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VEGF Expression in Esophageal cancer with Associated Demographic and Pathological Features as Seen in the Department of Pathology, Makerere University

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Abstract: Esophageal squamous cell carcinoma is the most prevalent type of esophageal cancer worldwide. Esophageal adenocarcinoma is the most common type in developed countries. Vascular Endothelial Growth Factor (VEGF), a signal protein produced by cells that stimulates the process of angiogenesis has been shown to be expressed in esophageal carcinoma tumor tissue. The study was a cross sectional laboratory-based study. Formalin fixed and paraffin embedded (FFPE) tissue blocks of 110 patients diagnosed with esophageal cancer at the department of Pathology. Data abstraction form forms were used to capture variables; that is age, sex, tumor histological subtype, grade and VEGF protein expression and then analysis was done using STATA version 14. For measures of association, 95% confidence interval was used and a p-value of less than 0.05 was considered statistically significant. The mean age of patients from which specimens had been collected was 59 years with a standard deviation of 11 years. Most of the patients were males, 71(64.6%). Majority of the specimens were of conventional squamous cell histological type, 101 (91.8%) and of moderately differentiated histological grade, 58 (52.7%). The prevalence of VEGF expression was 51.8% (57/110). There was no statistically significant association between VEGF expression and age, histological type and grade of esophageal carcinoma. There is a 51.8% of expression of VEGF in esophageal cancer in our study. Our study may help better patient management and improve knowledge gap of VEGF expression in esophageal cancer in Uganda.

IndexTerms*: esophageal cancer, Vascular endothelial growth factor*

1.INTRODUCTION

According to the GLOBOCAN estimates of 2020, esophageal cancer was the tenth most common cancer affecting people globally [1]. There were an estimated 604,100 new cases of esophageal cancer diagnosed in 2020 [1]. The incidence of esophageal cancer in East Africa is 8.4 and 6.4 per 100,000 of the population among males and females respectively [2]. In Uganda the incidence of esophageal cancer among males and females is 22.6 and 13.9 per 100,000 of the population respectively [3]. The most common histological types of esophageal cancer include squamous cell carcinoma (SCC) and adenocarcinoma (AD). Vascular Endothelial Growth Factor (VEGF) is a signal protein produced by cells that stimulates the process of angiogenesis (formation of blood vessels) [4]. This has been demonstrated in physiological processes like embryogenesis, skeletal growth and reproductive functions. Also, it has been demonstrated to play a role in angiogenesis in pathological processes like tumor growth and intraocular neovascular disorders [5]. Previous studies on the expression

of VEGF have reported that its expression varies by histology grade and histological type of esophageal cancer. In Uganda, the prevalence of VEGF protein expression in esophageal carcinoma and its role in treatment and determining prognosis is not yet known. Therefore, the aim of our study was to determine prevalence of VEGF protein expression in esophageal cancer and its associated clinicopathological features at the Department of Pathology, Makerere University, and Kampala, Uganda.

2. Materials and Methods

2.1 Patients and tissue samples

A total of 110 archived tissue blocks with a histological diagnosis of esophageal cancer were analyzed. This was a cross-sectional laboratory-based study.

The study was conducted in the Department of Pathology laboratory, Makerere University located in Kampala, Uganda. The Department of Pathology hosted within the School of Biomedical Sciences at Makerere University College of Health Sciences. The department receives about 2000-3000 biopsies per year samples across Uganda. Tissue blocks from 1st January 2009 to 31st December 2021 where a diagnosis of esophageal cancer was made were considered in the present study. Sample size was determined using Kesh Leslie1965 formula.

