Correlation between lymphangiogenesis and clinicopathological parameters in renal cell carcinoma

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INTRODUCTION Lymphangiogenesis has been reported to be important in the prognosis of several tumours. The aim of this study was to assess the correlation between lymphangiogenesis and clinicopathological prognostic parameters in patients with clear cell renal cell carcinoma.

METHODS 62 patients with renal cell carcinoma were included in the study. The D2-40 antibody, assessed immunohistochemically for each patient, was used as a marker. Light microscopy was used to determine the presence of intratumoral lymphatic vessels (ILVs) and the number of peritumoral lymph vessels (PLVs)/mm^2 or PLV density (PLVD). Correlation between the numbers and the Fuhrman nuclear grade, tumour stage, distant metastasis status, presence of lymph node metastasis and lymphovascular invasion was assessed.

RESULTS A significant correlation was found between the presence of ILVs and distant metastasis ($p = 0.033$) and lymph node metastasis ($p = 0.024$). However, no significant correlation was found between the Fuhrman nuclear grade ($p = 0.553$), tumour stage ($p = 0.464$) and lymphovascular invasion ($p = 0.242$). Mean PLVD was 20.8, and no significant difference was found between the patients with PLVD below average and those with PLVD above average in terms of distant metastasis ($p = 0.337$), lymph node metastasis ($p = 0.792$), the Fuhrman nuclear grade ($p = 0.566$), tumour stage ($p = 0.795$) and lymphovascular invasion ($p = 0.942$).

CONCLUSION We found a significant correlation between ILVs and lymph node and distant metastases in patients with renal cell carcinoma.

Keywords: D2-40, lymphangiogenesis, renal cell carcinoma
Singapore Med J 2012; 53(5): 332–335

INTRODUCTION

Renal cell carcinoma (RCC) is the most common tumour of the kidney.[1] In RCC, tumour dissemination occurs mainly with haematogenous spread, and metastasis is seen in about one-third of patients. Hilar and regional lymph node metastasis has been reported in 10%–25% of patients.[2-5] Metastasis to lymph nodes in patients with RCC is associated with a poor prognosis.[1,6,7] It has been reported in numerous studies that lymphangiogenesis has prognostic importance in many tumours. Recent studies have investigated lymphangiogenesis in RCC.[8-12] D2-40 is a novel monoclonal antibody that is routinely used as a marker for tumours of lymphatic origin. D2-40 has been used in practice as a selective immunohistochemical marker for lymphatic endothelium, and it is especially used in demonstrating lymphatic invasion by the primary tumour and in differentiating lymph vessels from blood vessels.[13,14]

The aim of this study was to assess the correlation between the presence of intratumoral lymphatic vessels (ILVs) and peritumoral lymphatic vessel (PLV) counts and the clinicopathological prognostic parameters in patients with RCC using D2-40.

METHODS

A total of 62 patients diagnosed with clear cell RCC during the period 2002–2010 at the Department of Pathology, School of Medicine, Harran University, Sanliurfa, Turkey, were included in this study. 34 (54.8%) patients were women and 28 (45.2%) were men. The mean age was 60.9 (range 29–85) years. Pre-operative stage and tumour disseminations were assessed using the radiological data of the patients. All patients underwent a radical nephrectomy, but lymph node dissection (LND) was not routinely performed. Limited LND was performed during surgery for 15 patients due to suspicion of metastasis.

Data on the clinicopathological prognostic parameters such as tumour stage and the presence of distant and lymph node metastases were retrieved from medical records and pathology reports. The haematoxylin and eosin-stained slides of the patients were retrieved from the archive and re-examined to determine the Fuhrman nuclear grade and the presence of lymphovascular invasion. Two paraffin blocks containing sufficient tumour tissue and the peritumoral region were selected for each patient. Sections of 4 µm were obtained from these blocks, deparaffinised by xylol and rehydrated by passing through the ethyl alcohol series. The antigen retrieval procedure was performed for 40 minutes at 95°C in 0.01 M sodium citrate solution. All preparations were kept in 3% hydrogen peroxide for 30 minutes for endogenous peroxidase blockage. D2-40 (code N1607, DakoCytomation, Carpinteria, CA, USA) was administered for two hours as the primary antibody. Thereafter, biotin-added anti-immunoglobulin and streptavidin-peroxidase-conjugated sections were incubated for ten minutes. To visualise the immunoreactions, the sections

