

ORIGINAL ARTICLE

Vascular Endothelial Growth Factor Expression in Renal Cell Carcinoma

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ABSTRACT

Introduction: Renal cell carcinoma (RCC) is the most common renal malignancies. In advanced stage, it is highly resistant to systemic therapies. RCC is a highly vascular tumour and angiogenesis pathway has been postulated in its carcinogenesis. Novel drug targeting Vascular Endothelial Growth Factor (VEGF) and advanced surgical interventions have shown to increase the overall patients' survival. In this study, we evaluated the VEGF expression of RCC using immunohistochemistry technique and its potential correlation with the tumour grades. **Methods:** 40 RCC cases that underwent nephrectomy were selected. The archived samples of formalin fixed, paraffin-embedded (FFPE) were retrieved. The tumour tissue blocks were carefully chosen, sectioned and stained with VEGF using immunohistochemistry technique. The intensity of VEGF expression was scored as 0 (negative), 1+ (weak), 2+ (moderate) or 3+ (strong). **Results:** Majority of the RCC cases were male, with male to female ratio of 2.1:1. Mean patient age was 56.2 years (age ranged between 16 to 74 years). Most of the cases were Malays (42.5 %). VEGF was expressed in 36 (90%) of RCC cases. Among the 36 cases that were immunopositive, 8 (16.7%) were grade 1, 20 (55.6%) grade 2 and 8 (16.7%) grade 3. There was no significant association between VEGF expressions score and grades of RCC ($p=0.39$). **Conclusion:** VEGF was expressed in majority of RCC cases although there was no significant association with tumour grades.

Keywords: VEGF, Angiogenesis, Renal cell carcinoma, Tumour grade

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INTRODUCTION

Renal cancer is the 13th most common malignancy globally, and has increased by 2% annually during the last two decades (1). RCC or previously known as hypernephroma accounts for 90% of all renal malignancies. In the United States, RCC represented 5% of all cancers in men and 3% of all cancers in women in 2013. It is a cancer of adult and most often occurs between the sixth and seventh decades of life. The incidence of RCC is 1.6 to 2.0 times higher in men than in women; men account for almost two thirds of all deaths (2, 3). In Malaysia, RCC accounts for 43.8% of all renal cancer. The incidence is higher in men (1.7%) than in women (0.6%). It is most commonly found in Chinese followed by Malays and Indian (4). RCC originates from renal tubular epithelium and has a

wide variation of histopathological patterns. Three major types of RCC are clear cell carcinoma, papillary RCC and chromophobe RCC. The most common type is clear cell carcinoma, which accounts for 80% of all RCCs (5). RCC has unpredictable behaviour and its mortality rate is higher than other urological malignancies. It can metastasize to other organs such as lung, liver and adrenal gland (6). Approximately only 50-60% of RCC is localized and can be cured by surgical treatment.

RCC is a highly vascular solid tumour and acquires nutrients for survival by forming new blood vessels (neovascularization) or sprouting new blood vessels from old existing vessels (angiogenesis) (7, 8). Angiogenesis is the prime event to the onset of early tumorigenesis and it is found to be regulated by a number of angiogenic factors (9). One such factor is vascular endothelial growth factor (VEGF). Its angiogenic capability allows the tumour advancement and metastasis hence increases the grade and stage of the tumour.

VEGF is a dimeric 46-Kd, endothelial cell specific,

glycosylated, and heparin-binding cytokine. It is synthesized by both normal and tumour cells and acts specifically on endothelial cells by stimulating vascular endothelial growth (10). It is potent and has high specific angiogenic factor that is capable of stimulating tumour growth through its autocrine property. It also exerts paracrine effects by binding to specific tyrosine kinase-receptors on vascular endothelial cells and has vascular permeability factor functions that promote metastasis (11, 12).

