 90% of cases of renal artery stenosis. It usually involves the ostium and the proximal third of the main renal artery and perirenal aorta [15, 16].

Arterial FMD is a non-inflammatory, non-atherosclerotic, occlusive condition of systemic arteries with predilection for renal and internal carotid arteries [19]. FMD of

renal arteries is bilateral in nearly half of patients, and more common on the right side (3:1); it is usually diagnosed in the fourth decade, with female predominance and most frequently affecting the distal two thirds of the renal artery and its branches [15, 16]. The exact incidence of FMD is not known due to a large number of asymptomatic cases, however, an autopsy study showed an incidence of 1% [9, 12]. Intimal, medial and adventitial FMD are three major pathologic subtypes of FMD.

Although RCC is connected to hypertension and there are some suggestions that renal artery changes could be responsible for tumor necrosis in patients with RCC, the exact nature of this relationship is not fully understood.

The aim of this study was to analyze morphometric parameters of renal arteries (longest diameter and thickness of arterial tunica media) in patients with RCC, and to compare them with a control group of patients without RCC to gain additional, more objective, information about relationship between RCC and renal arteries.

MATERIALS AND METHODS:

We analyzed archival cases of RCC diagnosed in 2003 at Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia, which also contained samples of renal arteries. Out of the consecutive series of 61 (M:F=38:23) cases, 21 (14 male and 8 female) cases were excluded because of the tangentially cut or artificially damaged renal artery specimens, especially in cases of right simple nephrectomy when renal artery is cut at 50% to 75% of arterial length. The final sample consisted of 39 (M:F=24:15) RCC cases, patient age 35-74 (mean 58.4) years. Tumor size was in the range of 2-16 (mean 7.37) cm. All cases included in the study contained routinely sampled specimens of distal segments of renal artery as well as renal vein and ureteral margin. The presence and extent of tumor necrosis were characterized as absent, less than 50% tumor necrosis, and more than 50% tumor necrosis according to similar studies of RCC [11] and studies of soft tissue tumor grading [21].

Control group consisted of specimens from both renal arteries obtained during the 2004-2005 period from 16 (M:F=11:5) patients aged 32-85 (mean 62.3) years on routine autopsy at Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia. Autopsy as well as further histological analysis did not disclose any malignant disease in the control group, and causes of death were non-tumour diseases (heart failure, pneumonia, pulmonary thromboembolism). Measurements of renal arteries in the control group were performed on sections from 32 renal arteries. One block from distal part and one block from proximal part was morphometrically analysed in all 32 renal arteries.

FMD was classified into three principal pathological types: intimal, medial and

adventitial FMD [12]. Medial type (type II) was subdivided into three subtypes: medial "muscular" hyperplasia (type IIa), medial fibroplasia with aneurysms (type IIb) and perimedial fibroplasia (type IIc) [4, 13, 19, 23].

Specimens from both groups of patients were equally processed; first routinely fixed in 10% buffered formaldehyde, embedded in paraffin, cut at 5 μm and stained with haematoxylin-eosin and orcein, and examined by light microscopy.

Morphometric analysis of longest diameter and thickness of renal artery tunica media on adequate sections was performed by use of a JVC TK-1270 video camera, Leica Diaplan microscope and Issa 3.1 computer software (Vamstek 2002, Zagreb, Croatia). The longest diameter was measured from adventitia to adventitia, and thickness of tunica media from elastica interna to elastica externa using slides stained with orcein (Figure 1A and Figure 1B). In the group of patients with RCC one bloc per case was evaluated, because after nephrectomy only short distal segment of renal artery was available for histologic analysis. In both groups we used 3 measurements per case and then counted mean value.

Mann-Whitney U test was used on statistical analysis. The level of significance was set at $p<0.05$.

RESULTS

In the study sample of 39 RCC cases, there were 24 (61.5%) male patients aged 38-74 (mean 57.2) years and 15 (38.5%) female patients aged 35-74 (mean 53.6 years). Pathologic changes of renal arteries were found in 24 renal artery sections (M:F=12:12), whereas 15 (M:F=11:4) cases were free from pathologic changes of renal artery samples. Out of 24 cases with renal artery changes, renal artery atherosclerosis was recorded in 6 (M:F=4:2) and FMD in 18 (M:F=8:10) cases (Figure 2A, Figure 2B and Figure 2C).