2.2 H&E and VEGF immunohistochemistry procedure

The sections were stained using routine hematoxylin and eosin (H & E) staining method in accordance with standard operating procedures. Sections were then prepared for immunostaining. They were dewaxed in 3 changes of xylene, hydrated in alcohol, placed in the epitope retrieval buffer solution (Leica Novocastra) at pH 9, incubated with peroxidase, washed with Tris Buffer Saline, incubated with mouse monoclonal primary antibody (clone VG1, M7273), diluted 1:100 for 4 hours, washed with Tris buffer saline solution, incubated with novolink post primary antibody, stained with Mayers hematoxylin, cleared in 3 changes of -xylene, mounted with DPx and then cover slipped. Colon adenocarcinoma was used as positive control, the negative control constituted sections without the primary antibody.

2.3 VEGF Immunohistochemistry scoring method

The intensity was scored as 0 (negative), 1 (weak staining), 2 (moderate staining) or 3 (strong staining) [6]. The percentage of positive tumor cells was scored as 0 (none), 1 (1-10%), 2 (11–50%), 3 (51–80%) or 4 (>80%) [6]. The scores for intensity and percentage was multiplied with the results giving a semi quantitative immunoreactive score (IRS) ranging from 0 to 12 [6]. IRS greater or equal to 4 was considered positive and a value less than 4 was considered negative. Two independent observers were used to evaluate the tumor staining. Differences were discussed at a double-header microscope to achieve a final consensus.

2.4 Statistical method:

Data collected was entered into Microsoft Excel software. It was then double cross- checked, cleaned and edited for any mistakes. The analysis was performed using STATA version 14, and data for the following variables analyzed: age, sex, and histological subtype, grade of tumor and VEGF protein expression status.

Variables were analyzed using both univariate and multivariate analysis. Associations between categorical variables were assessed using Pearson's chi-square test, A 95% confidence interval was used and P-Value of less or equal to 0.05 was considered statistically significant. Continuous variables were summarized as means and standard deviations.

Categorical variables were summarized as proportions and the findings were presented as frequency tables Photomicrographs were used to display both H & E and IHC features.

2.5 *Ethical considerations*: Permission to carry out a study was obtained from the school of Biomedical Sciences Research and Ethics committee. A waiver of consent to use biopsy specimens was obtained from the School of Biomedical Sciences Research and Ethics Committee (SBSREC). For confidentiality, patient names and biopsy numbers were not used, instead unique identification numbers were assigned and used for each specimen.

3. Results

3.1: Patient Characteristics

The mean age of patients from which specimens had been collected was 59 years with a standard deviation of 11 years. Majority of the patients were males, 71(64.6%). Most of the specimens were of conventional histological subtype (91.8 %), adenocarcinoma had 5.4% of the cases, basaloid squamous cell carcinoma had 1.8% and adenosquamous carcinoma had 0.9%.

3.2: Association between VEGF expression and with patient's age and sex and histopathologic features

In regards to the age, sex, histological grade, and histological type, there were no statistically significant association with expression.

Table 1: Characteristics of patients from which specimens were collected (n = 110).

Characteristics	Frequency(n=110)	Proportion (%)
Age: mean =59±11		
35-44	10	9.1
45-54	31	28.2
≥55	69	62.7
Sex		
Male	71	64.6
Histological grade		
Well Differentiated	22	20
Moderately Differentiated	58	52.7
Poorly Differentiated	30	27.3
Histological type		
Conventional SCC	101	91.8
Adenocarcinoma	6	5.4
Basaloid squamous cell carcinoma	2	1.8
Adenosquamous	1	0.9

Table 2: Comparison of clinicopathological characteristics between cases positive for VEGF and those that are negative

Characteristic	VEGF expression		P-value
	Positive (%)	Negative (%)	
Age			0.607
35-44	4(40)	6(60)	
45-54	15(48.4)	16(51.6)	
≥55	38(55.1)	31(44.9)	
Sex			0.752
Male	36(50.7)	35(49.3)	
Female	21(53.9)	18(46.1)	
Histological grade			0.949
Well Differentiated	12(54.6)	10(45.4)	
Moderately Differentiated	30(51.7)	28(28.3)	
Poorly Differentiated	15(50)	15(50)	
Histological type			0.352
Conventional SCC	51(50.5)	50(49.5)	
Other types	6(66.7)	3(33.3)	

