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ORIGINAL ARTICLE

Association of E-Cadherin expression with different histopathological grading of Oral Squamous Cell Carcinoma (OSCC) in Hyderabad Sindh.

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ABSTRACT… Objective: To evaluate association of E-cadherin with different histopathological grading of oral squamous cell carcinoma in Hyderabad region. **Study Design:** Cross-sectional study. **Setting:** Department of Oral and maxillofacial surgery and Oral Pathology, Isra Dental College (IDC) Hyderabad. **Period:** 25th November 2021 to 25th November 2022. **Material & Methods:** Total 58 patients of OSCC and 10 normal mucosa samples were enrolled in this study, patient's age, gender, habit and site of lesion was recorded on proforma. Total 68 paraffin embedded tissue fragments were processed and stained with Eosin & Hematoxylin stain. Further the tissue sections were immune-stained with E-cadherin and slide evaluated by pathologists. **Results:** Total 58 diagnosed patients of OSCC were characterized into three groups well differentiated, moderatly, and poor ly differentiated OSCC. Total 46 (79%) were male and 12 (21%) were female. The cruel age of a female to male was 36.14 VS 43.21 years. The mean age of a female to male was 36.14 VS 43.21 years. Study reported decrease level of E-cadherin appearance with increasing "histopathological grading of OSCC", expression of E-cadherin was high in well differentiated OSCC followed by moderately differentiated OSCC and poorly differentiated OSCC. **Conclusion:** study concluded that loss of E-cadherin has been associated with incresed invasiveness and metastatic potential in various cancers, including oral cancers. The level of E-cadherin expression recorded decrease with increasing severity and histopathological grading of OSCC. This would be proven a useful marker tool for the prediction of OSCC grading and oral cancers to metastasize, providing valuable information for cancer diagnosis and treatment planning.

Key words: E-cadherin, Histopathological Grading, OSCC.

INTRODUCTION

Oral cancer of the squamous cells is a major cause of fatalities in all communities and a significant source of sickness worldwide.

[1]According to the World Health Organization OSCC, An estimated 3.5 million new cases of OSCC are identified annually, which makes it the sixth most prevalent cancer globally. OSCC is more common in emerging nations due to the occurrence of risk features like alcohol and tobacco use, betel quid chewing, and poor oral hygiene. One of OSCC is most distinctive clinical characteristics is its propensity to invade nearby tissues and spread locally. Thus, it is essential to predict the invasive and metastatic potential of OSCCs early on in the therapeutic process. Recent years have seen the discovery of several biological markers that may control tumor development and offer helpful prognostic data for the treatment of OSCC.[2] Despite the fact that medical professionals are constantly looking for an early detection tool that would be useful in the early detection and treatment of OSCC. Many targets genes and markers have been discovered, but an early detection tool is still being sought. The conducted study focus on E-cadherin and its association with OSCC, it is hypothysed that loss of E-cadherin in tissues indicating development of oral malignancies.

E-cadherin is not only essential for cell adhesion, but it also plays a role in signal transmission networks that control cell proliferation, segmentation, and

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apoptosis. Loss of E-cadherin expression or function has been related with the progression of several types of cancers, particularly those that originate in epithelial tissues.[3] This is because the loss of E-cadherin can lead to reduced cell-cell adhesion and increased cell motility, which are both key features of cancer metastasis.[4] Loss of E-cadherin expression is a common feature of many types of cancer, including breast, stomach, and colon cancer. This loss of expression can lead to the breakdown of cell-cell adhesion, which can promote tumor invasion and metastasis. Thus, E-cadherin is a tumour suppressor, and its transcript is frequently used as a predictive as well as diagnostic indicator in cancer studies. Overall, the study of E-cadherin expression in OSCC and its correlation with histopathological grading may have important clinical implications, including the development of new therapeutic strategies and the identification of novel prognostic markers.[5] Therefore, the focus of this study is to determine the correlation of E-cadherin with OSCC and its expression variation in different histopathological grading of OSCC.

MATERIAL & METHODS

Samples for this cross-sectional research were collected from the "Oral Surgery Department" at Isra Dental College (IDC), Hyderabad, Sindh. Study was conducted from 25th November 2021 to 25th November 2022, after approval from Ethical research committee (IU/RR-10/AQK/2021/1956) Isra University Hyderabad. 58 clinically OSCC diagnosed patients and 10 normal mucosal specimens were enrolled after taken a written consent in study by following inclusion criteria and normal oral mucosa was taken as control group. Patient's age, gender, habit and site of lesion were recorded. 68 samples were fixed in 10% formalin and dehydrated by ascending grades of xylene and alcohol. Tissues were embedded in paraffin wax block and two sections of each tissue samples about 4um thin sections were taken by the microtome. One section of tissue samples were stained with hematoxylin stain, and latter with Eosin stain for the histopathological evaluation of slide and send to the pathologist, diagnosed cases were categorized into three groups on the basis of cellular differentiation and histopathological grading of "oral squamous cell carcinoma" according to Broder's classification as well differentiated (WD) OSCC, "moderately differentiated" (MD) OSCC, and poorly differentiated OSCC. Second set each tissue sections were rehydrated and prepared for immuno-stained with monoclonal antibodies against E-cadherin (Biogenex USA, 6ml, commercially available) Kushwaha, et al.