Results of morphometric measurements of renal artery longest diameter and tunica media thickness in the group of RCC patients are shown in Table 1. Statistical analysis yielded no significant difference in renal artery longest diameter or media layer thickness between the patients with renal artery lesions (FMD or atherosclerosis) and patients without renal artery lesions (p=0.885 for diameter and p=0.828 for media thickness, p=0.213 for longest diameter and p=0.087 for media thickness, respectively). Comparison of cases with FMD and atherosclerotic changes in renal artery yielded no significant difference either (p=0.205 for longest diameter and p=0.096 for tunica media thickness).

Tumor necrosis was found in 31 (79.5%) RCC cases, of which 23 (M:F=15:8) had less than 50% necrosis and 8 (M:F=4:4) tumors contained more than 50% of necrotic areas.

Sections of renal arteries without tumor necrosis had median diameter of 3492.27 μm (range 2757.30-3852.82 μm) and media thickness of 499.41 μm (range 353.37-643.03 μm). Median diameter of renal arteries with less than 50% of necrotic areas was 3448.18 μm (range 837.30-5248.28 μm) and tunica media thickness was 531.37 μm (range167.86-1153.30 μm). Median diameter of renal arteries with tumors containing more than 50% of

necrotic areas was 4233.75 μm (range 2377.35-5180.04 μm) with thickness of tunica media measuring 657.32 μm (range 365.45-969.52 μm). Comparison of diameter and thickness of tunica media of renal arteries from cases without necrosis to those with necrotic areas and between cases with different extent of tumor necrosis produced no statistically significant differences ($p>0.05$).

In the control group, pathologic changes of renal arteries were found on 17 (53.1%) sections from proximal parts of renal arteries and only 6 (18.8%) sections from distal segments of renal arteries. All changes observed on both distal and proximal sections were atherosclerotic, whereas FMD was not found at all. Measurements of distal and proximal sections in the control group are presented in Tables 2 and Table 3. Statistical analysis revealed no significant gender differences in renal artery diameter and tunica media thickness in either distal or proximal segments of renal arteries ($p>0.05$).

Comparison of renal artery diameter and tunica media thickness between sections without lesions and those with atherosclerosis within the control group yielded no statistical significance for either distal or proximal segments ($p>0.05$).

Longest diameter of distal segments of renal arteries in the control group was slightly wider when compared to renal arteries with RCC but the difference did not reach statistical significance ($p>0.05$). However, comparison of tunica media thickness showed renal arteries from RCC cases to be significantly thicker when compared to distal segments of renal arteries in the control group ($p=0.0002$). Comparison of tunica media thickness between renal artery sections with atherosclerosis from RCC group (median 622.63 μm) and distal sections with atherosclerosis from the control group (median 434.37 μm) yielded a statistically significant difference ($p=0.019$). A statistically

significant difference was also found when tunica media thickness of sections without pathologic changes on renal arteries from RCC group was compared to sections of distal parts without pathologic changes in the control group (p=0.010).

DISCUSSION

Although the present as well as previous studies [11, 20] pointed to a high rate of renal artery lesions in RCC patients, we found no significant differences in renal artery diameter or media layer thickness between renal arteries without changes and those with FMD or atherosclerotic lesions. This finding was not unexpected since our study of the control group as well as other studies [4, 12, 16, 19] showed renal artery changes to be mostly segmental, with only a small part of renal artery being available for sampling and histological examination after radical nephrectomy due to RCC.

However, when renal artery sections from the group of RCC patients were compared to distal segments of renal arteries from the control group, we found that tunica media was significantly thicker in the group of RCC patients. The difference in media layer thickness was also present when only sections with atherosclerosis from both groups were compared, as well as on comparison of sections without pathologic changes. These results suggest that renal arteries in RCC patients are changed, with a thicker tunica media that can narrow arterial lumina and influence arterial blood flow.

