EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 12
15-21
Year: 2018

IMMUNOHISTOCHEMICAL EXPRESSION OF VEGF IN RENAL CELL CARCINOMA AMONG SUDANESE PATIENTS

Hayat Ali Ahmed Fadul*[1] and Dr. Ageeb Mohammed Hassan[2]

[1]Department of Histopathology Collage of Postgraduate Studies Alneelain University, Khartoum, Sudan.
[2]Assistant Professor-Department of Pathology-Elrazy University.

***Corresponding Author: Hayat Ali Ahmed Fadul**
Department of Histopathology Collage of Postgraduate Studies Alneelain University, Khartoum, Sudan.

Article Received on 27/09/2018 **Article Revised on 17/10/2018** **Article Accepted on 07/11/2018**

ABSTRACT

Background: Renal cell carcinoma (RCC) is the most common adult renal epithelial cancer in the United States and the ninth most common malignancy in Europe in 2008 until recently. Renal cell carcinoma continues to be a difficult malignancy to treat because of its ability to spread asymptomatically and its inherent resistance to conventional chemotherapy. **Objective**: To evaluate immunohistochemical expression of vascular endothelial growth factor in renal cell carcinoma in our population and correlate its expression with different variables such as age and grade, also determination the specificity of vascular endothelial growth factor in renal cell carcinoma by using immune-histochemical technique. **Materials and methods:** this descriptive cross sectiona studyl for analyzing data from 40 formalin-fixed of paraffin-embedded tissues. These sections from all cases were subjected to stain with haematoxylin and eosin (H&E) for histopathological examination and immunohistochemical technique for VEGF. **Results**: The results show that 22 of 40 (55%) with grade 1 and 3 each one had 11(27.5%) frequencies and 12 (30%) frequencies with grade 2 while grade 4 had 6 (15%) frequencies, also we found that 32 (80%) diagnosed with clear cell renal cell carcinoma ccRCC, 2 (5%) chromophope, 5 (12.5%) papillary and 1(2.5%) was collecting duct, the expression of vascular endothelial growth factor marker in renal cell carcinoma were 29 (72.5%) out of 40 samples, the expression rate of ccRCC, papillary and chromophope were 24 (60%), 3 (7.5%) and 2 (5%) respectively for vascular endothelial growth factor marker with significant (.211), the expression rate of vascular endothelial growth factor marker in grade 1,2,3 and 4 were 3 (7.5%), 9 (22.5%), 11 (27.5%) and 6 (15%) respectively with significant (0.000). **Conclusion:** In this study we conclude that the expression of VEGF marker in grade 1 and 2 were 30% while in grade 3 and 4 was 70% so it's had a higher microvessele density than those without or low expression. Also we show that the specifity of vascular endothelial growth factor marker were 72.5% and must be used in investigations as prognostic value.

KEYWORDS: *Vascular endothelial growth factor, Immunohistochemistry, Histopathology.*

INTRODUCTION

Renal-cell carcinoma occurs nearly twice as often in men as in women, and in the United States its incidence is equivalent among whites and blacks, its originates in the renal cortex and accounts for 80 to 85 percent of malignant kidney tumors. Other histologic types include transitional-cell carcinoma of the renal pelvis, making up 15 to 20 percent of kidney cancers in adults, and Wilms' tumor (nephroblastoma) in children.[1] A. 40-year-old man's lifetime risk of renal-cell carcinoma is 1.27 percent, and the risk of death is 0.51 percent. 1 The incidence of renal-cell carcinoma has been rising steadily, increasing 38 percent through 20 years.[2] R.enal cell carcinoma (RCC) is a cause of significant morbidity and mortality, with an estimated 35,000 new cases and 12,480 deaths in the United States in 2003.[3] Vascular endothelial growth factor (VEGF) plays a crucial role in angiogenesis in development of cancer and in other important diseases. VEGF regulates multiple endothelial cell functions including mitogenesis, permeability, vascular tone, and the production of vasoactive molecules. The diverse biological activities of VEGF are mediated through at least two receptor protein, Because of VEGF's central importance for pathophysiological angiogenesis and the development of antagonists for VEGF in the treatment of cancer.[4] Vascular endothelial growth factor serum level or tumor expression is prognostic in renal cell carcinoma (RCC), So elevated serum VEGF level is associated with worse overall survival (OS), disease-specific survival (DSS), and progression-free survival(PFS) while tumor expression is only associated with worse DSS.[5] tumorigenesis and metastasis are dependent on the formation of new blood vessels (angiogenesis) and that blocking angiogenesis

