EUROPEAN ACADEMIC RESEARCH
Vol. IX, Issue 3/ June2021

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

ISSN 2286-4822
www.euacademic.org

Immunohistochemical Expression of P53 and Epidermal Growth Factor Receptor in Oral Lesions of Sudanese Patients in Gezira Sudan 2020

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Abstract

Background: *Epidermal Growth Factor Receptor and P53 tumor suppressor protein are involved in the etiology and prognosis of Oral Squamous Cell Carcinoma (OSCC). The aim of this study to investigate the immunohistochemical expression of EGFR and P53 proteins in cancerous and benign oral squamous tissues.*

Material and methods: *Forty-nine benign oral tissues and fifty-one cancerous oral squamous tissues were studied. The immunohistochemistry technique was used to investigate the expression of EGFR and P53 proteins in the benign and cancerous oral squamous tissues. The t-test percent, odd ratios, sensitivity and specificity were analyzed by using SPSS program.*

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Results: *The EGFR positive tissues in the benign and cancerous oral squamous tissues were 8 and 3 tissues, respectively, while the positive tissues for the p53 in the benign and cancerous tissues were 22 and 28, respectively. There was insignificant variation between the expression of EGFR and p53 in the benign and cancerous oral squamous tissues. The odd ratios of the EGFR and p53 were 0.3 and 1.49, respectively. The sensitivity and specificity of EGFR were 27.3% and 46.1%, respectively and those of p53 were 56% and 54%, respectively.*

Conclusion: *The p53 was had more susceptibility to be a tumor marker for OSCC than the EGFR.*

Keywords: Oral Squamous Cell Carcinoma, immunohistochemistry, Epidermal Growth Factor Receptor, P53, tumor tissue markers

INTRODUCTION

The Oral squamous cell carcinoma arises form oral mucosa in the floor of the mouth, tongue alveolar, buccaland labial mucosa, lower and upper lips and gingiva. Every year, approximately 275000 are diagnosed as OSCC and 128000 die from the disease. Risk factor of OSCC include usage of tobacco; smokeless and smoking, drinking alcohol, usage of Marijuana infection (bacterial and human papilloma virus) and inflammation [1-3]. Epidermal Growth Factor Receptor (EGFR) is a member of the epidermal growth factor receptor family which use the tyrosine kinase as a second messenger. They are of four types; EGFR, ErbB2, ErbB3 and ErbB4. They participate in cell proliferation, differentiation and migration [4,5]

The p53 gene is a tumor suppressor gene which located on chromosome 17. The transcription of the gene produces the p53 protein which in collaboration with the p21 protein and the cell division stimulating protein (cdk2) stops the cell division. Mutant forms of p53 protein are not capable of stimulating the production of p21 protein and stopping the cell division which leads to formation of tumors [6]The p53 is a tetramer protein with identical polypeptide chains. It has a central domain that binds the four polypeptide chains, an arginine rich domain

for binding the DNA to control the cell division and a transactivation domain [7].

Oral cancer (OC) which includes cancers of the lip, tongue and rest of the oral cavity, but not cancers of the major salivary glands [8], is responsible for sizeable morbidity and mortality rates worldwide especially in developing countries. While it is estimated that cancer incidence 14 million new cases, oral cancer alone claims about 300.000 deaths (2.1%) annually with 1.8% mortality worldwide [9, 10]. Oral cancer in Sudan is ranked as the sixth amongst all cancers types (6.1 per 100.000) [11]. This is strongly attributed to the use of local type of smokeless tobacco (SLT) known as Toombak, which is popular in the Sudanese community. Toombak is made from finely ground leaves of *Nicotianarustica*, Tobacco-specific nitrosamines (TSNA) levels in Sudanese Toombak were found to be unusually high compared to the reported levels in any other SLT.

The aim of this study was to investigate the immunohistochemistry staining of EGFR and p53 in benign and cancerous oral squamous tissues and to carry out a DNA scanning for a possible mutation in the p53 gene.

