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Immunohistochemical Expression of VEGF in Gastric Tumors

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Abstract:

Background:- The purpose of this study was to examine the VEGF expression in gastric tumor.

Objective:- The aim of the study was to investigated and evaluate the effect of vascular endothelial growth factor on angiogenesis in gastric tumor tissue and a correlation between VEGF expression and gastric tumors.

Methods:- In this study we depended on the formalin fixed tissue sample from patients diagnosed with gastric tumors and the cases obtained by random selection method. In this study also we depended on two methods seen as haematoxylin and eosin and Immunohistochemical techniques. Ethical clearance for this study is provided by ethical. Committee of Faculty of medical laboratory sciences – AL-Neelain University, Khartoum, Sudan.

Result:- The analysis of 40 patients with gastric tumors showed that there is no correlation between VEGF expressions and gastric tumors P.Value (0.192). The expression of VEGF in 10 patients

with Benign (Adenoma) showed positive= 6 (15%) and Negative = 4 (10%), 23 patients with Adenocarcinoma showed Positive = 20(50%) and Negative = 3 (7.5%), 5 patients with Lymphoma showed Positive = 1 (2.5%) and Negative = 4(12.5%), 1 patient with Leiomyosarcoma showed Negative = 1(2.5%), 1 patient with gastrointestinal stroma tumor showed Negative = 1(2.5%) P.Value (0.0).

The age of the involved patients with gastric tumors ranged between 20 to 85years with mean age 60 years.

Conclusion:- In this study there is no correlation between VEGF expression and type gastric tumor.

Key words: Immunohistochemical Expression of VEGF, Gastric Tumors

INTRODUCTION:-

A malignant tumor of the stomach. Gastric cancer can develop in any part of the stomach and can spread from the stomach to other organs. The incidence of gastric cancer was 7.4per 100,00 men and women per year these rates are age –adjusted and based on 2008- 2012 cases in United States (1). Stomach cancer is the fifth most common cancer in the world, with 952,000 new cases diagnosed in countries with the top 20 highest incidence of stomach cancer in 2012, eg: Korea, Republic of 41.8, Mongolia 32.5 , japan 29.9 (2). The incidence of gastric cancer was estimated at 934, 000 cases, 56%of the new cases being derived from Eastern Asia, 41% from china (3). Vascular Endothelial growth factor VEGF promotes vascular endothelial cell proliferation and its high expression in tumors is significantly associated with advanced disease and poor prognosis. (4-6). we investigated VEGF expression levels in gastric tumor specimens by immunohistochemistry, and examined their relationship to prognostic factors of gastric tumor.

The Haematoxylin and Eosin Staining in gastric tumor Figure (1).

In 30 cases of gastric tumor which the VEGF. Immunoreaction was positive the final product of reaction was found in the cytoplasm and membrane of tumor cells in the P.Value (<0.05). Figure (2).

The gastric tumor which the VEGF Immunoreaction was Negative the final product of reaction. Figure (3).

The gastric Benign tumor which the VEGF Immunoreaction was positive the final product of reaction. Figure (4).

MATERIAL AND METHOD:-

Tissues specimens, a total of 40 patients 28 males and 12 females, age >60 years n=25 and≤ 60years n= 15 who received surgery for gastric tumor. hospitals of alribat university and ministry of defense hospital and Ibn Sina and Radiation an isotope center- Khartoum (RICK), paraffin embedded specimens collected , we used in diagnosis the Haematoxylin and Eosin such as Mayer's H which it's chemically oxidized by used Sodium iodate and it is good nuclear stain for (7min),while the Eosin is rose or red crystalline stain used as counter stain to the cytoplasm for (1min), and in the advanced technique the immunohistochemistry procedure with done as follows: sections (3µm)from formalin-fixed, paraffin embedded tumors was cut and mounted onto positively charged slides (fisher brand) following deparaffinization in xylene ,slides with rehydrated through a graded series of alcohol and with placed in D.W. samples with steamed for Antigen retrieval for VEGF used water bath .briefly, slides with placed in slide coplin jar containing enough sodium citrate buffer (PH 9.0) to cover the sections ,then with boiled at high temp for 40 minutes them will allow sections to cool at RT Endogenous peroxidase activity