According to some authors, necrosis in RCC is attributed to a decrease in microvessel density and immaturity of microvessels with the possibility that tumor necrosis is a consequence of acute hypoxia [10, 17]. However, the exact pathogenesis of tumor necrosis as well as the cause and nature of renal artery changes and the possible impact

on tumor necrosis have not yet been fully clarified [11, 17]. We observed an increase in media layer thickness in the group of RCC patients, with the presence and increased extent of tumor necrosis (no necrosis: less than 50% necrosis: more that 50% necrosis= 499.4 μm: 531.4 μm: 657.3 μm), however, the differences were not statistically significant. Concerning a relatively small number of cases without tumor necrosis and a small number of cases with more than 50% tumor necrosis in the present study, the hypothesis that renal artery changes induce necrosis or influence the extent of tumor necrosis should be further analyzed (in larger series and in comparison with microvessel density).

In the hypertensive population, high blood pressure is manly attributed to atherosclerosis, whereas renovascular FMD is considered to be the cause of hypertension in less than 2% of patients [23]. In patients with renovascular hypertension, FMD is the underlying cause in 20%-50% of cases [12]. Hypertension has been implicated as a risk factor for the development of RCC, but also as a paraneoplastic manifestation of RCC [1, 7, 18]. Hypertension has also been implicated as a risk factor for increased mortality from RCC [5, 8].

Due to the asymptomatic nature in a large number of cases and inadequately sensitive imaging methods, the true incidence and prevalence of FMD in the general, healthy population and in patients with hypertension or RCC remains unknown. The best method for detecting renal artery stenosis is digital subtraction angiography, however, the usage of this method in daily routine is very rare, in patients with renal tumor in particular [14, 22].

Based on our results, we may conclude that significantly thicker tunica media

characterized renal arteries in the group of patients with RCC when compared to the control group, without significant difference between arteries with pathologic changes and without changes. These findings suggest that renal arteries in all patients with RCC are changed, but since distribution of FMD and atherosclerosis could be only segmental, their presence may not be identified in all cases because whole renal artery is not available for pathologic examination due to operative technique. Yet, it remains unknown whether they are the consequence of tumor or represent a risk factor for hypertension or even tumour growth. The morphometric results of our study encourage further studies to elucidate the cause for the high rate of renal artery lesions and the association of these lesions with the development and extent of tumor necrosis as well as the exact relationship of hypertension and RCC.

REFERENCES

1. W.H. Chow, G. Gridley, J.F. Fraumeni, B. Järvholm, Obesity, hypertension, and the risk of kidney cancer in men. N. Engl. J. Med. 343 (2000) 1305-1311.
2. H.T. Cohen, F.J. McGovern, Renal-Cell Carcinoma. N. Engl. J. Med. 353 (2005) 2477-2490.
3. J.N. Eble, K. Togashi, P. Pisani, Renal cell carcinoma, In: J.N. Eble, G. Sauter, J.I. Epstein, I.A. Sesterhenn (Eds.), Tumors of the urinary system and male genital organs, World Health Organization classification of tumors, IARC Press, Lyon, (2004), pp12-14.
4. A.Z. Fenves, V.S. Ram, Fibromuscular dysplasia of the renal arteries. Curr. Hypertens. Rep. 1 (1999) 546-549.
5. A.E. Fletcher, D.G. Beevers, C.J. Bulpitt, E.C. Coles, C.T. Dollery, J.G. Ledingham, A.J. Palmer, J.C. Petrie, J. Webster, Cancer mortality and atenolol treatment. BMJ. 306 (1993) 622-623.
6. I. Frank, M.L. Blute, J.C. Cheville, C.M. Lohse, A.L. Weaver, H. Zincke, An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy used on tumor stage, size, grade and necrosis: the SSIGN score. J. Urol. 168 (2002) 2395-2400.
7. P.J. Gold, A. Fefer, J.A. Thompson, Paraneoplastic manifestations of renal cell carcinoma. Semin. Urol. Oncol.14 (1996) 216-222.
8. E. Grossman, F.H. Messerli, V. Boyko, U. Goldbourt, Is there an association between hypertension and cancer mortality? Am. J. Med. 112 (2002) 479-486.
9. M.J. Heffelinger, K.E. Holley, E.G. Harrison JR, J.C. Hunt, Arterial fibromuscular

dysplasia studied at autopsy. Am. J. Clin. Pathol. 54 (1970) 274.

