



## **The identification of HPV, CMV, HSV and EBV Infections and P53 Expression in Esophageal Squamous Cell Carcinoma in Gadarif Teaching Hospital (2014- 2017)**

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### **Abstract**

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#### **Background;**

Esophageal carcinoma is not common disease in Sudan especially in eastern Sudan although it is neglected and patients usually presented at late stages of the disease. The main aim of this study is to identify the demographic, clinical and laboratory risk factors in Gadarif state and to determine their association with this disease and to evaluate the role of HPV, CMVs, HSV and EPV viruses and assess the expression of P53 protein in Squamous cell carcinoma of the esophagus.

#### **Materials and Methods;**

This was a prospective analytical cross sectional study of one hundred and fifty patients for whom endoscopic examination was done, esophageal tissue biopsy was taken and examination was performed for these biopsies. Tissue sections prepared and were subjected to detection of HPV, HSV, CMV, EBV infections and P53 by using immunohistochemistry techniques (IHC).

#### **Result;**

HPV infection was detected in Fifty eight (38.7%) it was positive in 21(24.1%) diagnosed as poor differentiated esophageal carcinoma and negative in 66(75.9%) of them P value 0.01. Twenty one (14%) was diagnosed positive for CMV infection 5 of them was diagnosed as well differentiated esophageal carcinoma and it was positive in 16(18.4%) diagnosed as poor differentiated esophageal carcinoma P value 0.09 . Twenty (13.3%) of them were diagnosed positive for HSV infection 3 (4.8) of them diagnosed as well differentiated esophageal carcinoma and it was positive

in 17(19.5%) diagnosed as poor differentiated esophageal carcinoma P value 0.01. Fifteen (10%) of them were diagnosed positive for EBV infection 1 (1.6) of them was diagnosed as well differentiated esophageal carcinoma and it was positive in 14(16.1%) diagnosed as poor differentiated esophageal carcinoma P value 0.004. Twenty five (16.7%) of them were diagnosed positive for P53 expression 6(9.5%) of them was diagnosed as well differentiated esophageal carcinoma and at P value 0.07.

### Conclusions;

Here, we summarize there was association between HPV and HSV infections and the grade of esophageal carcinoma.

**Keywords:** Esophageal carcinoma HPV, CMVs, HSV and EPV viruses, IHC.

## Introduction

Esophageal cancer is among the ten most common malignancies worldwide and ranks as the sixth leading cause of death from cancer. It constitutes 7% of all gastrointestinal cancers and is one of the most lethal of all cancers [1]. Globally esophageal cancer ranks as the sixth most common cancer among males and ninth most common cancer among females. However, in India, it is the second most common cancer among males and the fourth most common cancer among females [2] The incidence of esophageal cancer varies greatly between developed and developing countries and a 50-fold difference has been observed between high and low-risk populations. It is more common in Asian than in western countries. The esophageal cancer belt is a geographic area of high incidence, which stretches from north-central China westward through Central Asia to northern Iran [1] esophageal cancer exists in two main forms with distinct etiological and pathological characteristics, squamous cell carcinoma (SCC) and adenocarcinoma. More than 90% of esophageal cancers worldwide are SCCs, although adenocarcinomas are more prevalent in the USA. SCC is often preceded by increased proliferation of esophageal epithelial cells leading to basal cell hyperplasia, dysplasia, and carcinoma in situ [3]. Tobacco smoking and alcohol drinking are strongly associated with the risk of esophageal SCC and to a lesser degree with the risk of esophageal adenocarcinoma. Reports from Southern India suggest that ESCC occurs in more than 80% of cases in chronic tobacco smokers that is further potentiated by heavy use of alcohol and additional prevailing risk factors, including nutritional factors and

vitamin deficiencies[1]. Viral infection like HPV, CMV, HSV and EBV also are risk factors [4].

