EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 11/ February 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Expression of CD34 in Gastric Tumors among Sudanese Patients using Immunohistochemisty

TARIG AWAD ELSHAREEF MOHAMMED
Faculty of Medical laboratories Sciences
AL-Neelain University, Sudan
AGEEB MOHAMMED HASSAN
Department of Pathology
Sudan International University, Sudan
MAHMOUD IBRAHIM OSMAN
Department of Histopathology and Cytology
Sharq El Niel College, Sudan
IBRAHIM BAKHIT YOUSIF ELEMAM
Department of Histopathology and Cytology
Shendi University, Sudan

Abstract:

Several studies have showed that the CD34 was over expressed in gastric cancer and play role in development of gastric cancer and metastasis of. This retrospective study was conducted in Khartoum State –Sudan, in the period from October 2015 to January 2016. Aim of this study to investigate expression of CD34 in malignant gastric cancer and benign gastritis. The study includes fifty (50) Formalin – fixed paraffin embedded biopsies from which forty one (41) of malignant gastric cancer (study group) and nine (9) of benign gastritis (control group). CD34 was detected in (83.3%) (35/41) among malignant tissues and (66.7%) (6/9) of benign gastritis, the (P.value =0.63), this result showed an insignificant difference between case and control groups. The study concluded that CD34 has positive immunostaining in malignant gastric cancer and in benign gastritis and no significant difference between them.

***Conclusion**: The study concluded that, there is no sufficient evidence for the association of between the rate of CD34 expression and*

gastric subtype's tumor. The study recommended adapting to more deep study by increasing sample size.

Key words: CD34, Gastric tumors, Benign gastritis

INTRODUCTION

Gastric Cancer (GC) is one of the most common cancers in the world, which accounts for 7% of all cancer the GC also accounts for 9% of all cancer-related deaths in the world [1]. The GC mortality rate is increasing in the world and its estimated incidence is more than one million new cases per year in the world [2].According to World Health Organization (WHO) gastric tumors can be classified into two main categories as epithelial tumors and non-epithelial tumors [3]. In Sudan gastric cancer is the fifth commonest cancer among males [4].

The CD34 molecule is a 110 kDa transmembrane cell surface glycoprotein which was originally described as a marker for human hematopoietic stem cells [5] [6].CD34 is closely correlated to angiogenesis and is a marker of endothelial cells. CD34 has a stable and sensitive expression in capillaries and small vascular endothelial cells in tumors. Angiogenesis evaluation depending on CD34 is an important clue to estimate metastasis and prognosis of malignant tumors [7]

MATERIALS AND METHODS:

This retrospective study was conducted in Khartoum State – Sudan, in the period from October 2015 to January 2016, the geographical and histoclinical data were achieved from the patients files, and Formalin –fixed paraffin embedded biopsies of forty one (41) of malignant gastric cancer (study group) and nine (9) of benign gastritis (control group) samples were used to

investigate the positive rate of CD34 by the streptavidin–biotin method using the (thermo- EnVision kit) protocol.

From each tissue sample paraffin block was prepared two paraffin sections were cut into 3µm thick sections floated into preheated floating water bath at 50˚c, one section was placed in positively charged glass slide for Immunohistochemistry, other section was placed in clean microscopical slide for Haematoxylin and Eosin, incubated in oven at 65˚c. The staining procedure will be as follows. sections were stained with haematoxylin and eosin as described by Mayer's in Bancroft (2003) then slides were examined primary by consultant of histopathologists and then results were confirmed by histopathologist to verify that an adequate number of gastric cancer cells were present and to assess morphological classification.

IMMUNOHISTOCHEMICAL STAINING:

Procedure will be carried out using the following antibodies:-

CD34	Monoclonal mouse anti human CD34

The immunehistochemical procedure will be done as follows:

One section (3µm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto positively charged slide following by deparaffinization in xylene; slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval for CD34 using water bath at 95ºc for 30 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 min, and then Slides were incubated with 100-200 µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then was rinsed in phosphate buffer saline (PBS). After washing with phosphate-buffered saline for

3 min, binding of antibodies were detected by incubating for 20 minutes with dextran labeled polymer (thermo- EnVision kit). Finally, the sections were 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 min. Slides were counterstained with haematoxylin. For each run of staining, positive and negative control slides was also prepared. The positive control slides were contain the antigen under investigation and the negative control slides were prepared from the same tissue block, but was incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist and scored (according to Raica *et al* criteria)
The data was analyzed, using the statistical programs software Statistical Package for the Social Sciences (SPSS) version (20), Chi square test and different statistical measures were calculated.

RESULT:

Males were the dominant gender, (62%) (31/50), and female represented (38%) (19/50) with male: female ratio (1.6:1). The frequency of malignant gastric tumor (82%) (41/50) and benign represented (18%) (9/50). The frequency of malignant gastric tumor according to the age groups appeared in (figure 1). As the following (28%) (11/41) (23%) (9/41) (23%) (9/41) for the age groups (above the 60 yrs), (above the 50 yrs) and (above the 40 yrs) respectively. Considering the subtypes of malignant gastric tumor, forty one (38%) (15/41) were gastrointestinal stromal tumor, (28%) (11/41) were moderately differentiated adenocarcinoma, (18%) (7/41) were poorly differentiated adenocarcinoma, (10%) (4/41) were adenocarcinoma and (10%) (4/41) were poorly differentiated adenocarcinoma signet ring.