10. B. Hemmerlein, A. Kugler, R. Olisik, R.H. Ringert, H.J. Radzun, P. Thelen, Vascular endothelial growth factor expression, angiogenesis, and necrosis in renal cell carcinomas. Virchows Arch. 439 (2001) 645-652.
11. B. Kruslin, K. Tomic, D. Tomas, D. Mladinov, D. Trnski, M. Belicza, The correlation between the tumor necrosis and renal artery changes in renal cell carcinoma. Int. J. Surg. Pathol. 14 (2006) 312-319.
12. T.F. Luscher, J.T. Lie, A.W. Stanson, O.W. Houser, L.H. Hollier, S.G. Sheps, Arterial fibromuscular dysplasia. Mayo Clin. Proc. 62 (1987) 931-952.
13. V. Nickeleit, S. Moll, E. Cynke, F.P. Brunner, M. Michatsch, A young woman with high blood pressure on haemodialysis: it is never too late to evaluate hypertension. Nephrol. Dial. Transplant. 14 (1999) 2734-2737.
14. R. Parasuraman, N. Attallah, K.K. Venkat, A. Yoshida, M. Abouljoud, S. Khanal, A. Greenbaum, Rapid progression of native renal artery fibromuscular dysplasia following kidney donation. Am. J. Transplant. 4 (2004) 1910-1914.
15. J. Rosai, Rosai and Ackerman's surgical pathology, Mosby, St. Louis, 2004.
16. R.D. Safian, S.C. Textor, Renal artery stenosis. N. Engl. J. Med. 344 (2001) 431-442.
17. S. Sengupta, C.M. Lohse, B.C. Leibovitz, I. Frank, R.H. Thompson, W.S. Webster, H. Zincke, M.L. Blute, J.C. Cheville, E.D. Kwon, Histologic coagulative tumor necrosis as a prognostic indicator of renal cell carcinoma aggressiveness. Cancer. 104 (2005) 511-520.
18. J.A. Shapiro, M.A. Williams, N.S. Weiss, A. Stergachis, A.Z. LaCroix, W.E.

Barlow, Hypertension, antihypertensive medication use and risk of renal cell carcinoma. Am. J. Epidemiol. 149 (1999) 521-530.

19. J.C. Stanley, B.L. Gewertz, E.L. Bove, V. Sottiurai, W.J. Fry, Arterial fibrodysplasia. Histopathologic character and current etiologic concepts. Arch. Surg. 110 (1975) 561-566.
20. K. Tomic, D. Tomas, I. Tomaskovic, M. Kos, M. Belicza, B. Kruslin, Renal artery changes in patients with primary renal cell carcinoma. Virchows Arch. 448 (2006) 24-28.
21. M. Trojani, G. Contesso, J.M. Coindre, J. Rouesse, N.B. Bui, A. de Mascarel, J.F. Goussot, M. David, F. Bonichon, C. Lagarde**,** Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. Int. J. Cancer. 33 (1984) 37-42.
22. G.B. Vasbinder, P.J. Nelemans, A.G. Kessels, A.A. Kroon, J.H. Maki, T. Leiner, F.J. Beek, M.B. Korst, K. Flobbe, M.W. de Haan, W.H. van Zwam, C.T. Postma, M.G. Hunink, P.W. de Leeuw, J.M. van Engelshoven, Accuracy of tomographic angiography and magnetic resonance angiography for diagnosing renal artery stenosis. Ann. Intern. Med. 141 (2004) 674-682.
23. S.P. Youngberg, S.G. Sheps, C.G. Strong**,** Fibromuscular disease of the renal arteries. Med. Clin. North. Am. 61 (1977) 623-641.

Table 1. Median, minimum and maximum values of measured parameters on renal arteries from cases with renal cell carcinoma

		Median (μm)	Minimum (μm)	Maximum (μm)
Diameter	All	3783.82	837.30	5248.28
	No changes	3808.79	859.95	5203.66
	FMD	3407.20	837.30	5214.47
	ATH	4154.71	3197.46	5248.28
Media layer thickness	All	531.37	167.86	1153.30
	No changes	503.61	167.86	969.52
	FMD	529.34	353.37	1153.30
	ATH	622.63	520.73	835.70

Legend: All=all cases with renal cell carcinoma; No changes=cases of renal cell carcinoma without renal artery changes; FMD=cases of renal cell carcinoma with fibromuscular dysplasia of renal arteries; ATH=cases of renal cell carcinoma with atherosclerosis of renal arteries.