Several studies have found significant differences in serum VEGF level between normal and RCC and significant correlations between serum VEGF levels with tumour volume. These findings showed that circulating VEGF has a potential as a biomarker for RCC (13, 14). Nevertheless, despite the significant correlation between serum VEGF levels with tumour volume, correlation between VEGF serum levels and tumour stage, patients' survival, or tumour metastasis were still unable to be determined (15). Serum VEGF level has limited value as a tumour marker; hence FFPE of RCC cases were used in this study (15-17)

In this study, we examined the VEGF protein expression in FFPE RCC samples from nephrectomy specimen using immunohistochemistry technique. The correlation between VEGF expression and tumour grades was also studied.

MATERIALS AND METHODS

Sample collection

This cross sectional study was conducted by retrieving RCC cases diagnosed within five years from the departmental archive. Cases of missing blocks or incomplete blocks were excluded. This study was approved by the Human Research Ethic Committee, Universiti Putra Malaysia (UPM/FPSK/PADS/T7-MJKEtikaPer/F01) and the Ministry of Health Malaysia (NMRR-08-481-1636).

Fuhrman grading system

Two independent pathologists reviewed all the selected RCC slides under light microscopy. The RCC grades were determined using Fuhrman grading system. The grading was based on the nuclear size, shape and nucleoli and was divided into 4 grades (grade I-IV).

Age and ethnic groups

Demographic data of RCC patients' undergone nephrectomy were obtained from the histopathological examination request forms submitted to the Department of Pathology.

Immunohistochemistry staining for VEGF

The FFPE blocks that showed RCC were selected. The blocks sectioned at 4µm thick using a microtome. The tumour tissues were put on Poly-L-Lysine coated slides.

Subsequently they were subjected to deparaffinization and rehydration using a series of xylene and alcohol solution at decreasing concentration. Antigen retrieval was carried out via microwave treatment at 100°C for 20 minutes before stained with primary antibody. The tissue sections were then treated with a 1:100 dilution of a monoclonal mouse anti-human VEGF antibody (Clone VG1; DAKO, Denmark). Detection was performed using the Dako Envision Detection system, Peroxidase/DAB+ (K4065; Dako). As part of a quality assurance, normal kidney tissue was selected as a positive control. The RCC cases were stained concurrently with the positive control tissue. The presence of cytoplasmic staining of the tubular cells of the kidney indicated that the positive control was working. The cases that showed optimum staining with minimum background staining were selected in this study.

Scoring for VEGF

The RCC slides that were stained with VEGF antibody were reviewed under light microscopy. The positive or negative staining was determined by the presence or absence of cytoplasmic staining. The intensity of VEGF expression was scored as 0 (negative), 1+ (weak), 2+ (moderate) or 3+ (strong). The cut off point for positive expression was presence 5% or more of VEGF expression of the tumour cells. If the staining was weak or equivocal, the slides were re-evaluated; and were regarded as negative if an equivocal result was obtained again. Only cytoplasmic with or without cytoplasmic membrane staining was considered as positive staining. Scoring for VEGF have been done by two qualified pathologists.

Statistical analysis

The demographic distribution and VEGF expression of the cases were analysed using SPSS version 21. The spearman correlation test was used to analyse correlation between VEGF expressions score with grades of RCC.

RESULTS

Forty cases of histologically confirmed renal cell carcinoma were included into the study. The mean age of the patients was 56.2 years (age ranged between 16 to 74 years). Of forty cases, there were 27 (67.5%) males and 13 (32.5%) females with a M: F ratio of 2.1:1. There were 17 (42.5%) Malays, 15 (37.5%) Chinese and 8 (20%) Indian. Table I illustrates the demographic profile of RCC in the study.

