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Immunoexpression of Vascular Endothelial Growth Factor among Sudanese Patients with Bladder Cancer

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Abstract: BACKGROUND: *Vascular endothelial growth factor (VEGF) is a signaling protein which is important for formation of the circulatory system and growth of blood vessels from pre-existing vasculature. Cancers that can express VEGF are able to grow and metastasize, because many solid cancers require an adequate blood supply to grow beyond the limited size. Aim: To determine the Immunoexpression of Vascular Endothelial Growth Factor among Sudanese Patients with Bladder Cancer.* ***METHODOLOGY:*** *This was an analytical cross-sectional study, included forty patients with (TCC); study patients from different Histopathology laboratories in Khartoum state- Sudan, VEGF was detected by using IHC method.* ***RESULTS:*** *VEGF immunoexpression was reported positive in 20 out of 40 patients (50%), the study conducted that; there was an association of VEGF immunoexpression and higher tumor grade of (TCC) as the frequency of VEGF expression was 16 out of 27 patients (59.2%) with high grade, while the frequency of VEGF expression was 4 out of 13 patients of low grade (30.8%) P value = 0.010.* ***CONCLUSSION:*** *according to obtained results we concluded that; the, there was a significant association between VEGF and bad prognosis of (TCC).*

Keywords: VEGF, Bladder Cancer, TCC, IHC, Sudan.

INTRODUCTION

Carcinoma of the bladder is the seventh most common cancer worldwide. It comprises 3.2% of all cancers. Bladder Cancer starts when the normal cells that lining the bladder start unceasing growth out of control to form a tumor. Bladder Cancer is classified into three major types, TCC which is represent 90% of total bladder cancer recently called Urothelial Carcinoma, and second type is Squamous Cell Carcinoma (SQCC) and third is Adenocarcinoma of bladder.(1). The common type of Bladder Cancer in Sudan is (TCC), which is fetal disease in absent of early detection and proper diagnosis, therefore early detection of (TCC) can decrease the rate of death and saving money of patients. Also identifying the correlation between VEGF Immunoexpression and (TCC) could increase the chances of cancer detection, which can help a Sudanese scientist and pathologist to deal with this type of cancer.

MATERIALS & METHODS

Forty cases of Transitional cell carcinoma were included in this study, from both genders. The patients' age ranged between 25 and 80 years old, [Mean age 60.1 years], this was analytical cross-sectional study and subjected in different histopathology laboratories in Khartoum state.

Data collection tools; by using Master Sheets were used to record all patients' data and samples results; VEGF was detected by using IHC method.

Sample processing: After samples collection, these samples were processed as a block of tissue microarray (TMA), according to Bancroft (2). Three microns section from microarrayed block was mounted on IHC positive charge slide using Lieca microtome (model: 5179/10.2019, S.N:050038604-Germany) then the section was stained by IHC method according to the manufacture (Thermo Scientific- Italy) to detect the expression of VEGF in bladder cancer tissues.

Method of detection: by using immunohistochemistry technique. Section (3µm) from formalin-fixed, paraffin-embedded tumors were cut and mounted onto salinized slide (thermo). Following deparaffinization in xylene, slide was rehydrated through a graded series of alcohol and was placed in running water. Samples were steamed for antigen retrieval using PTlink water bath (model: PT/2012, USA) at 90°c for 15 minutes. Briefly, slide was placed in Coplin jar containing enough sodium citrate buffer (pH 9.0) to cover the section, then was boiled at high temperature 90°c for 20 minutes, then allowed section to cool at room temperature (RT). Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 minutes, and then slide was incubated with 200 µl of primary antibodies for 20 minutes at RT in a moisture chamber, and then rinsed in phosphate buffer saline (PBS). The primary antibody was ready to use (Dako, Carpintera) added for 15 minutes. After that washed with PBS for 3 minutes, binding of antibodies were detected by incubating for 20 minutes with dextrane polymer. After that, the section was washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the

characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 minutes, slide was counterstained with haematoxylin for 3-5 minutes, after that slide was blued in running tap water for 10 minutes, dehydrated in ascending grades of ethanol, cleared in xylene and mounted in Dixtrene Plasticizer and Xylene (DPX).

RESULTS

The study results showed that; the commonest frequency of TCC was observed among age group from (70-79) years old (13 cases=32.5%). Regarding correlation of VEGF immunoexpression with TCC we summarized that; VEGF was positive in 20 samples (50%) and negative in 20 samples (50%). VFGF was positive in 16 out of 24 cases of high grade (59.2%) and 4 out of 16 cases of low grade (30.2%) *P* value = 0.010 as indicated in (table 1). The most age group positive with VEGF was those of (70-79) 9 cases followed by age group (60-69) 5 cases with P value =0.590 (table 2). VEGF was positive in 14 out of 27 male cases (51.9%), while VEGF was positive in 6 out of 13 females (46.2%) with P value 0.736 as indicated in (table 3).The study conducted that; there was an association of VEGF immunoexpression and higher tumor grade as the frequency of VEGF expression was16 out of 27 patients (59.2%) with high grade, while the frequency of VEGF expression was 4 out of 13 patients (30.8%).

Table1: Correlation of VEGF immunoexpression with TCC tumor grade

	VEGF		Total	P.value
Grade	Positive	Negative		
High	16	8	24	0.010
Low	4	12	16	
Total	20	20	20	

Table 2: Distribution frequency of VEGF immunoexpression among patient's age groups.

Age group	VEGF		Total	P.value
	Positive	Negative		
20-29	1	1	2	0.590
30-39	0.00	0.00	0.00	
40-49	1	4	5	
50-59	4	4	8	
60-69	5	6	11	
70-79	9	4	13	
80-89	0.00	1	1	
Total	20	20	40	

Table 3: correlation of VEGF immunoexpression with patient's gender.

		VEGF		Total	P.value
		(+)	(-)		
sex	Male	14	13	27	0.736
	female	6	7	13	
total		20	20	40	

Discussion

TCC affects the older people more than younger, as occurred in this study, the high rate of incidence among those of age ranged between (70-79) years old, the result is agreed with that which obtained by Sir Elkhatim and Salwa [3] who concluded that; the commonest age affected by TCC in Sudan was the age group between (50-70) years old.

During collection of TCC samples from archival samples in the targeted hospitals , the most common gender affected by TCC was male than female (27:13) (67.5%) and (32.5%) the same to the finding of Pakzad [4] who reported that; in Asia (67.0% in men and 32.9% in women, gender ratio was 2.03:1).

In this study, VEGF immunoexpression was reported in 20 patients (50%). this result is relative similar to that; reported by Chu [5] who concluded that; the positive expression of VEGF was observed in (54.7%) from 161 patients with TCC. Also our results were near to that study of Xia [6] who summarized that; the positive VEGF expression was 58% in patients with TCC.

Regarding association of VEGF expression with TCC tumor grade, our results similar to that study of Yang and Donmez [7] they concluded that; the over expression of VEGF was observed in high grade tumor samples.

CONCLUSION

According to the obtained results it was concluded that; there was a significant correlation between VEGF immunoexpression and a higher tumor grade. VEGF associated with bad prognosis of bladder cancer.

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