MATERIAL AND METHODS

Study design, ethical clearance and community

This research is classified as descriptive and case control study design. It was conducted after an ethical and academic approval was obtained from the faculty of medicine- University of Gezira-Wad medani- Sudan. A license to use the archival oral squamous tissues was obtained from the national cancer institute (NCI)- University of Gezira- Wad medani- Sudan. This study involved 49 benign oral squamous tissues and 51 oral squamous cell carcinoma tissues from Sudanese participants.

IHC staining

Sections were obtained from paraffin wax blocks and heated in oven for 30 minutes at 60 ℃ for removal of the wax and heated in water bath for 10 minutes at 90 ℃ for retrieval of EGFR-P53 in the tissue as follows: the sections were taken to water and treated with hydrogen peroxide solution for 20 min. After that, the sections were washed in tap water

for 5 minutes and phosphate buffered saline for 5 minutes, then treated with primary antiserum for 30 minutes and washed in phosphate buffered saline for 5 minutes. The primary antiserum was treated with secondary antiserum for 30 minutes followed by rinsing in phosphate buffered saline. At the end the sections were treated with Diaminobenzidine (DAB) for 15 minutes, washed in tap water then the nuclei were stained using Mayer's hematoxylin for 7 - 10 min then blued with tap water, dehydrated, cleared and mounted. The immunohistochemistry results were approved by two histopathologists.

Statistical analysis

The Independent samples t-test of the SPSS version 20 was used to compare the EGFR and p53 staining results in the benign and cancerous oral squamous tissues. The odd ratios, sensitivity and specificity were calculated manually.

RESULTS

General results: description of the study population is presented in **[Table 1].** It includes the total number of samples, the number of benign and OSCC samples, and the age groups

IHC staining results: The EGFR was positively stained in 3 OSCC and 8 benign tissues while the positive tissues for p53 in the benign and OSCC tissues were 28 and 22, respectively (Table.2). There were insignificant variations between the IHC staining of the EGFR and p53 in the benign and cancerous oral squamous tissues **[Table 2]**.

The odd ratios calculations showed that being positive for EGFR is 0.32 having OSCC compared to being negative for EGFR, where as being positive for p53 is 1.49 times having OSCC compared to being negative for it. Being negative for EGFR has more probability of being OSCC **[Table 3]**

The sensitivity and specificity testing showed that the p53 was more sensitive in the detection of OSCC. A negative tissue for EGFR was 46.1% benign tissue while a negative tissue for p53 was 54% benign tissue **[Table 4].**

Table [1] Description of the study population

Age group			Study subject		Total
			Normal	OSCC	
20-40	Sex	Female	6	3	9
		Male	9	5	14
	Total		15	8	23
41-60	Sex	Female	10	14	24
		Male	6	14	20
	Total		16	28	44
≥ 61	Sex	Female	7	6	13
		Male	11	9	20
	Total		18	15	33
Total	Sex	Female	23	23	46
		Male	26	28	54
	Total		49	51	100

Table [2] Expression of EGFR and P53 in normal and cancerous oral squamous tissues

	Positive		p-value *	Negative		*p-value
	Normal	Cancerous		Normal	Cancerous	
EGFR	8 (16.3%)	3 (5.88%)	0.097	41 (83.7%)	48 (94.1%)	0.098
P53	22 (44.9%)	28 (54.9%)	0.32	27 (55.1%)	23 (45.1%)	0.32

Table [3] The odd ratios of the immunohistochemistry results of EGFR and P53 for the detection of OSCC

		EGFR	P53
Numerator	Cancer positive	5.88	54.9
	Normal positive	16.3	44.9
	Ratio	0.36	1.22
Denumerator	Cancer negative	94.1	45.1
	Normal negative	83.7	55.1
	Ratio	1.12	0.82
Odd ratio (OR)		0.32	1.49

Table [4]: Sensitivity and specificity of the EGFR and P53 detected by IHC

	EGFR	P53
Positive cancerous tissuess	3	28
Total positive tissues (normal and cancerous)	11	50
Sensitivity %	27.3	56
Negative normal tissues	41	27
Total negative tissues (normal and cancerous)	89	50
Specificity%	46.1	54