## Materials and Methods

This was a prospective analytical cross sectional study. The study population consisted of 150 esophageal tissues diagnosed as esophageal squamous cell carcinoma. One section from each block measures four micrometers was cut and then stained in H&E to confirm diagnosis of each block. Then five sections were cut from each recruited block. Each section from the remainder three sections (measuring four microns) was floated in 70% ethanol and water bath (Electrothermal ser NO.18861434-China) at 40c<sup>0</sup>, consecutively. Each floated section was mounted on positive charge immune slide (Thermo Scientific- Italy) to detect immune expression of HPV, HSV, CMV, EPV and P53 in each sample all slides contained sections were dried in dry oven (WTC binder 7200 TUTTLINGEN, B28, NO.88485-USA) at 60c<sup>0</sup> for 30 minutes. Paraffin wax sections were detected using immunohistochemistry. For IHC Ab-3 (Clone K1H8) mouse monoclonal antibody biomarker was used to detect presence of HPV type (6, 11, 16, 18, 31, 33, 42, 51, 52, 56 and 58). LMP-1 biomarker was used to detect expression of EBV. Ab-1 rabbit polyclonal antibody biomarker was used to detect HSV primary antibody used to detect CMV and P53. All used biomarkers come from (Dako, Carpintera) was used to detect HR- HPV, HSV, CMV, EPV infections in paraffin sections using specific primer to each virus. Each primer used was specific to only one virus and primary antibody specific for P53 was used.

## Statistics:

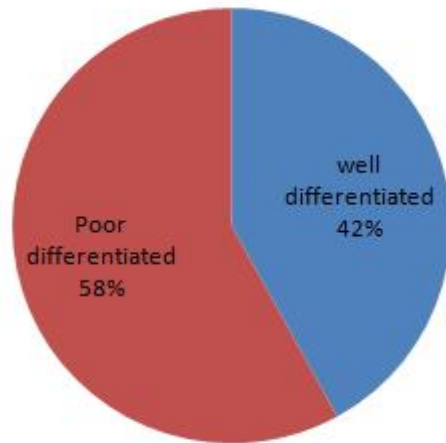
All obtained results were analysed by Statistical Package for the Social Sciences (SPSS) version 20, with Pearson's chi-square test used to assess intergroup significance. Ninety five (95%) confidence interval and p value was calculated (level of significance was set at P. value of 0.05).. Other variables, frequencies were calculated.

## Results

A total of 150 samples from cases patients with histopathologically confirmed esophageal squamous cell carcinomas. The age of patients was ranged between 60 years and 87 years. Patients below 60 years of age were 41 (27.3%) while 109 (72.7%) their ages were 60 years and above. Sixty three (42%) were well differentiated, while 87 (58%) were poor differentiated esophageal carcinoma diagnosed, (figure 1). Using IHC techniques, IHC, HPV was found to be positive in 58(38.7%) cases while the majority of patients 92(61.3%) negative for as HPV infection (figure 2). The majority of patients 129(86%) negative for CMV infection, while 21(14%) positive for CMV infection, (figure 3). The majority of patients 130(86.7%) negative for as HSV infection, while 20(13.3%) positive for HSV infection, (figure 4). The majority of patients 135(90%) negative for as EBV infection, while 15 (10%) positive for CMV infection, (figure 5). The majority of patients 125(83.3%) negative for P53 expression, while 25(16.7%) positive for P53 expression (figure 6). According to esophageal squamous cell carcinoma grade HPV infection were positive in 37(58.7%) of patients diagnosed as well differentiated esophageal carcinoma and negative in 26(41.3%) of them. HPV was positive in 21(24.1%)