Table 2. Median, minimum and maximum values of measured parameters on distal parts of renal arteries from control group

		Median (μm)	Minimum (μm)	Maximum (μm)
Diameter	All	4109.88	2386.33	7304.33
	No changes	4068.42	2386.33	7304.33
	ATH	4237.82	3723.11	4821.75
Media layer thickness	All	386.30	171.19	651.28
	No changes	363.89	171.19	651.28
	ATH	434.37	266.22	547.84

Legend: All=all sections from distal segments of renal arteries; No changes=sections from distal parts of renal arteries without pathological changes; ATH=sections from distal parts of renal arteries with atherosclerotic lesions of renal arteries.

Table 3. Median, minimum and maximum values of measured parameters on proximal parts of renal arteries from control group

		Median (μm)	Minimum (μm)	Maximum (μm)
Diameter	All	4999.14	2506.33	9045.64
	No changes	4940.09	2765.93	6757.04
	ATH	5052.17	2506.33	9045.64
Media layer thickness	All	368.22	138.31	721.66
	No changes	328.66	214.77	522.95
	ATH	407.77	138.31	721.66

Legend: All=all sections from proximal segments of renal arteries; No changes=sections from proximal parts of renal arteries without pathological changes; ATH=sections from proximal parts of renal arteries with atherosclerotic lesions of renal arteries.

Figure 1. Morphometric analysis of renal artery: (A) longest diameter, magnification 25X; (B) thickness of tunica media, magnification 40X; both microphotographs for morphometric analysis were stained with orcein.

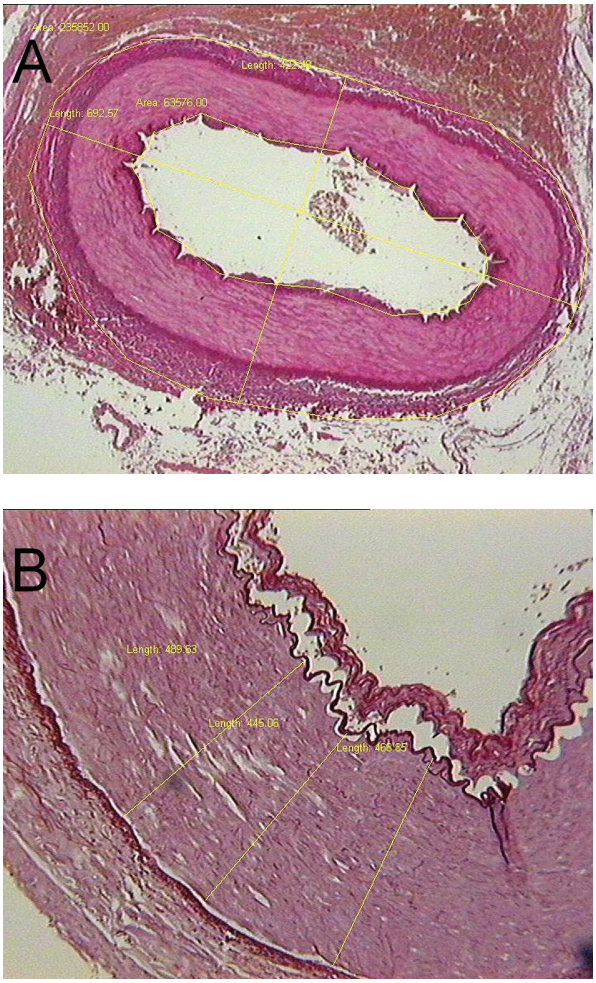

Figure 2. (A) Macroscopic appearance of renal cell carcinoma with atherosclerotic lesions of renal artery; (B) microphotograph of a renal artery with fibromuscular dysplasia IIb; (C) microphotograph of a renal artery with atherosclerotic lesions; microphotographs were stained with haematoxylin and eosin, magnification 40X.