could be a strategy to inhibit tumor progression. Vascular endothelial growth factor (VEGF) is one of the most important growth factors to promote angiogenesis and changes in the tumor microenvironment.[6] The development of renal-cell carcinoma from normal renal epithelium may also involve alterations in genes whose products control cell division. These include genes that participate directly in controlling the cell cycle, such as the retinoblastoma (Rb) gene, the p53 tumor-suppressor gene, and the ras gene family. Expression of growth factors or their receptors may also enhance cellular replication in renal cell carcinoma. In such carcinomas, overexpression of the epidermal growth factor receptor, its ligand (transforming growth factor a) and insulin-like growth factor receptors has been reported. Angiogenic growth factors, such as basic fibroblast growth factor and vascular endothelial growth factor, may contribute to the hypervascularity characteristically found in clear-cell carcinomas and to the development of metastases, and they may have prognostic importance.[7]

MATERIALS AND METHODS

(4 µm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto positively charged slides (Thermo) dried overnight at 37oC. Following deparaffinization in xylene 10 minutes, slides was rehydrated through a graded series of alcohol 100%, 90%, 70% distelled water 3 minutes in each one. Samples was steamed for antigen retrieval to restore the original structure of antigens (unmasking) so as to be recognized by the primary antibody for vascular endothelial growth factor. Heat-induced unmasking: using waterbath the citrate-based buffer (pH 6-7) was used. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and methanol for 10 min, then washed in PBS for 2-3 minutes, and then Slides were incubated with 100-200 µl of primary antibodies for 20 min at room temperature in a moisture chamber. After that the slides were washed with PBS 3 times, 2-3 minutes each., binding of antibodies will be detected by incubating for 20 minutes with dextran labelled polymer (Thermo -ultra vision) the slides were washed with PBS 3 times, 2-3 minutes each., followed by adding 100 µl of pre-warmed (~25°C) 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex resistant to alcohol and xylene. For up to 5 min. The slides were washed with H2O and stained with hematoxylin for 4-8 seconds and washed with H2O for 5 minutes. Slides were dehydrated with 40% - 70% - 96% - 100% ethanol and xylene and finally mounted using xylene-based mounting media. For each run of staining, positive and negative control slides were also prepared. The positive control slides was contain the antigen under investigation by reference methods, and the negative control slides was prepared from the same tissue block, but will be incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results was confirmed by consultant histopathologist.

RESULTS

In our study we found that 34 patients were above the age 40 while 6 patients below age 40 of total 40 samples. And it's supported by (Pierre. I 2008) study who said that the risk of renal cell carcinoma–specific mortality RCC-SM was lowest among patients younger than 50 years. Our finding show that 22 of 40 (55%) with grade 1 and 3 each one had 11(27.5%) frequencies and 12 (30%) frequencies with grade 2 while grade 4 had 6 (15%) frequencies, data collection show that 24 were male and 16 were female of total 40 patients, we found that 32 (80%) diagnosed with clear cell renal cell carcinoma ccRCC, 2 (5%) chromophope, 5 (12.5%) papillary and 1(2.5%) was collecting duct. Also agreement other studies that found Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma (RCC), and is frequently accompanied by the genetic features of von Hippel-Lindau (VHL) loss including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF). In our finding the expression of vascular endothelial growth factor marker in renal cell carcinoma were 29 (72.5%) out of 40 samples, also we found that the expression rate of ccRCC, papillary and chromophope were 24 (60%), 3 (7.5%) and 2 (5%) respectively for vascular endothelial growth factor marker with significant (.211), we also found that the expression rate of vascular endothelial growth factor marker in grade 1,2,3 and 4 were 3 (7.5%), 9 (22.5%), 11 (27.5%) and 6 (15%) respectively with significant (0.000).