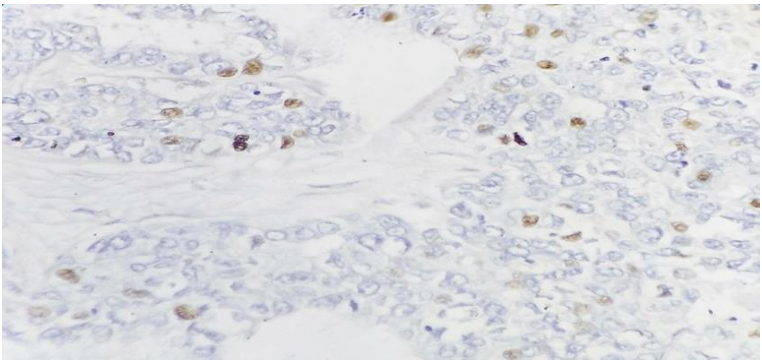

Figure (1): Photograph Show positive nuclear expression p 53 (Grade 2 moderately differentiated). 40x

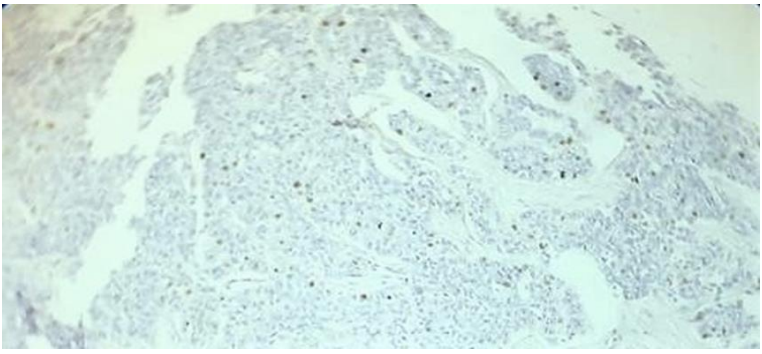

Figure (2): Photograph show nuclear expression of p53 (grade 1 well differentiated) 10x

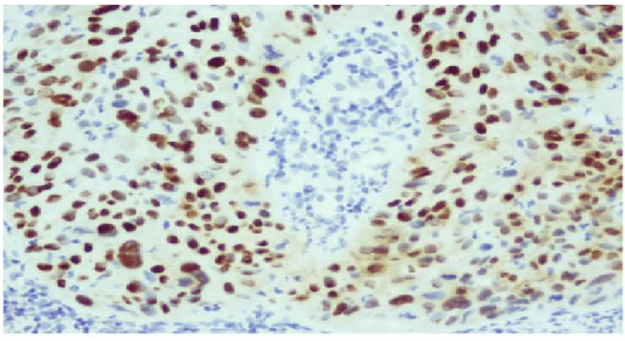

Figure (3) shows strong positive expression p 53

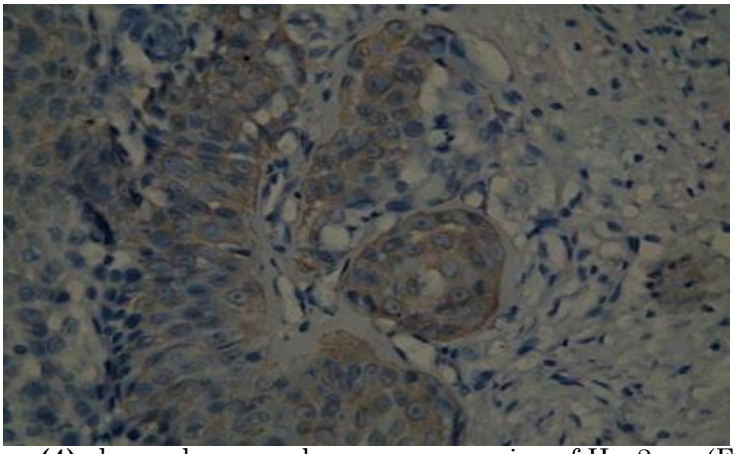

Figure (4) shows show membranous expression of Her2neu (EGFR) in oral squamous cell carcinoma

DISCUSSION:

Early diagnosis of oral cancer and treatment aims at increasing the survival rate of a patient. The gold standard method of revealing the oral lesion is histopathological analysis of a biopsy taken from suspicious oral lesion. The biomarker investigation becomes popular after the introduction of cellular and molecular studies about benignities in protein expression that have shown promising results in early diagnosis of oral cancer. The etiologic association between Toombak use and oral cancer has been investigated by several studies [12–13]. Study conducted by **(Eltayeb, A.S. et al, 2017)**[14] on oral cancer awareness and knowledge, the study revealed a level of around 66.6% of oral cancer awareness in different states of Sudan, however, there is a continuous practice of high risk habits despite the knowledge. It can be noted that there are no relevant studies in this discussion in Sudan, which, although it deprives our study from the benefit of comparison with previous studies in the Sudanese context, but at the same time leads to considering our study a pioneer in this field and needs more studies to be supported and evaluated.

The most popular predictive biomarkers related to oral cancer development includes P53 and EGFR **(Polanska, H. et al, 2014)** [15]. Therefore, this study aimed to investigate the expression of EGFR and

P53 proteins in cancerous and benign oral squamous tissues in Khartoum, Sudan. The immunohistochemistry technique was used to investigate the expression of EGFR and P53 proteins in the benign and cancerous oral squamous tissues.The study population composed of 49 healthy humans and 51 Oral Squamous cell carcinoma patients (OSCC). Our study reported male gender dominance, in benign subjects it involved 23 females and 26 males while the OSCC patients were 23 females and 28 males. **(T. Kirita et al, 2015)** [16] on the oral cancer in Japan, also reported age-adjusted male-to-female ratio is 3:2, which is higher in males than in females. Moreover, in Israel, **(Avraham Zini et al, 2009)** [17] reported the majority of patients with OSSC were males "had higher percentages of OSCC than females (71.4% vs. 47.2%, respectively)". Furthermore, **(Dundy G et al, 2016)** [18] Nepal, the majority (86.5%) of oral SCC were males with buccal mucosa being the most common site.

The Age of the study population was divided to three groups; (20-40), (41-60) and (≥ 61). The most frequent age group was 41-60 yrs. old, followed by the more elderly group ≥ 61 yrs. old. Our result was similar to previous study conducted in Arab countries by **(Abeer Al-Jaber et al, 2016)**[19]they reported that Yemenis have an alarming high prevalence of OC among people younger than 45 years. Eleven studies explored determinants or prognosis of OC. Behavioral determinants such as smokeless tobacco (Shamma and Qat), and cigarette smoking were strongly associated with OC. Alcohol drinking and solar radiation exposures were cited as possible risk factors. However, in Israel, **(Avraham Zini et al, 2009)** [17] the frequency of OSCC was higher among people aged 55 years or older (73.3%) and the youngest group had higher percentages of sarcoma and lymphoma (31.0% and 15.5%, respectively). Moreover, **(Dundy G et al, 2016)** [18]out of 40 biopsies of oral mucosa, 3 showed benign oral mucosa and 37 were diagnosed as squamous cell carcinoma (SCC), most patients were in 5th and 6th decade.

Many molecular markers are coming up and have been studied and have given new understanding of pathogenesis in oral squamous cell cancer. EGFR and P53 gene have emerged as critical mediators of signal transduction pathways. Over expressions of these proteins have been known to be associated with tumor cell proliferation, decreased or

resistance to apoptosis, angiogenesis, resulting in tumor progression and metastasis **(Arteaga, C.L., 2001)**[20].Various retrospective studies have correlated their over expression with decreased likelihood of survival and poor prognosis in oral cancers.

In this study EGFR was positive in 8 (16.3%) of benign and 3 (5.88%) of cancerous tissues. It showed negative result in 41 (83.7%) of benign and most of the cancerous tissue 48 (94.1%) were negative. P53 in benign tissues showed positive expression 22 (44.9%) and negative expression in 27 (55.1%) of the cases. Moreover, regarding cancerous tissue; P53 expression was positive in 28 (54.9%) a little more than half the cases and negative in 23 (45.1%) a little less than half the cases. There was no significant association between IHC expression of the markers with benign and cancerous tissue, p value 0.097 and 0.32, respectively.Furthermore, the odds of having the OSCC were 0.32 and 1.49 higher given being positive for EGFR and P53, respectively compared to being negative for them. Concerning the sensitivity and specificity of the EGFR and P53 detected by IHC, P53 was more sensitive than the EGFR in detecting the OSCC by IHC. A negative tissue for EGFR and P53 was 46.1% and 54% benign tissue, respectively.