VEGF immunohistochemistry

Table II shows distribution of grades and VEGF positivity. 36 (90%) RCC were immunopositive for VEGF (Figure 1). Among the 36 cases that were immunopositive, 8 (16.7%) were grade 1, 20 (55.6%) grade 2, and 8 (16.7%) grade 3. There was no grade 4 RCC case in this study. Spearman's correlation test showed correlation coefficient -0.14 with the p value of 0.39, which is

Table I: Demographic data of renal cell carcinoma cases (n=40)

Sex distribution	Male	27 (67.5%)
	Female	13 (32.5%)
Ethnic distribution	Malay	17 (42.5%)
	Chinese	15 (37.5%)
	Indian	8 (20%)
Age distribution	0-49 years	12 (30%)
	50-80 years	28 (70%)
	Range	16-74 years
	Mean	56.2 years

Table II: Distribution by grades of RCC and VEGF intensity

Grade of RCC	Intensity of VEGF				TOTAL (n=40)
	0 (no staining)	1 (weak)	2 (moderate)	3 (strong)	
1	1	2	1	5	9
2	1	3	10	7	21
3	2	3	1	4	10
4	0	0	0	0	0
	4	8	12	16	40

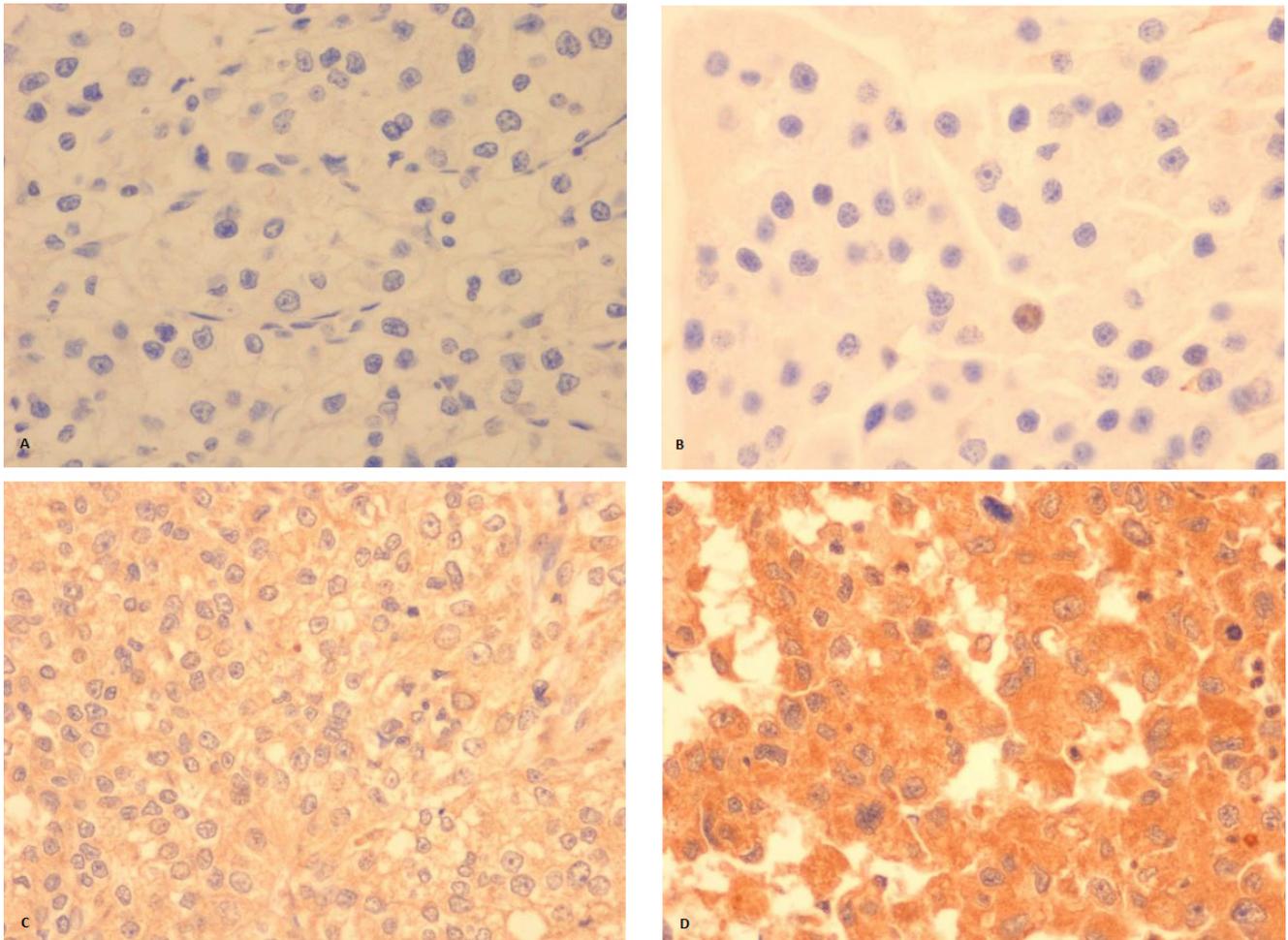


Figure 1: VEGF expressions in renal cell carcinoma. A) Immunonegative (0), B) Weak immunopositive (1+), C) Moderate immunopositive (2+) and D) Strong immunopositive (3+). (VEGF stain, Original magnification x400)

insignificant. As a result, the null hypothesis not rejected VEGF expression is not associated with higher grades of renal cell carcinoma.

DISCUSSION

RCC is an adult cancer and frequently occurs in sixth to seventh decades. Unhealthy lifestyle such as smoking and diseases like obesity and hypertension which are common among elderly have been implicated in the development of RCC (18). Cancer Registry report by Ministry of Health Malaysia (2007) reported that the

most affected race was Chinese and men was more commonly affected compared to women (4).

Expressions of VEGF antibody were mainly present on the cytoplasmic regions of the tumour cells and occasionally been expressed along the cell membranes. In this study, immunopositive expression was documented on the cytoplasmic regions with or without cell membranes expression.

This study showed high expressions of VEGF in most of RCC cases as 90% of the RCC samples demonstrated