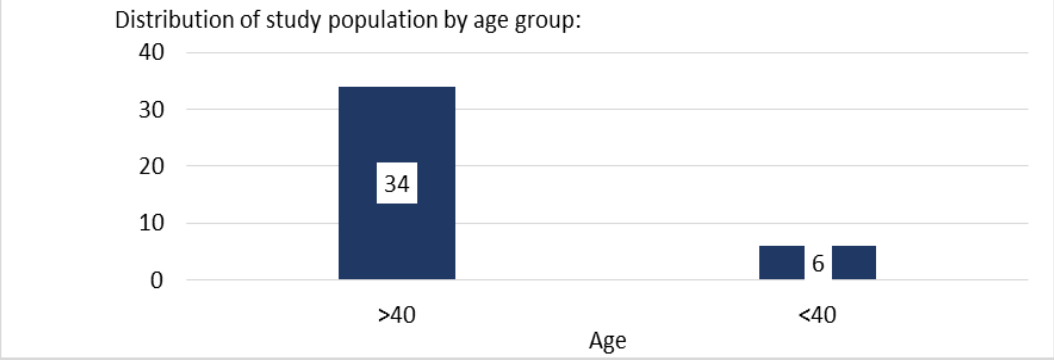

Figure 1: Distribution of study population by age group.

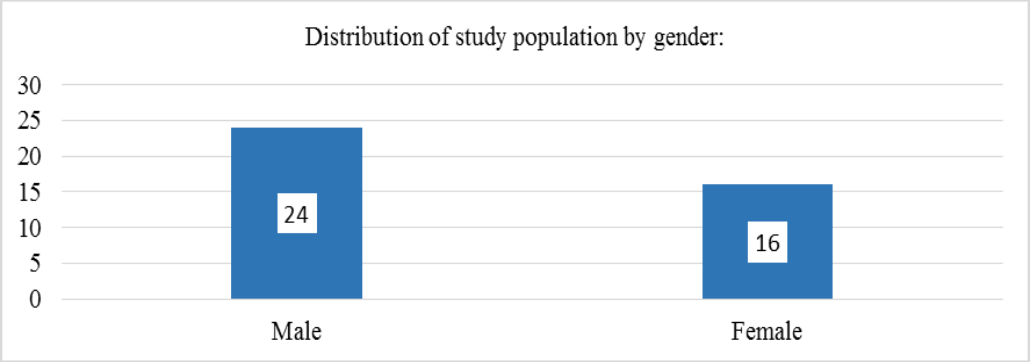

Figure 2: Distribution of study population by gender.

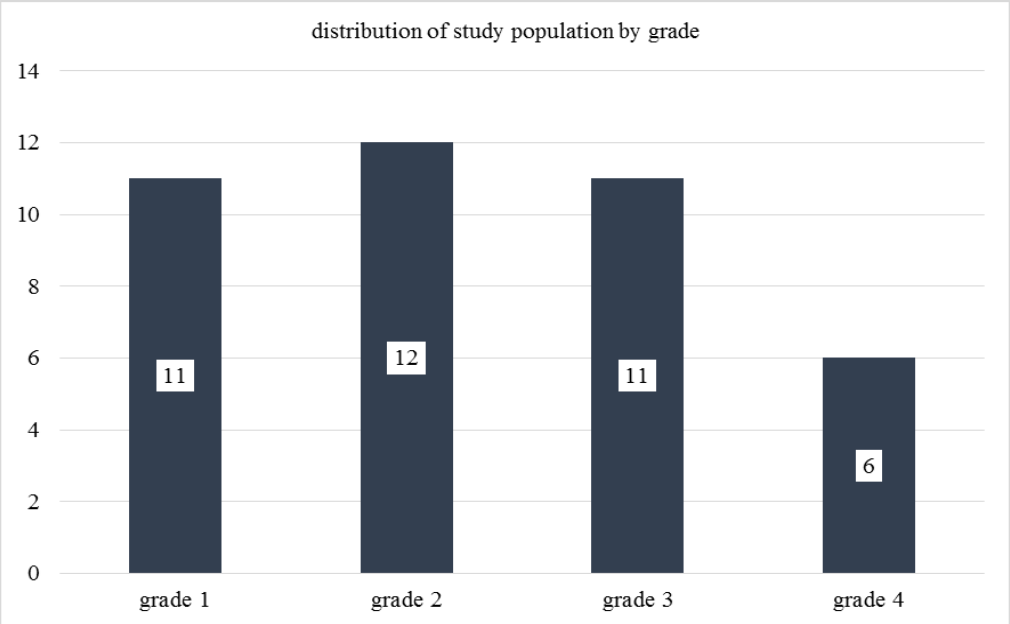

Figure 3: Distribution of study population by grade.