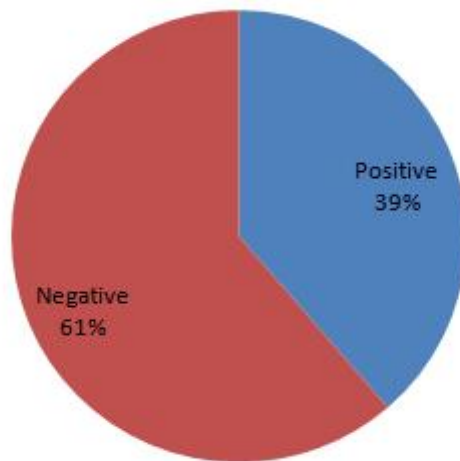
diagnosed as poor differentiated esophageal carcinoma and negative in 66(75.9%) of them, table (1). Association between malignancy grades with HPV infection was positive to be significant at P value 0.01 and 95% CI 4.4 (2.2 – 9.0). CMV infection positive in 5(7.9%) of patients diagnosed as well differentiated esophageal carcinoma and absent in 58(92.1%) of them. However it was positive in 16(18.4%) diagnosed as poor differentiated esophageal carcinoma and negative in 71(81.6%) of them, table (2). Association between malignancy grade with CMV infection was positive to be insignificant at P value 0.09 and 95%CI 0.3 (0.1 – 1.1). HSV infection positive in 3(4.8%) of patients diagnosed as well differentiated esophageal carcinoma and negative in 60(95.2%) of them. However it was positive in 17(19.5%) diagnosed as poor differentiated esophageal carcinoma and negative in 70(80.5%) of them. table (3) Association between malignancy grade with HSV infection was positive to be significant at P value 0.01 and 95%CI 0.2 (0.05 – 0.7). EBV infection positive in 1(1.6%) of patients diagnosed as well differentiated esophageal carcinoma and negative in 62(98.4%) of them. However it was positive in 14(16.1%) diagnosed as poor differentiated esophageal carcinoma and negative in 73(83.9%) of them, table (4). Association between malignancy grade with EBV infection was positive to be insignificant at P value 0.004 and 95%CI 0.08 (0.01 – 0.6). P53 expression positive in 6(9.5%) of patients diagnosed as well differentiated esophageal carcinoma and negative in 57(90.5%) of them. However it was positive in 19(21.8%) diagnosed as poor differentiated esophageal carcinoma and negative in 68(78.2%) of them, table (5) Association between malignancy grade with P53 expression was positive to be insignificant at P value 0.07 and 95%CI 0.3 (0.1 – 1)

**Grade of malignancy**



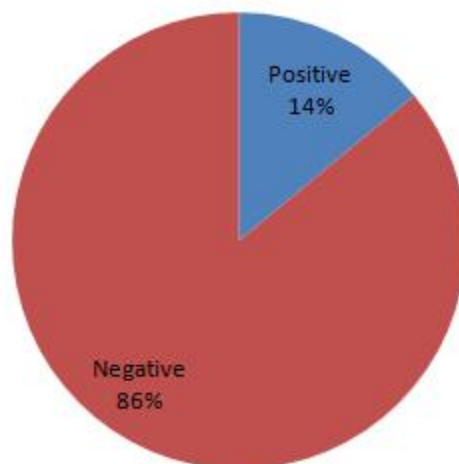
**Figure (1) Show the frequency of grade of malignancy among study population**

**HPV infection of the patients**



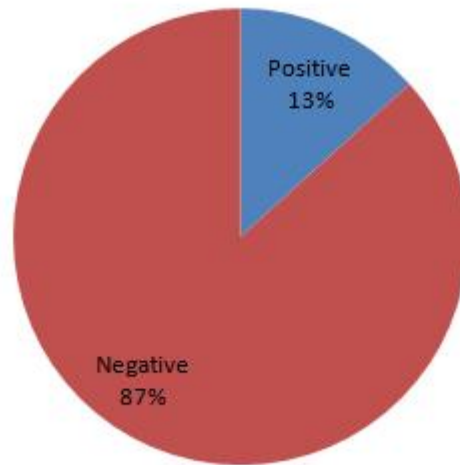
**Figure (2) Show the frequency of HPV infection of the patients.**

**CMV infection of the patient**



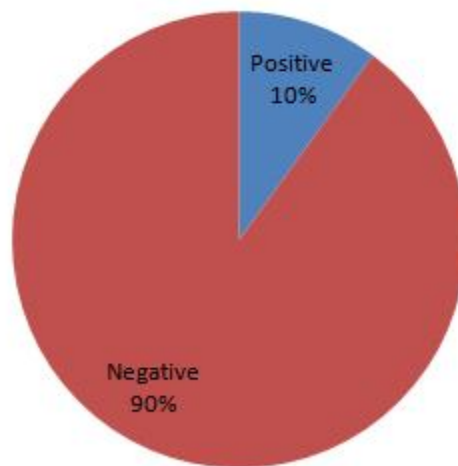
**Figure (3) Show the frequency of CMV infection of the patients.**

**HSV infection of the patients**



**Figure (4) Show the frequency of HSV infection of the patients.**

**EBV infection of the patient**



**Figure (5) Show the frequency of EBV infection of the patients.**

**Table (1) show grade of malignancy \* HPV infection of the patients Cross tabulation**

Grade of malignancy	HPV infections		Total	P.value
	Positive	Negative		
Poor differentiated	21 (24.1%)	66 (75.9%)	87 (100%)	0.01
Well differentiated	37 (58.7%)	26 (41.3%)	63 (100%)	
Total	58 (38.7%)	92(61.3%)	150 (100%)	