Initially, the antigen was retrieved from a tissue section using a microwave in a citrate buffer solution with a pH of 6.0. The power level was high for the first 15 minutes, and then low for the remaining 10 minutes. Tris-buffered water was used to clean the sample of tissue. Tissue slices were handled with 4% hydrogen peroxide for thirty min to stop the tissue's natural antioxidant properties. After that, the tissue pieces were placed in a moist compartment at 37 degrees Celsius for 60 minutes with a main specific antibody against the E-cadherin protein.

Afterward, the tissue segments were treated with a secondary antibody ("biotinylated anti-immunoglobulins/superenhancer") for 30 minutes at standard temperature to improve the outcome of the succeeding polymer step. After room temperature for 30 minutes, the sections had protected with a weak secondary antibody or chemical (enzyme-conjugated streptavidin). Afterward, the antigen was observed by counterstaining the tissue slices with Mayer's hematoxylin and diaminobenzidine chromogen treatment.

Using the immunoreactive score (IRS)[15], the immunoreactivity of E-cadherin in each group was semi quantitatively evaluated. IRS = immunostaining intensity (A) percentage of immunopositive cells (B).[6]

RESULTS

Total 58 patients of OSCC and 10 normal mucosa from buccal were enrolled in this study, 46 (79%) were male OSCC patients and 12 (21%) were female. The mean age of female was 36.14 while male was 43.21 years. Information about oral habits was obtained from 58 patients, among

those smokeless Tobacco user were 47 (81%), Smokers were 8 (14%), alcohol consumers were 02 (3%) and 1(2%) patient was reported with non-Tobacco use in study (shown in Table-I)

Site of OSCC was recorded on proforma, the most common site of lesion found buccal mucosa 24 (42%), other sites including, alveolar area 15 (26%), tongue 06 (10%), retro molar area 05 (8%), floor of the mouth 05 (8%) and lip 3 (6%) reported in study. (shown in Table-I)

Total 58 samples were diagnosed after clinical and histological examination of patients, out of them 39 (67%) cases were reported as well differentiated OSCC, 12 (21%) were moderately differentiated OSCC, and 07 (12%) cases were reported as poorly differentiated OSCC (as shown Table-II).

The mean percentage of immunopositive cells was greater in "well-differentiated squamous cell carcinoma" (WDSCC, 6.8300 %) compared to moderately and "poorly-differentiated squamous cell carcinomas" (MDSCC, 1.9800% and PDSCC, 0.588%), well differentiated OSCC and normal epithelium had the highest immunostaining amplitude, followed by moderately distinguished OSCC and poorly distinguished OSCC (shown in Table-IV).

In normal mucosa and well differentiated OSCC cells, E-cadherin was strongly expressed as a homogenous membrane structure. It was diverse in moderately differentiated OSCC (both membrane and cytoplasmic), but in poorly differentiated OSCC cells, it acquired a weak or negative expression (n-3), which was significantly expressed ($P < 0.05$).

1. Variables	**N (%)**	
no tobacco use	1 (2%)	
Smokeless tobacco	47 (81%)	
Smoking	8 (14%)	
Alcohol	2 (3%)	
2. Site of Lesion		
Buccal mucosa	24 (42%)	
Alveolar area	15 (26%)	
Tongue	06 (10%)	
Retro molar area	05 (8%)	
Floor of the mouth	05 (8%)	
Lip	3 (6%)	
3. Gender		**SD**
Male	46 (79%)	43.11±21.6
Female	12 (21%)	36.12 ±14.2
Total		59 (97.8)

Table-I. Demographical features of OSCC patients

Histological Grading	**Frequency of Patients (%)**
WD OSCC	39 (67%)
MD OSCC	12 (21%)
PD OSCC	07 (12%)
Total	58 (100%)

Table-II. "Histological grading of oral squamous cell carcinoma"