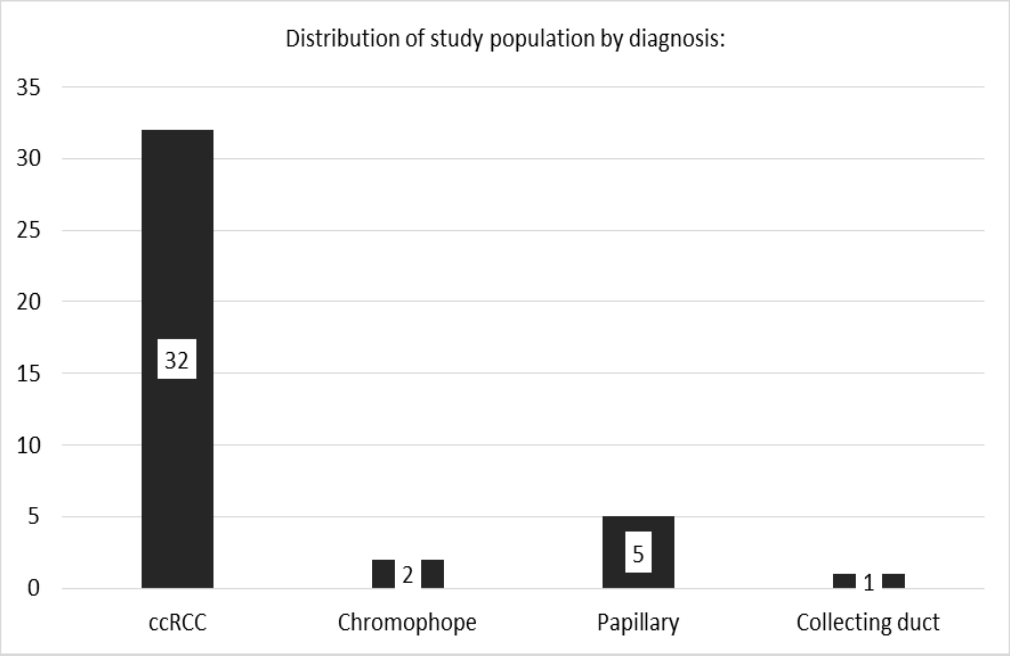

Figure 4: Distribution of study population by diagnosis.

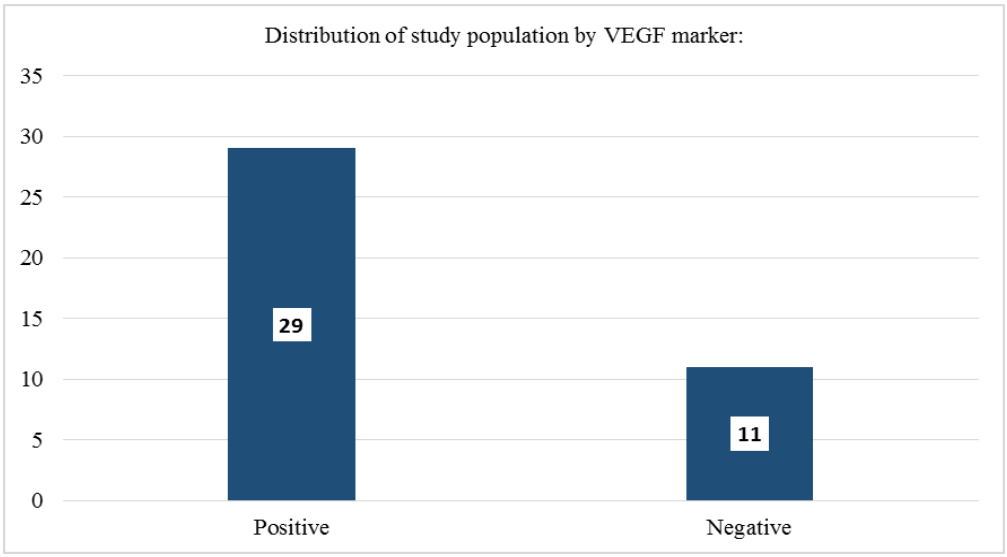

Figure 5: Distribution of study population by vacular endothelial growth factor marker.