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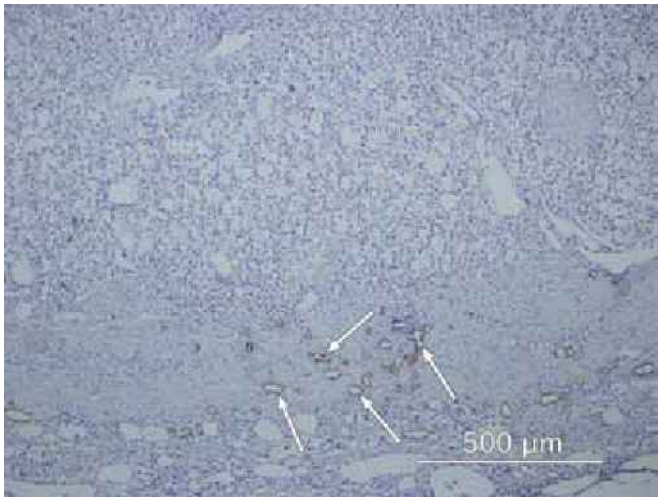

Fig. 1 Photomicrograph shows several lymph vessels (arrows) in the peritumoral area (D2-40, × 100).

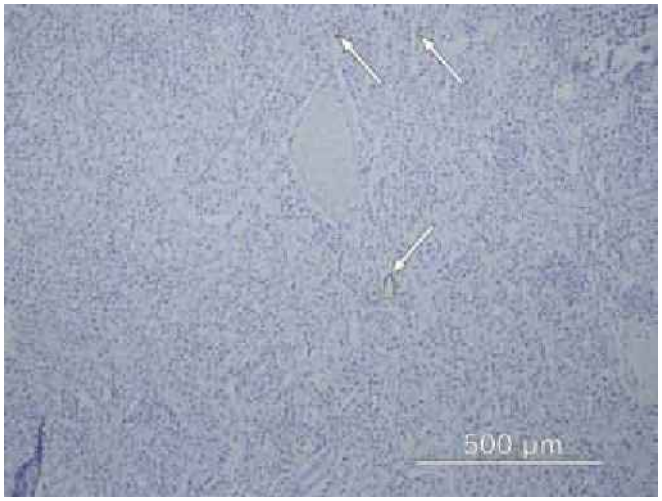

Fig. 2 Photomicrograph shows a few intratumoral lymph vessels (arrows) (D2-40, × 100).

Table I. Correlation between clinicopathological prognostic parameters and ILVs.

Parameter	ILV-positive	ILV-negative	p-value	Total
Distant organ metastasis				
M0	6	40	0.033	46
M1	6	10		16
Lymph node metastasis				
N0	1	11	0.024	12
N1-2	2	1		3
Fuhrman nuclear grade				
I	0	5	0.553	5
II	4	21		25
III	6	17		23
IV	2	7		9
Tumour stage				
I	2	16	0.464	18
II	6	18		24
III	3	15		18
IV	1	1		2
Lymphovascular invasion				
Yes	6	16	0.242	22
No	6	34		40
Total	12	50		62

ILV: intratumoral lymphatic vessel

were treated with 3,3'-diaminobenzidine tetrahydrochloride. Mayer's haematoxylin was then used for 60 seconds for counterstaining. Tonsillar tissue was used as the positive control in the immunohistochemical examination. Sections that had been treated with phosphate-buffered saline instead of the primary antibody were used as the negative control. The slides were examined with an Olympus BX51 microscope. Renal parenchyma extending to 500 µm from the tumour border was accepted as the peritumoral area. The Chalkley count method was used to count the lymph vessels in the peritumoral area. Three different areas where the lymphatics were dense were defined with low magnification (hotspots). PLV density (PLVD) was determined by counting the number of lymphatic vessels in an area of 1 mm^2 at 200 times magnification.[15] Due to the lower number of lymphatic vessels in patients with ILVs, these patients were assessed only for the presence of ILVs.

Correlation between the presence of ILVs and clinicopathological prognostic parameters such as the presence of distant organ and lymph node metastases, Fuhrman nuclear grade, tumour stage and lymphovascular invasion were analysed. Mean PLVD was calculated, and the patients were divided into two groups: those with PLVD below average and those with PLVD above average. These two groups were assessed with regard to the presence of distant organ and lymph node metastases, the Fuhrman nuclear grade, tumour stage and lymphovascular invasion. The chi-square test was used for statistical analyses. A p-value < 0.05 was considered statistically significant.