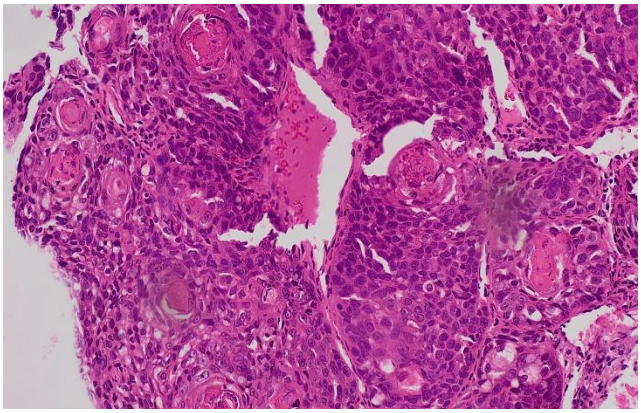

Figure 1: A

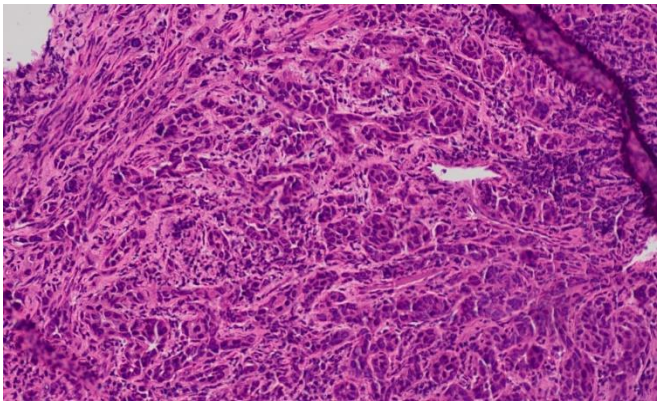

Figure 1: B

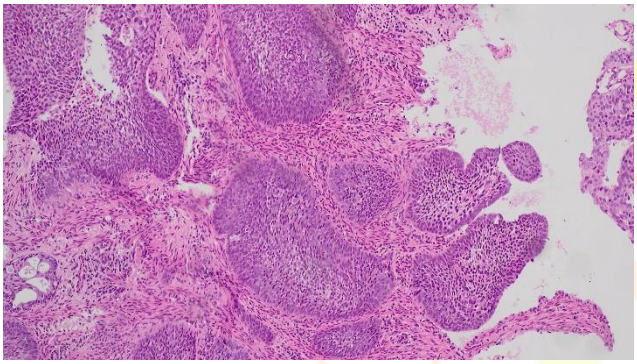

Figure 1: C

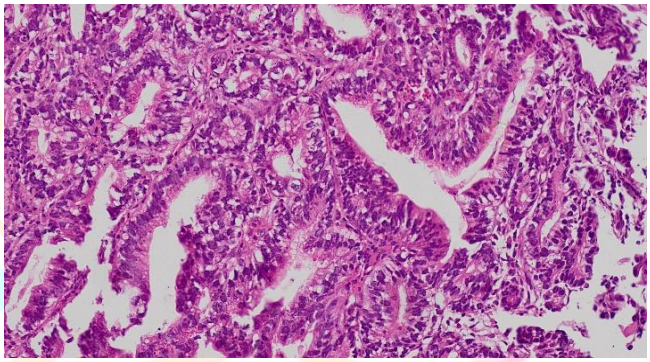

Figure 1: D

FIGURE1: A: Showing well differentiated conventional squamous cell carcinoma (200X). B: poorly differentiated squamous cell carcinoma (200X). C: basaloid squamous cell carcinoma (100X). D: adenocarcinoma (200X)