*Key word:
VEGF = Vascular endothelial growth factor

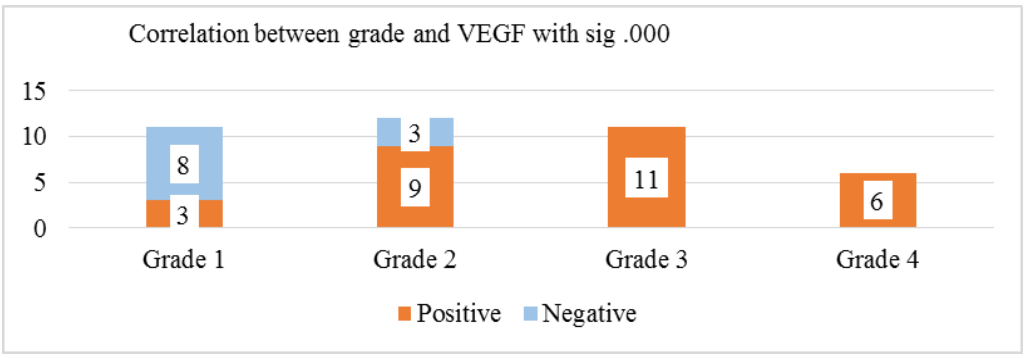

Figure 6: Correlation between grade and VEGF with sig level (0.000).

*Key word:
VEGF = Vascular endothelial growth factor
Sig = significant

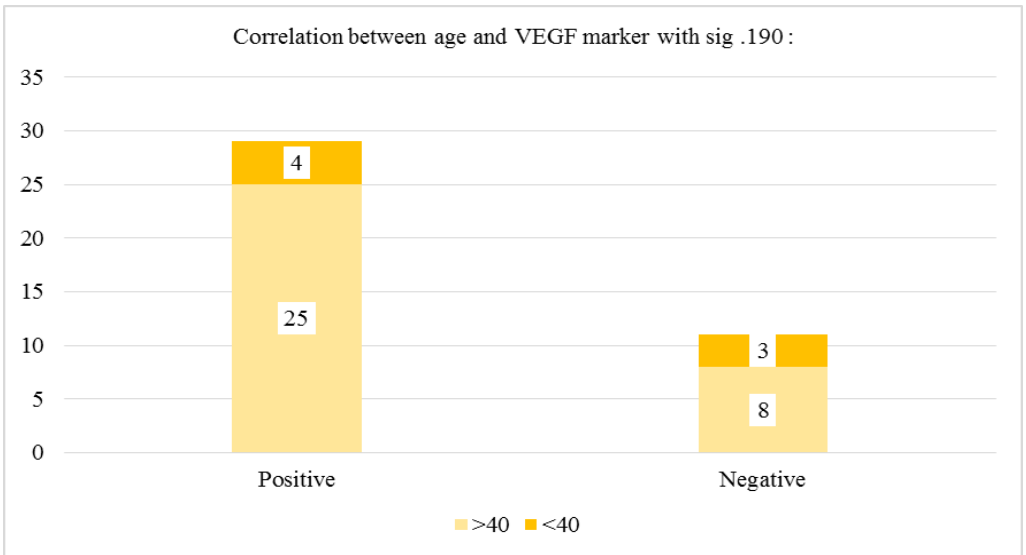

Figure 7: Correlation between age and VEGF marker with sig level (.190).