immunopositive results. Brown et al. and Takahashi et al. also reported significant expressions of VEGF in RCC cases; 91.7% and 90% cases respectively (19, 20). Yang et al., suggested that high expressions of VEGF were interrelated with elevated activity of cancer development (21). VEGF helps tumour progression by increasing vascular permeability to plasma protein, induces endothelial division and migration, promotes endothelial cell survival by inhibiting apoptosis and reverses endothelial senescence (22-25).

Cancer progression is highly related to angiogenesis as uncontrolled cancer cells tend to exhaust their current blood supply. Larger tumour size tends to have inadequate blood supply thus inducing hypoxic condition leading to upregulation of VEGF expression (26). This suggests that VEGF could be manipulated to be a useful predictive tumour marker in RCC. Nevertheless many studies have found that the role of VEGF as a predictive biomarker are not conclusive (27, 28).

VEGF positive expressions were seen in other cancers such as in breast, lung cancer as well as in prostate cancer with a promising results as a tumour marker (6, 9). Nonetheless, this study was unable to show any positive relationship between VEGF expression and RCC grades. Our result concurs with a study conducted by Minardi et al., (2005) (29). They concluded that VEGF expressions could not be the only parameter used to predict survival in RCC. To date, Fuhrman grading appears to be the only predictive factor of survival and mortality in RCC. In this study, the insignificant value obtained might be due to the small sample size used, a larger sample size might produce a different outcome. Other factors that might affect our finding are sampling bias and uneven distribution of the grades.

CONCLUSION

This study showed high expression of VEGF in RCC although there was no positive correlation with the tumour grades. VEGF has a potential role as an alternative target inhibitor and prognostic marker in RCC as VEGF expressions are highly correlated with vascularity, tumour proliferation and metastasis. Further study with a larger sample size and even distribution of all grades are required to confirm its association with the tumour grades.

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