Comparing the association of expression of CD34 in malignant gastric tumors and benign gastric tumors, CD34 were detected (85.3%) (35/41) and (66.6%) (6/9) respectively, the (P.value =0.63) (Table 1).

Considering the subtypes of gastric tumors the CD34 showed immunostaining according to scoring strong, moderate and weak immunostaining as following (51.4%) (18/35), (34.2%) (12/35), (14.2%) (5/35) respectively, the (P.value= 0.122), there is no statistically significant difference between the gastric tumors subtypes (Table 2). Comparing the association the rate expression of CD34 and malignant gastric tumor and benign gastritis CD34 showed immunostaining according to scoring strong, moderate, weak and negative immunostaining as following (36.6%) (15/41), (11.1%) (1/9), (29.2%) (12/41) (11.1%) (1/9), (12.1%) (5/41) (44.4%) (4/9), (21.9%) (9/41) (33.3%) (3/90) respectively, the (P.value= 0.069), there is no statistically significant difference of rate of CD34 expression between malignant gastric cancer and benign gastritis.

(Table 1): CD34 Immunostain comparison between benign and malignant gastric tumor:

Study group	IHC (CD34) result		Total	p. value
	Positive	Negative		
Benign gastric tumor	6	3	9	0.63
Malignant gastric tumor	35	6	41	
Total	41	9	50	

(Table 2): CD34 Immunostain rate comparison between malignant gastric subtype's tumors:

Malignant gastric subtypes tumors	Expression					Total
	Strong	Moderate	Weak	Negative	Nil	
Moderately differentiated adenocarcinoma	7	2	0	1	1	11
Poorly differentiated adenocarcinoma	4	3	0	0	0	7
Poorly differentiated	1	2	0	0	1	4

adenocarcinoma signet ring						
Gastrointestinal stromal tumor	**6**	**3**	**4**	**1**	**1**	**15**
Well differentiated adenocarcinoma	**0**	**2**	**1**	**1**	**0**	**4**
Total	**18**	**12**	**5**	**3**	**3**	**41**

DISCUSSION:

The current study showed positive immunostaining in malignant gastric cancer and benign gastritis according to the rate of CD34 expression and relation of it to tumor prognosis their study showed there is no significant value between malignant gastric cancer and benign gastritis ((P.value =0.069) this finding is disagreement with study conducted by Li Chen *et* al who concluded that (CD34 in gastric cancer tissues is associated with development of the cancer and prognosis of gastric tumor) [8]. The variation of our result may be due to serial reducing sample size.

The findings point out that, positive immunostain of CD34 were reported in thirty five biopsies (83.3%) (35/41) among malignant tissues, compared with six positive (66.7%) (6/9) of non malignant tissues, indicating an insignificant statistical difference, the (P.value =0.63) so there is no sufficient evidence for the relation between malignancies of gastric tumors and rate of CD34 expression this finding is supported by Shigang Ding, *et al* [9], their conclusion CD34 was universally expressed in blood vessels within benign and malignant gastric tissues. Also other supporting came from TIAN Nan *et al* [10], they concluded the expression of CD34 was found in gastric cancer and benign gastritis.

REFERENCES:

1- Kamangar, F., G.M. Dores and W.F. Anderson, 2006. Patterns of cancer incidence, mortality and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic regions of the world. J. Clin. Oncol. 24: 2137-2150.

2- De Martel, C., D. Forman and M. Plummer, 2013. Gastric cancer: Epidemiology and risk factors. Gastroenterol. Clin. North Am., 42: 219-240.

3- Tumors of stomach – chapter 3- WHO publication available at www.iarc.fr/en/publications/pdfs-online/pat-gen/bb2/bb2-chap3.pdf

4- Incidence of Digestive Cancers on the Rise in Sudan-Sudan Vision-Issue #: 3754, Issue Date: 21st January, 2016

5- Greaves MF, Brown J, Molgaard HV, *et al*. Molecular features of CD34: a hematopoietic progenitor cell associated molecule. *Leukemia* 1992; **1:31**–6.

6- Simmons DL, Satterthwaite AB, Tenen DG, *et al*. Molecular cloning of a cDNA encoding CD34, a sialomucin of human hematopoietic stem cells. *J Immunol* 1992;**148**:267–

7- Weidner N: Intratumormicrovessel density as a prognostic factor in cancer. Am J Pathol, 147: 9-19, 1995.

8-Li Chen, Xinyu Li, Guei-lan Wang, Yu Wang, York-Yuan Zhu, and Jianwei Zhu-Clinicopathological significance of overexpression of TSPAN1, KI67 and CD34 in gastric carcinoma-Tumori, 2008-94: 531-538

9-Shigang Ding, *et al*-Comparative evaluation of microvessel density determined by CD34 or CD105 in benign and malignant gastric lesions. 2006; 37 (7) doi:10.1016/j.humpath.

10- TIAN Nan *et al*- Expression and significance of CD34 in gastric precancerous lesions and gastric carcinoma- Med. J. Chin. Peop. Armed Poli. Forc., 2015, Vol. 26, Issue (4): 347-349.