*Key word
VEGF = Vascular endothelial growth factor
Sig = significant

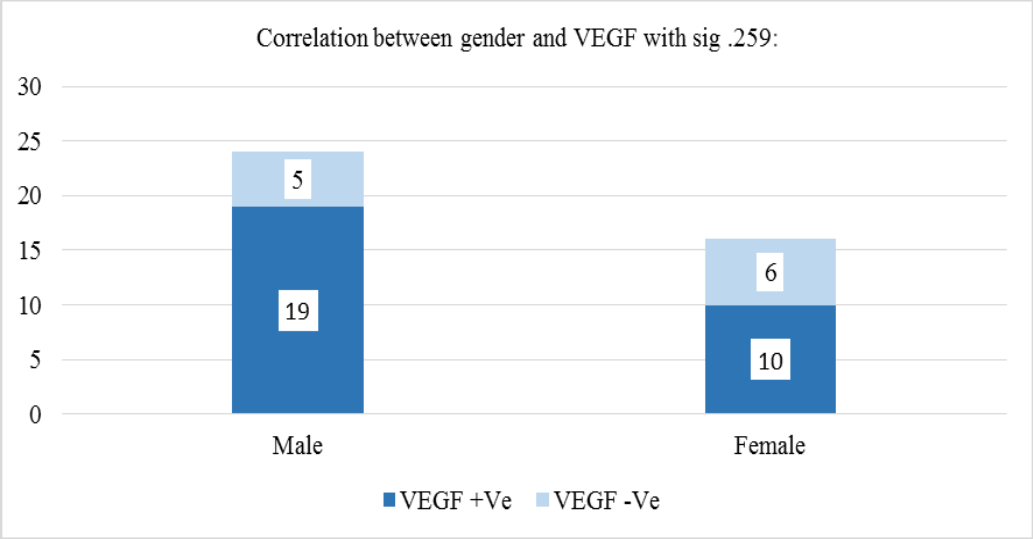

Figure 8: Correlation between gender and VEGF marker with sig level (.259).

*Key word:
VEGF = Vascular endothelial growth factor
Sig = significant

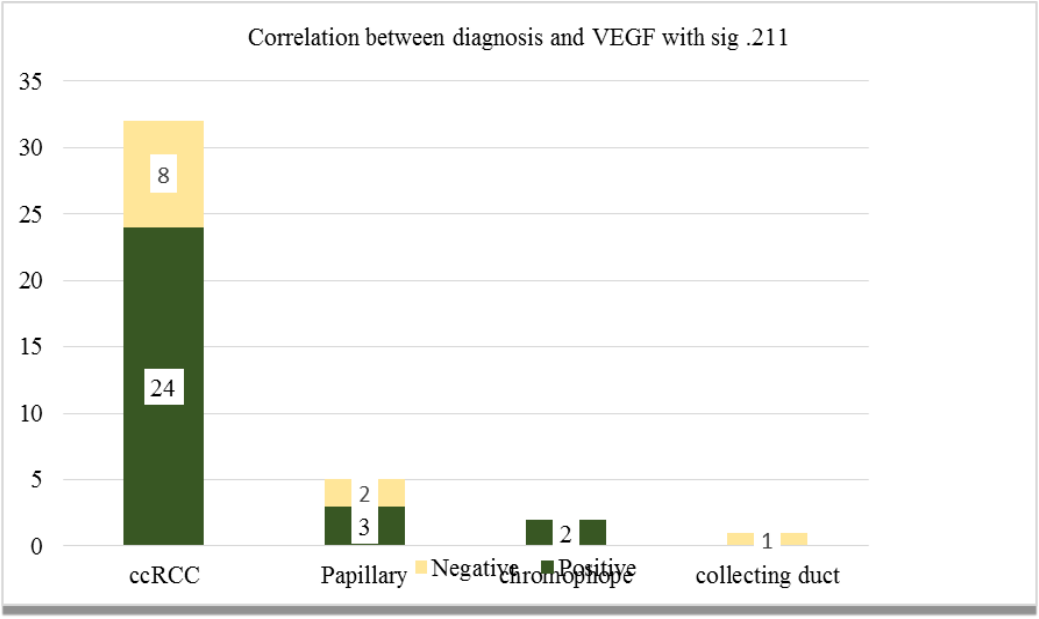

Figure 9: Correlation between diagnosis and VEGF marker with sig level (.211).