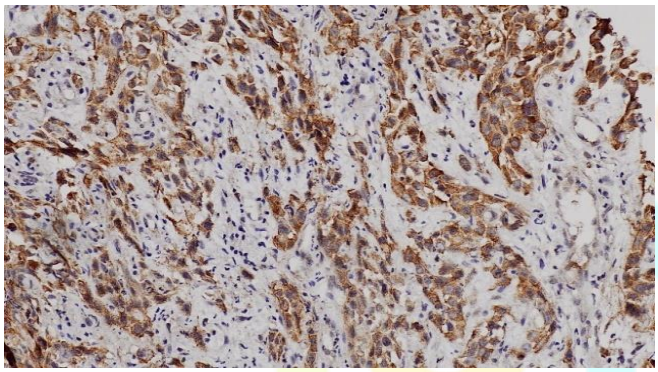

Figure 2: A

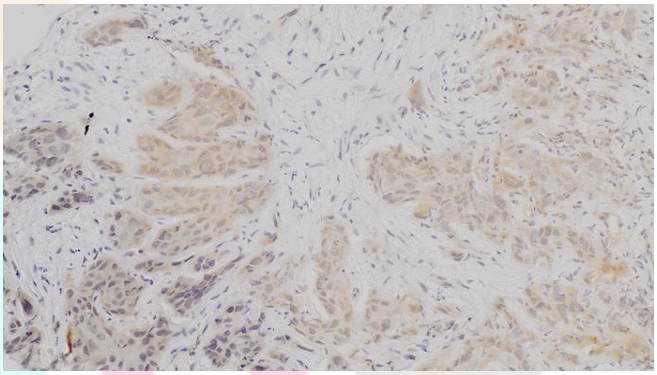

Figure 2: B

FIGURE2: Photomicrograph showing positive VEGF expression: A. numerous tumor cells are showing strong brown VEGF IHC staining (100X). B: photomicrograph showing weak expression of VEGF in esophageal carcinoma. The tumor cells are showing weak expression for VEGF immunohistochemistry.

4. Discussion

Esophageal cancer has a high burden globally including in low- and middle-income countries (LMICs) like Uganda. VEGF plays a role in the angiogenesis of many solid tumors including esophageal cancer. High expression of VEGF protein in esophageal cancer patient could be a potential prognostic biomarker that may help in the management of patients with esophageal cancer (Kleespies, 2004) In our study, we aimed to determine the prevalence of Vascular Endothelial Growth factor (VEGF) expression and its association with histological grade of esophageal cancer and demographic factors such as age and sex. The prevalence of VEGF expression among tissue blocks of esophageal cancer in our study was 51.8%. This was slightly higher than findings from a study in Japan that reported that 46.7% of esophageal carcinoma expressed VEGF [7]. There were lower rates of expression of VEGF from a study conducted among a population in Brazil where 40% of tumors of esophageal carcinoma expressed VEGF [8]. However, this contrasts with another study from Japan that reported that 59.7% of esophageal carcinomas expressed VEGF [5].

The differences in the prevalence of VEGF protein expression between the studies could be explained by the following: The studies were conducted in different populations i.e., populations in Uganda, Brazil and Japan. These are very different populations and have varying genetic makeup and environmental exposure. This could contribute to the variations in prevalence of VEGF protein expression reported by the different studies. Also, the differences in prevalence could have been contributed to by the different antibodies used in staining for VEGF protein expression. Our study used the primary mouse monoclonal antibodies (Clone VG1 Ref M7273). The study conducted in Brazil used a rabbit polyclonal antibody A-20, which recognizes VEGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA) [8]. The study conducted among the population of Japan used a rabbit monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) [5]. The different antibodies used could have varying uptake by the tissues during the process of immunohistochemistry staining. These could have contributed to the variations in the prevalence of VEGF protein expression reported. In addition, the variations could have been due to the methodological differences in determining the levels of expression of VEGF protein expression in tissues. Our study used the semi quantitative immunoreactive score (IRS) methods of determining the levels of expression of VEGF protein [6]. The study conducted in the population of Brazil used the percentage of cells stained to determine the levels of expression of VEGF protein [8]. The study conducted in Japan also used the percentage of cells stained to determine the levels of expression of VEGF protein [5].