S= % of immunopositive cells (A) × intensity of immunostaining (B)
0–1: Nil 2–3: Mild
4–8: Moderate
9–12: Strongly positive
A – Percentage of E–cadherin immunopositive cells
The ratio of E–cadherin immunopositive cells was assessed and graded, in five arbitrary fields, on a measure of 0–4 asfollows
0. points: No immunopositive cells
1. points: <10% immunopositive cells
2. points: 10%–29% immunopositive cells 3 points: 30%–59% immunopositive cells 4 points: 60%–100% immunopositive cells
B – E–cadherin immunostaining Intesity
E–cadherin immunostaining intensity was graded on ascale of 0–3 points.
0. points: No staining
1. point: dull staining intensity
2. points: Mild staining intensity
3. points: extreme staining intensity

Table-III. Immunoreactive score

Description	n	Mean (per sample)	SD	F	P
Normal mucosa	10	3.9300	0.67329	96.995	<0.0001
Well differentiated OSCC	39	6.8300	0.45361		
Moderately differentiated OSCC	12	1.9800	0.72748		
Poorly Differentiated OSCC	07	0.5881	0.58578		
Total	68	16.3281	0.97975		

Table IV. Mean % of E–cadherin positive cells in the control and research groups

DISCUSSION

OSCC is a most common frequent neoplasm especially in Asian regions such as Pakistan, India, and Bangladesh and worldwide as well[7], due to consumption of smokeless and smoking tobacco and other substances that promote oncogenesis.[8] Perhaps there is a critical need of early diagnosis and effective treatment of such neoplasm that would be play an important role in decreasing morbidity and saving human lifes. Medical science continuously searching for tools that could be effective in early diagnosis of neoplasm, this current study is also conducted to determine the association of e-cadherin with histological grades of OSCC.[9] According to study hypothesis and other studies, e-cadherin play important role in cell adherence and damage of e-cadherin has reported in many neoplasm, a loss of intercellular junction is thought to occur before metastasis and tumor invasion since E-cadherin appearance is typically diminished or may be absent in a type of epithelial malignancies.[10] So the current study is determining the e-cadherin expression in histological grading of OSCC.

Total 58 number of oral squamous cell carcinoma patients and 10 normal mucosa from healthy individuals was enrolled in study. 46 (79%) were male, while 12 (21%) were female OSCC patients. Study found smokeless tobacco like betal nut chewing, naswar and other substances were major cause of carcinoma, smoking and alcohol was also reported in study (as shown in Table-I).

A study was conducted in Room, according to G. Tenore at el (2020) tobacco is most communal cause of “oral squamous cell carcinoma” and study also reported smoking is also common risk factor in oncogenesis.[11]

Maximum communal site of lesion was “buccal mucosa” following by alveolar area of oral cavity, tongue, retro molar area and less common site of lesion was lip. According to Pires FR at el (2013) study tongue was most common site of OSCC following by alveolar area and floor of the mouth were less common reported.[12]

Total 58 OSCC patient were clinically and histopathological diagnosed, the majority of the patients had well differentiated OSCC (67%), followed by moderately differentiated OSCC (21%) and poorly differentiated OSCC (12%). Another study conducted in Pakistan Waqas at el (2021) also reported high prevalence of well differentiated OSCC followed by moderately differentiated and poorly differentiated OSCC.[13]

The current study recorded major correlation with histopathological grading of oral squamous cell carcinoma, expression of e-cadherin had seen increase in normal oral mucosa and well differentiated OSCC 6.8300% while it is recorded less in moderately differentiated OSCC1.9800% and much less observed in poorly differentiated OSCC 0.5881%. There is a considerable difference among all OSCC scores. These conclusions are also concordance with an Indian research Sandhya Singh at el (2019)[14], and Butt SA at el (2019)15 reported high e-cadherin expression in normal mucosa and well differentiated oral squamous cell carcinoma followed by moderately differentiated OSCC and poorly differentiated OSCC.

CONCLUSION

Study concluded Loss of E-cadherin expression has been linked to a higher risk of malignancies spreading and becoming aggressive, including oral cancers. The level of e-cadherin expression recorded decrease with increasing severity and histopathological grading of OSCC. This would be proven a useful marker tool for the prediction of OSCC grading and oral cancers to

metastasize, providing valuable information for cancer diagnosis and treatment planning.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Khushbu Lohana	Study design, Patient select, Data collection.	
2	Shahzaman Memon	Data analysis, suggestions, data interpretation, experimental work and histopathological evaluation and immunohistochemistry.	
3	Shafquat Hussain Khuwaja	Data analysis and result interpretation.	
4	Waqas Iqbal	Study design, Patient selection, data collection, experimental work and histopathological evaluation and immunohistochemistry.	
5	Hafiz Mahmood	Data collection, drafting in literature search.	
6	Farah Tasleem	Drafting discussion chapter, Data analysis, Drafting the manuscript