*Key word:
VEGF = Vascular endothelial growth factor
Sig = significant

DISCUSSION

The human VEGF-A gene is located on chromosome 6 and contains eight exons separated by seven introns. There are six different isoforms named after the amino acids number of the peptide chain, VEGF–121, VEGF–145, VEGF–165, VEGF–183, VEGF–189 and VEGF–206.[12,13] VEGF–165 is the most abundantly expressed isoform[8] and VEGF–206 the rarest.[9,10] VEGF mediates its biological effects by binding to more than two receptors belonging to the family of tyrosine kinases.[11] The VEGF A receptors initially described were VEGFR–1 (Flt–1) and VEGFR–2 (KDR, flk–1) localized on the microvascular endothelium of the normal kidneys and tumors, healing wounds and inflammatory sites.[12–13] The third receptor for VEGF A has been later demonstrated to be neuropilin–1[14,15], VEGF has been demonstrated to have an important role in kidney organogenesis, especially glomerulogenesis, acting as a paracrine messenger molecule between capillary endothelial cells and Bowman's capsule, in order to maintain the glomerular integrity.[24] In our study we found that 34 patients were above the age 40 while 6 patients below age 40 of total 40 samples. And it's supported by (Pierre. I 2008) study who said that the risk

of renal cell carcinoma–specific mortality RCC-SM was lowest among patients younger than 50 years. Our finding show that 22 of 40 (55%) with grade 1 and 3 each one had 11(27.5%) frequencies and 12 (30%) frequencies with grade 2 while grade 4 had 6 (15%) frequencies. While (Pierre. I 2008) found grade distribution was as follows: (53.3%) had grade 1 disease, (10.8%) had grade 2, (24.9%) had grade 3 and (11.0%) had grade 4 disease. In our data collection we found that 24 were male and 16 were female of total 40 patients. And it's like (Woldrich JM 2008) study that found a total of 89,243 (37.7%) were female and 147,687 (62.3%) were male. In this study we found that 32 (80%) diagnosed with clear cell renal cell carcinoma ccRCC, 2 (5%) chromophope, 5 (12.5%) papillary and 1(2.5%) was collecting duct. And its agreement with (Gordana Đ 2008) who found that 70% were ccRCC and 9% other type. Also agreement with (Lai, Zhao et al. 2018) that found Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma (RCC), and is frequently accompanied by the genetic features of von Hippel-Lindau (VHL) loss including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF). In our finding the expression of vascular endothelial growth factor marker in renal cell carcinoma were 29 (72.5%) out of 40 samples. And this agree with (Berger DP 1995) who found 70% were positive. In this finding we found that the expression rate of ccRCC, papillary and chromophope were 24 (60%), 3 (7.5%) and 2 (5%) respectively for vascular endothelial growth factor marker with significant (.211). And this agree with (Eronat O 2018) who concluded that this marker have no relation with pathologic prognostic parameters and would not provide additional information in the immunohistochemical examination. And their tendency of expression in chromophobe and papillary type RCC is remarkable which should be evaluated with a larger number of cases. Also (Jacobsen J 2004) said VEGF protein was present in most RCC cells. There was no difference in VEGF expression among the different RCC types. In our results we found that the expression rate of vascular endothelial growth factor marker in grade 1,2,3 and 4 were 3 (7.5%), 9 (22.5%), 11 (27.5%) and 6 (15%) respectively with significant (0.000). And it's agree with (Jacobsen J 2004) who said that The correlation between VEGF expression and tumour stage and with prognosis indicates the significance of VEGF within tumour growth and progression in RCC. Some authors have pointed-out controversy on VEGF expression in primary tumor tissues as prediction of outcome, and intratumoral levels of VEGF have not been shown to predict survival outcome of anti-VEGF therapy.[16] Porta *et al.* found that serum levels of VEGF were significant predictors of PFS in patients with renal carcinoma treated with sunitinib and Bernard *et al.* showed that the levels of VEGF soluble isoforms (VEGF121 and VEGF165) were associated with the response to sunitinib in patients affected by metastatic RCC.

6. CONCLUSION

Renal cell carcinoma (RCC) is the most common adult renal epithelial cancer in the United States and the ninth most common malignancy in Europe in 2008 until recently. Renal cell carcinoma continues to be a difficult malignancy to treat because of its ability to spread asymptomatically and its inherent resistance to conventional chemotherapy. In this study we conclude that the expression of VEGF marker in grade 1 and 2 were 30% while in grade 3 and 4 was 70% so it's had ahigher microvessele density than those without or low expression. Also we show that the specifity of vascular endothelial growth factor marker were 72.5% and must be used in investigations as prognostic value.