**Table (2) show grade of malignancy \* CMV infection of the patients Cross tabulation**

Grade of malignancy	CMV infections		Total	P.value
	Positive	Negative		
Poor differentiated	16 (18.4%)	71 (81.6%)	78 (100%)	0.09
Well differentiated	5 (7.9%)	58 (92.1%)	63 (100%)	
Total	21 (14%)	129(86%)	150 (100%)	

**Table (3) show grade of malignancy \* HSV infection of the patients Cross tabulation**

Grade of malignancy	HSV infections		Total	P.value
	Positive	Negative		
Poor differentiated	17 (19.5%)	70 (80.5%)	87 (100%)	0.01
Well differentiated	3 (4.8%)	60 (95.2%)	63 (100%)	
Total	20 (13.3%)	130(86.7%)	150 (100%)	

**Table (4) show grade of malignancy \* EPV infection of the patients Cross tabulation**

Grade of malignancy	EPV infections		Total	P.value
	Positive	Negative		
Poor differentiated	14 (16.1%)	73 (83.9%)	87 (100%)	0.04
Well differentiated	1 (1.6%)	62 (98.4%)	63 (100%)	
Total	15 (10.0%)	135(86.7%)	150 (100%)	

**Table (5) show grade of malignancy \* P53 expression Cross tabulation**

Grade of malignancy	P53 Expression		Total	P.value
	Positive	Negative		
Poor differentiated	19 (21.8%)	68 (78.2%)	87 (100%)	0.07
Well differentiated	6 (9.5%)	57 (90.5%)	63 (100%)	
Total	15 (10.0%)	135(86.7%)	150 (100%)	

## Discussion

It was noticed that the esophageal carcinoma was a frequent gastrointestinal cancer and it was seen in daily life in Gadarif Advanced Diagnostic Centre (GADC) endoscopic and histopathological units. The study included all patients presented to GADC with upper gastrointestinal tract symptoms and for whom endoscopic examination had been done, and diagnosed as squamous cell esophageal carcinoma patients at Gadarif teaching hospital at Gadarif State during June, 1. 2014- May, 31. 2017.

When factor such as age patient was considered, we found the occurrence of esophageal carcinoma to be higher in patients in the age group 60 years and above (72.7 % ), it did agree with a study led by *Kgomo* in South Africa in 2017 Jun 23 [5]. Similarly, a high prevalence of esophageal carcinoma was found in the 55 - 85 years age group (*Stahl*; 2013) [6].

The subjects of the study with esophageal carcinoma not diagnosed as HPV infection predominant which constituted 61.3%, it did not agree with (*Omrani* ; 2017) in Iran [7]. A similar study reported an increased incidence in HPV infection (*Wang*; 2016) [8], similarly a high prevalence of HPV infection was found in (*Wu*; 2001) study [9].

The patients with esophageal carcinoma not diagnosed as CMV infection predominant which constituted 86%, it did not agree with a previous study by (*Zhang et al 2011*) in China [10]. The patients with esophageal carcinoma not diagnosed as HSV and EBV infections predominant which constituted 86.7%, it did not agree with a study done by (*Zhang*; 2011) in China 2011. The patients with esophageal carcinoma not expressed P53 predominant which constituted 83.3%, it did not correlated with a previous study led by *Anwar et al 1992* [11].

## Conclusion

EC is one of the most serious malignancies of the gastrointestinal tract. The incidence rate of EC varies among different ethnic groups and geographic regions. Many risk factors, including

smoking, poverty and infection, may be involved in EC.

The esophageal carcinoma is not common cause of morbidity and mortality in Sudan especially in the Eastern states. Patients with this condition are frequently seen in the surgical department and referred to the GADC for further diagnosis and management.

It was found that there are association between grade of EC and HPV, HSV and EPV infections.

Further studies recommended for environmental risk factors and genetic predisposition of the disease since the disease is more seen in a particular area in Sudan and in certain tribes. Active case detection, if practically and financially feasible will provide early detection and management.

More studies and researches in esophageal carcinoma and others esophageal disease is recommended.

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