Among those who were positive for VEGF, the most common age group was for those aged 55 years or older (55.1%) and majority were male (50.7%). On bivariate analysis, the odds of VEGF positivity were 1.5 and 1.8 times among patients aged 45 to 54 and patients aged at least 55 years, respectively compared to the patients aged 35 to 44, this was not statistically significant. Regarding the association between age and expression of VEGF, the findings from our study are inconsistent with those reported in some other studies. One conducted among Japanese patients with esophageal carcinoma showed that age was associated with the expression of VEGF in patients with esophageal carcinoma [9]. In addition, another study also reported that advanced age was associated with a high expression of VEGF in patients with esophageal carcinoma [7]. However, some studies reported similar findings to our study in terms of the association between age and the expression of VEGF in tissues of esophageal cancer. A study conducted among Chinese patients of esophageal carcinoma show that there was no statistically significant association between age and the expression of VEGF in esophageal cancer tissue [10]. This is also like studies conducted in Japan and Brazil, which reported that they did not find a significant association between the age of the patient and the expression of VEGF in the esophageal cancer tissues [8, 11].

Regarding the association between sex and expression of VEGF, our study did not find any significant association. This is consistent with findings from other studies that showed that sex was not significantly associated with the expression of VEGF by the tissues of esophageal carcinoma [8, 11]. From our study, the most common subtype of esophageal cancer was conventional SCC 101 (91.8%) followed by adenocarcinoma 6(5.4%), basaloid squamous cell carcinoma 2 (1.8%) and adenosquamous carcinoma 1(0.9%). This is consistent with

findings from other studies conducted globally that reported that SCC was the most common subtype of esophageal carcinoma followed by adenocarcinoma of the esophagus [12-14].

Studies on the trends of esophageal carcinoma globally have overall shown that the annual incidence of SCC is declining while that of adenocarcinoma is rising, although the burden of SCC is higher than that of adenocarcinoma [15]. Adenocarcinoma has been associated with risk factors like gastro-esophageal reflux disease (GERD), obesity, smoking, and dietary factors[13], which are on a continuous rise [14]. Histological type was not found to be associated with the expression of VEGF among the tissues of esophageal carcinoma. However, the majority of the ESCC tissues (50.5%) expressed VEGF. This is consistent with findings from other countries where there was a high proportion of tissues of ESCC that expressed VEGF. For example, a study done in South Korea showed that 44% of the ESCC expressed VEGF [9]. Another study that was conducted in China showed that 53.4% of the ESCC expressed VEGF [10]. Among the cases that expressed VEGF in the current study, the most common histological grade was the well differentiated type (54.6%). However, histological grade was not associated with the expression of VEGF. This is in contrast to findings from studies conducted in populations of Japanese and Chinese patients where it was reported that high VEGF levels were significantly associated with well-differentiated tumors [7, 10, 16]. However, studies conducted in the population of Brazil and Japan reported that there were no statistically significant associations between tumor grade and VEGF expression in tumors of esophageal carcinoma [8, 11, 17].

5. Conclusion

There is a 51.8% of expression of VEGF among tissue blocks of esophageal cancer archived at the Department of Pathology, Makerere University, and Kampala, Uganda.

There is no association between VEGF protein expression and age, sex, histological grade, and histological type in the samples analyzed in the department of pathology, Makerere University.

6. Data Availability

The data that supports the findings are available on request from the corresponding author.

7. Conflict of Interests

The authors declared that there is no conflict of interests the publication of this paper.

8. Funding statement

None

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