RESULTS

All the patients in the study were diagnosed with clear cell RCC. When assessed according to the Fuhrman nuclear grade, five (8%) patients were grade I, 25 (40.2%) were grade II, 23 (37%) were grade III and nine (14.4%) were grade IV. There were distant metastases in 16 (25.7%) patients. There was metastasis in three of the 15 (20%) patients undergoing LND. Staging according to the 2004 TNM classification of malignant tumours showed that 18 (28.9%) patients were pT1, 24 (38.6%) were pT2, 18 (28.9%) were pT3 and two (3.2%) were pT4.[5] In our study, PLVs were observed in 60 (96.7%) patients (Fig. 1). ILVs were observed in 12 (19.3%) patients (Table I). In patients found to have ILVs, the lymphatic vessels were very few in number and mostly collapsed (Fig. 2). There was a significant relationship between the presence of ILVs, distant metastasis (p = 0.033) and lymph node metastasis (p = 0.024). There was no correlation between ILVs and the Fuhrman nuclear grade (p = 0.553), tumour stage (p = 0.464) and lymphovascular invasion (p = 0.242). The mean number of PLVs/mm^2 or PLVD was 20.8. There were 28 patients with PLVD > 20.8 and 34 with PLVD < 20.8 (Table II). There was no

Table II. Correlation between clinicopathological prognostic parameters and PLVD.

Parameter	PLVD > 20.8 (n = 34)	PLVD < 20.8 (n = 28)	p-value	Total
Distant organ metastasis				
M0	23	22	0.337	45
M1	11	6		17
Lymph node metastasis				
N0	7	5	0.792	12
N1-2	2	1		3
Fuhrman nuclear grade				
I	3	2	0.566	5
II	15	10		25
III	13	10		23
IV	3	6		9
Tumour stage				
I	11	7	0.795	18
II	13	11		24
III	8	10		18
IV	1	1		2
Lymphovascular invasion				
Yes	10	8	0.942	18
No	24	20		44
Total	34	28		62

PLVD: peritumoral lymphatic vessel density

significant difference between these two patient groups in terms of distant metastasis (p = 0.337), lymph node metastasis (p = 0.792), Fuhrman nuclear grade (p = 0.566), tumour stage (p = 0.795) or lymphovascular invasion (p = 0.942).

DISCUSSION

RCC is the most common renal tumour. There are numerous clinicopathological parameters that are known to affect the prognosis in these patients, including distant metastasis, lymph node metastasis, tumour stage, the Fuhrman nuclear grade and the presence of vascular invasion. Many studies have shown that intratumoral and peritumoral angiogenesis have an effect on the prognosis in patients with RCC.[8,11] However, despite the numerous studies on angiogenesis in patients with RCC, there have been few studies on lymphangiogenesis until recently. The latest spurt in such studies is mainly due to the recent identification of immunohistochemical markers specific to the lymphatic endothelial cells.[9] D2-40 is a recently developed monoclonal antibody directed against the M2A antigen, which is a sialoglycoprotein originally detected in association with germ cell neoplasia and foetal testicular gonocytes. D2-40 has been used in practice as a selective immunohistochemical marker for lymphatic endothelium.[8,9,14,16]

Several studies have recently been performed to assess the correlation between lymphangiogenesis and clinicopathological prognostic parameters using the immunohistochemical method with D2-40 in various organs.[17-28] Fernández et al found a significant correlation between ILV counts and an increased grade in invasive transitional bladder carcinomas.[17] Zeng et al showed that PLVD and PLV invasion were significantly associated with lymph node metastasis in prostatic adenocarcinoma.[18] Giorgadze et al detected more lymphatics in patients with malignant melanoma than in those with benign nevuses.[21] Roma et al found a significant correlation between increased PLV counts and increased Gleason scores in patients with prostate adenocarcinomas.[26] In contrast, Miyahara et al[27] and Franchi et al[28] found that there was no significant correlation between ILV and PLV counts and tumour grade in patients with squamous cell carcinomas. ILVs could not be detected in the majority of patients with RCC in studies on lymphangiogenesis. Iwata et al found that only six (5.6%) RCC specimens showed D2-40-positive lymph vessels within the tumour area. In addition, this study found no correlation between the presence of ILVs and PLVD and various clinicopathological parameters.[11] Similarly, Ishikawa et al did not find any significant correlation between PLVD and lymph node metastasis.[12] Quite to the contrary, Horiguchi et al found ILVs in 18.9% of patients and a significant correlation between the presence of ILVs and clinicopathological prognostic parameters.[8]

In our study on patients with RCC, 60 (96.7%) patients were found to have PLVs and 12 (19.3%) patients had ILVs. We did not find a correlation between PLV counts and clinicopathological prognostic parameters. Our results and those of previous studies indicate that the presence and number of PLVs are of no significance in patients with RCC. However, we found a significant correlation between the presence of ILVs and clinicopathological prognostic parameters such as distant metastasis (p = 0.033) and lymph node metastasis (p = 0.024) in the patients studied. This finding suggests that the presence of ILVs could be used as a prognostic parameter for RCC. Although studies have shown that the presence of ILVs may have a bearing on patient prognosis, further studies are required, as such reports in the literature are few and their findings often conflicting.

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