with block with 3%hydragen peroxide and methanol for 10 min with then slides incubated with 100-200µLof primary antibodies for 20min at room temperature in a moisture chamber. And then with rinsed in phosphate buffer saline the primary antibody VEGF (monoclonal Ab) with ready to use (thermo).after washing with PBS for 3min binding of antibodies with detected by incubating for 20min of secondary antibody with dextran labeled polymer(thermo kit),finally, the sections with washed in three changes of PBS, followed 3,3 diaminobenzidine tetrahydrochloride (DAB) as chromogen to produce the characteristic brown stain for the visualization of the antibody enzyme complex for up to 5 min .and wash in (PBS) slides with counter stain with haematoxylin. Each slide was evaluated with investigator and then confirmed by Histopathologist positive VEGF staining with identified inform of brown membranous staining. The obtained results and variables with arranged in standard master sheet, then with entered computer program SPSS and analyzed.

Ethical clearance for this study is provided by ethical committee of Faculty of medical laboratory sciences – AL-Neelain University, Khartoum, Sudan.

STATISTICAL ANALYSIS:-

Analysis was performed using SPSS version 21 for windows 10 and it's used for significant difference in the immunolabelling of VEGF depend on prognostic parameters such as age, sex, tumor size and Hsitopathological variant and VEGF expression were included in the univariate analysis to determine the predictors of lymph node metastasis and localized in gastric tumor and adenocarcinoma and gastro intestinal stroma tumor and Leiomyosarcoma univariate analysis using x^2 and chi-square, crosstabs and Frequencies to determine the in fluency of VEGF in different prognostic groups.

RESULT:-

The details of gastric tumor cases were summarized as 28 patients were Males while the Female's patients were 12 which effectively drawing Male: Female ratio (3:1)

We found there is no correlation between Immunohistochemical expression of VEGF and Histopathological types, Gender, tumor size, patients Age all that due to P.Value (<0.005).

Table (1) Description of expression of VEGF and Histopathological parameters:-

Description of correlation between reaction level and Gender male T= 28 (70%) cases and showed Positive =19 (47.5%) and Negative =9 (22.5%), Female T=12(30%) cases and showed Positive= 8 (20.0%) and showed Negative = 4 (10.0%). Statistically insignificant (P.Value = 0.570).

Description of correlation between reaction level and age >60 T=27 showed Positive = 18 (45.0%) and showed Negative = 7 (17.5%), <60 T=13 showed Positive 9 (22.5%) and showed Negative = 6 (15.0%). Statistically insignificant (P.Value=0.189).

Description of correlation between reaction level and tumor size number of >5 T= 11 showed Positive = 4(10.0%) and Negative =7(17.5%), number of <5 showed Positive = 20(50.0%) showed Negative = 9(22.5%).Statistically insignificant (P.Value= 0.232).

Description of correlation between reaction level and Histological grade number of poorly differentiated T=3 showed Positive =3(7.5%) and Negative = 0(0%), number of Moderate differentiated showed Positive =5(12.5%) and showed Negative =2(5.0%), Well differentiated showed Positive =3(7.5%) and Negative =0(0%). Statistically insignificant (P.Value= 0.220).

Relation between the expressions of VEGF and Histopathological diagnosis, and VEGF expression in number of Benign (Adenoma) T= 10 showed positive= 6 (15%) and

Negative = 4 (10%), number of Adenocarcinoma T=23 showed Positive = 20(50%) and Negative = 3 (7.5%), number of Lymphoma T=5 showed Positive = 1 (2.5%) and Negative = 4(12.5%), number of Leiomyosarcoma T=1 showed (Negative = 1(2.5%), number of gastrointestinal stroma tumor T=1 showed Negative = 1(2.5%) P.Value (0.0) Table (2). Statistically insignificant (P.Value= 0.192)

Table (1) Summary of Correlation of VEGF expression with Clinicopathological Characteristics:-