REFERENCES

1. FERRARA N., Vascular Endothelial Growth Factor: basic science and clinical progress, Endocrine Reviews, 2004; 25(4): 581–611.
2. SATCHELL S. C., ANDERSON K. L., MATHIESON P. W., Angiopoietin 1 and Vascular Endothelial Growth Factor modulate human glomerular endothelial cell barrier properties, J Am Soc Nephrol, 2004; 15: 566–574.
3. FERRARA N., KEYT B., Vascular endothelial growth factor. Basic biology and clinical implications. In: ROSEN E., GOLDBERG I. D. (eds), Regulation of Angiogenesis, Springer Verlag, New York, 1997; 209–232.
4. ROBINSON C. J., STRINGER S. E., The splice variants of vascular endothelial growth factor (VEGF) and their receptors, J Cell Sci, 2001; 114(5): 853–865.
5. KEYT B. A., NGUYEN H. V., BERLEAU L. T. et al., Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptorselective VEGF variants by site-directed mutagenesis, J Biol Chem, 1996, 8; 271(10): 5638–5646.
6. INOUE K., SAKURADA Y., MURAKAMI M. et al., Detection of gene expression of vascular endothelial growth factor and flk-1 in the renal glomeruli of the normal rat kidney using the laser microdissection system, Virchows Archiv, 2003; 442(2): 159–162.
7. FENG D., NAGY J. A., BREKKEN R. A., PETTERSSON A. et al., Ultrastructural localization of the Vascular Permeability Factor/Vascular Endothelial Growth Factor (VPF/VEGF) Receptor-2 (FLK-1, KDR) in normal mouse kidney and I the hyperpermeable vessels induced by VPF/VEGFexpressing tumors and adenoviral vectors, J Histochem Cytochem, 2000; 48: 545–556.
8. HARPER S. J., XING C. Y., WHITTLE C. et al., Expression of neuropilin by human glomerular epithelial cells in vitro and in vivo, Clin Sci (Colch), 2001; 101: 439–466.
9. OSTENDORF T., KUNTER U., EITNER F. et al., VEGF165 mediates glomerular endothelial repair, J Clin Invest, 1999; 104(7): 913–923.

10. ROBERT B., ZHAO X., ABRAHAMSON D. R., Coexpression of neuropilin-1, Flk1, and VEGF164 in developing and mature mouse kidney glomeruli, Am J Physiol Renal Physiol, 2000; 279: F275–F282.
11. Eble, J., N. Sauter, G. Epstein, J., I. Sesterhenn, I., A. (2004). "Pathology and genetics of tumours of the urinary system and male genital organs". Lyon, France: IARC Press.
12. Eronat, O., A. Onursever, D. Suren, M. Yildirim, O. Kandemir and H. Ali Kahya (2016). "VEGF receptor subtypes may serve as novel prognostic factors and putative indicators for anti VEGF receptor treatment response in renal cell carcinoma cases." J buon, 2016; 21(5): 1250-1258.
13. Godley, P. and S. W. Kim (2002). "Renal cell carcinoma." Current Opinion in Oncology, 2002; 14(3): 280-285.
14. Godley, P. A. and T. E. Stinchcombe (1999). "Renal cell carcinoma." Current Opinion in Oncology, 1999; 11(3): 213.
15. Guyot, M., C. Hilmi, D. Ambrosetti, M. Merlano, C. Lo Nigro, J. Durivault, R. Grepin and G. Pages (2017). "Targeting the pro-angiogenic forms of VEGF or inhibiting their expression as anti-cancer strategies." Oncotarget, 2017; 8(6): 9174-9188.
16. Lai, Y., Z. Zhao, T. Zeng, X. Liang, D. Chen, X. Duan, G. Zeng and W. Wu (2018). "Crosstalk between VEGFR and other receptor tyrosine kinases for TKI therapy of metastatic renal cell carcinoma." Cancer Cell Int., 2018; 18: 31.
17. Lane, B., R. and Kattan, M., W (2005)." Predicting outcomes in renal cell carcinoma". Curr Opin Urol, 2005; 15: 289–297.