The Epidermal growth factor receptor family of receptor tyrosine kinases (RTK) comprises of four distinct receptors: the EGFR (also known as ErbB-1/HER1), ErbB-2 (neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4). Several studies have been published on the role of EGFR in the pathogenesis of oral carcinoma. Immunohistochemical over expression of p53 is considered as a marker of poor prognosis in many cancers.**(Singla S et al, 2018)**[21]the EGFR expression of 33 (27.5%) patients were low and 87 (72.5%) were high while p53 expression of 29 (24.2%) were low and 91 (75.8%) were high. There was a significant correlation between the expression of markers p53 and EGFR in leukoplakia and SCC. The expression of p53 was correlated between leukoplakia, SCC, and control and was found to be significant. Similarly, EGFR expression was significant between cases of leukoplakia, SCCs, and controls.

(Aline Correa Abrahao et al, 2011)[22]supra-basal p53 immunoexpression was associated with severe grades of dysplasia. **(Solomon MC et al, 2016)**[23] EGFR was the most frequently

expressed 150/178 (84%) biomarker of the cases. **(Ali SM et al, 2019)**[24]reported EGFR overexpression was observed in 70% and p53 in 67% of OSCC patients. The findings emphasize the role of EGFR, COX and p53 overexpression in OSCC patients.

EGFR may represent a promising target for novel molecular cancer therapies. EGFR expression levels in the premalignant lesion appear to be a sensitive factor in predicting the neoplastic potential of dysplastic tissues. Study done by **(Mahendra A et al, 2014)**[25]positive EGFR staining was present in all the cases 100% (30/30) out of which 7 (46.7%) cases of OSCC showed >75% EGFR expression and 8 (53.3%) cases of oral leukoplakia showed 25% EGFR expression. This suggests that EGFR may serve as a biological marker to identify high-risk subgroups and guide prophylactic therapy.

Many studies have proved the prognostic significance of p53 and EGFR biomarkers owing to association of these markers overexpression with overall survival, **(Ribeiro FA et al, 2014)**[26] reported overexpression of EGFR results in a poor prognosis in oral cancer and its activation is associated with the malignant phenotype, inhibition of apoptosis and increased metastatic potential. EGFR variations and mutations have been correlated with tumor formation, and possibly alter the therapeutic efficacy of EGFR inhibitors. **(Bernardes VF et al, 2013)**[27]reported EGFR+ rates were 53.8% (28/52) by IHC, however, there is no association between EGFR expression and gene amplification in OSCC when the IHC is driven to external epitopes of the protein. Moreover, **(Hashmi AA et al, 2018)**[28]reported that on the basis of intensity, strong EGFR expression was noted in 13.9% (16 cases) while 16.5% (19 cases) and 23.5% (27 cases) revealed intermediate and weak EGFR expression respectively. Significant association of EGFR expression was noted with tumor stage and disease-free survival.

Furthermore, **(Sarkis SA et al, 2010)**[29]reported positive EGFR immunoreactivity in 35(87.5%) cases. There was a statistically significant correlation regarding EGFR extent score with respect to intratumoral lymphatic vessel density (ILVD) as well as EGFR intensity score with respect to ILVD and peritumoral lymphatic vessel density (PLVD). EGFR is expressed by most of the cases. Lack of

correlation among the studied markers suggests their independent effect on tumor behavior.

LIMITATIONS AND RECOMMENDATIONS:

- With a larger sample size and significant statistical power the associations are important: therefore we suggest more large scale studies to evaluate diagnostic importance of p53 and EGFR biomarkers.
- Further studies to evaluate the prognostic significance of p53 and EGFR expression in OSCC and its association with disease free survival in Sudanese population.

CONCLUSION:

The significance increase in expression of p53 and EFGR biomarkers in OSCC suggests their role as surrogate markers of malignant transformation. However, our findings revealed that, the p53 had more susceptibility to be a tumor marker for OSCC than the EGFR.

Financial support and sponsorship
Nil.
Conflicts of interest
There are no conflicts of interest.

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