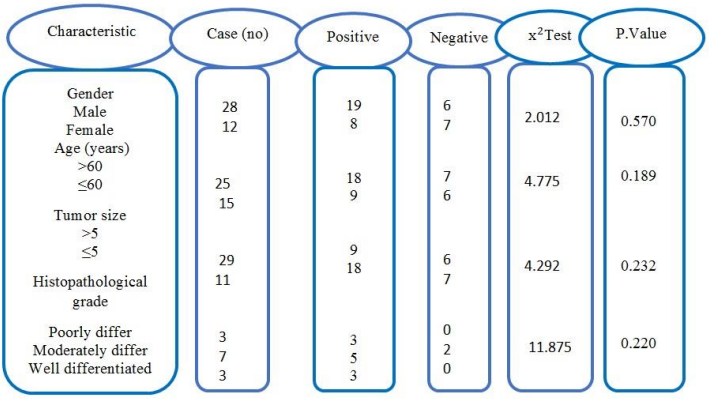
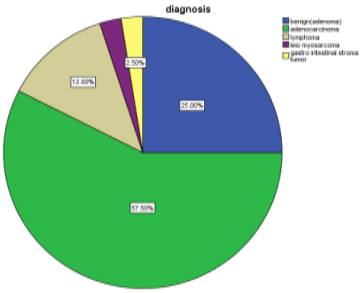


Table (2) Relation between the expressions of VEGF and Histopathological diagnosis:-

Diagnosis	**VEGF**		**Total**
	Positive	**Negative**	
Benign(adenoma)	6 (15%)	4(10%)	25
Adenocarcinoma	20(50%)	3(7.5%)	57.5
Lymphoma	1 (2.5%)	4(10%)	12.5
Leiomyosarcoma	0(0%)	1(2.5%)	2.5
Gastrointestinal Stroma tumor	0(0%)	1(2.5%)	2.5
Total	27	13	100.0
	40		

***(3) Descriptive pie chart for the diagnostic value:-**



(A)

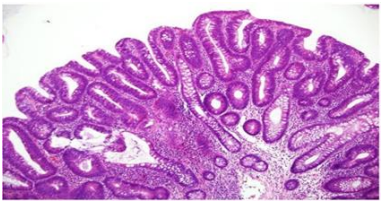


Figure1: Staining Haematoxylin in gastric tumor (SP x200)

(B)

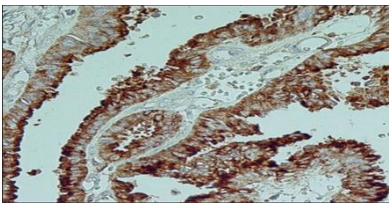


Figure 2. Positive expression of VEGF in gastric tumor (SP x200)

(C)

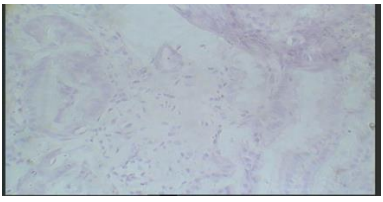


Figure 3.Negative expression of VEGF in gastric tumor (SP x200)

(D)

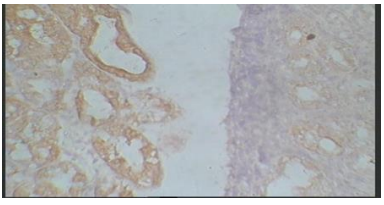


Figure 4. Positive expression of VEGF in benign tumor (SPx200).

DISCUSSION:-

Our data is close to those positive showed 67.5%. of the gastric tumors VEGF expression and this results are close to those of Raica MI et al [7] which their study demonstrated 70% of VEGF positively in gastric carcinoma cases, but less than those of Soo Jung Lee MD et al[8] , but more from for which demonstrated 74.9%.

In our study we are not agree with Shimada H, Takeda A, et al which P.Value (<0.01) (9), and Seo HY, Park JM, et al which P.Value (<0.05) (9-10), and Karayiannakis AJ, Syrigos KN which P.Value (<0.05),(11). they found there is correlation between VEGF expression and tumor size, pathological stage

and lymph node metastases .but we agree with them in the fact that there is no correlation between VEGF and patients age and gender P.Value (>0.05).

The difference between our study and other studies may be due to genetics or environmental factors.

CONCLUSION*:*

Our study indicated that the Immunohistochemical analysis of VEGF expression has no correlation with gastric tumors and prognostic parameters.

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