Association of Serum Soluble E-Cadherin Levels with Oral Potentially Malignant Disorders in Tobacco Users and Non-Users

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ABSTRACT

Background: Oral Potentially Malignant Disorders (OPMDs) transforms into Oral Squamous Cell Carcinoma (OSCC) but this transformation is not steadfast and depends on multiple factors. E-cadherin, a calcium-dependent cell-cell adhesion molecule plays pivotal role in epithelial cell behavior, tissue formation, and suppression of cancer and participates in signal transduction of various cellular events including polarity, differentiation, growth and migration. The serum E-cadherin level are raised in cancer patients

Objective: We designed the study to compare soluble E-cadherin levels in the serum of patients with OPMDs and healthy control among tobacco users and non-users.

Material and Methods: This comparative cross sectional study was conducted at Peshawar Dental College, Khyber College of Dentistry, and Bacha Khan Dental College on the serum samples of 80 subjects, comprising of 40 cases of OPMDs and 40 healthy controls, by adopting non-probability consecutive sampling technique.

The serum was analyzed for soluble E-cadherin levels/concentration by ELISA method. Data was recorded, evaluated and analyzed by SPSS version 23.

Results: In OPMDs (Group A) 29 (72.5 %) were tobacco user and 11 (27.5 %) were non-users while in healthy controls (Group B) only 7 (17.5 %) were tobacco user and 33 (82.5 %) were non-users

The mean serum soluble E-cadherin levels among OPMDs and healthy controls were 25.94 ng/mL (SD of ± 4.65), 24.10 ng/mL (SD of ± 6.21) respectively. It was statistically insignificant ($P > 0.05$) and were identified when independent samples T-Test were applied to compare the serum soluble E-cadherin levels between the two groups. Statistically significant ($P < 0.05$) relation has been established in our study between tobacco users and OPMDs. By using Chi square test both tobacco users and non-users, males and females showed highly significant association with OPMDs group and healthy controls with a ($P < 0.05$) respectively

Conclusion: The concentration of serum soluble E-cadherin were higher in OPMDs cases when compared with healthy controls both tobacco users and non-users but it was found statistically insignificant. Furthermore, the serum soluble E-cadherin with OPMDs group had a significant association with tobacco user. Risk estimate (odds ratios) showed that tobacco users were 12.43 times more at risk to develop OPMDs than non-users

Keywords: Oral Potential Malignant Disorders (OPMDs), Serum soluble E-cadherins, Oral Squamous Cell Carcinoma (OSCC).

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INTRODUCTION

Globally squamous cell carcinoma (SCC) of head and neck region is increasing alarmingly and is on sixth position with the annual incidence of six hundred thousand cases^{1,2}. Among head and neck cancer, OSCC is a leading cause of increased morbidity and mortality. As OSCC does not meet the criteria to be screened, this possess a challenge in early diagnosis but however in clinical practice and in literature it is suggested that most of the OSCC is preceded by asymptomatic lesions referred as OPMDs³⁻⁵. OPMDs, are defined as “the risk of malignancy being present in a lesion or condition either at the time of initial diagnosis or at a future date”⁶.

The persistent lesions or conditions within the oral cavity are OPMDs described by the potential for malignant transformation⁷. The following were recognized as OPMDs, leukoplakia, erythroplakia, oral lichen planus, oral submucous fibrosis, palatal lesion of reverse cigar smoking, actinic keratosis, and tobacco pouch keratosis by WHO working group on oral cancer⁴.

OPMDs transforms into OSCC but this transformation is not steadfast and depends on multiple factors like population, sex, behaviors and the dysplasia grade.

In another meta-analysis, it was concluded that serum E-cadherin levels increase in patients with carcinoma. This show serum E-cadherin being can be used as a sensitive indicator of tumor related proteolytic activity. But serum E-cadherin may also increase due to other causes. Furthermore, it may not be used as a screening marker like others due to its low specificity and sensitivity⁸. However, E-cadherin expression and degree of differentiation of tumor and dysplasia were not correlated⁹. But in certain other

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carcinomas serum E-cadherin level rises significantly in carcinoma of colon and rectum. It may be significant statistically but its clinical significance has not been established¹⁰.

In one study it was concluded that soluble E-cadherin in saliva of patients with OSCC is present in significant amount as compared to normal subject with statistical significance. Also, the authors delineated the significant inverse relationship between soluble salivary E-cadherin and membranous E-cadherin¹¹.

To our knowledge, in the scientific literature it is the first study reporting the serum E-cadherin levels among cases of OPMDs and healthy controls. The findings of this study may lead to opening of other important breakthrough in the oral malignancy research related to the role of E-cadherin.

The present comparative cross-sectional study was designed to compare the serum E-cadherin levels cases of OPMDs and healthy controls among tobacco users and non-users.

MATERIAL AND METHODS

This comparative cross sectional study was conducted in Peshawar Dental College, Khyber College of Dentistry and Bacha Khan Dental College on the serum samples of 80 subjects, comprising of 40 cases of OPMDs and 40 healthy controls, by adopting non-probability consecutive sampling technique. The study duration was six months from April 20th to October 19th 2019.

The sampling was done by non-probability consecutive random sampling including both genders excluding patients with OSCC and alcoholics.

Detailed history OPMDs and Healthy controls were recorded on specific proforma after informing them about purpose of the study and obtaining their written consent on proforma.

5 ml blood was collected from diagnosed patients of OPMDs and healthy controls under aseptic conditions and spun in a bench centrifuge (Hettich EBA 20 Germany) at 4000rpm for 5 minutes for collection of serum. Serum samples were aspirated by micropipettes and placed in serum cuvettes and were stored at -20 °C until analyzed. The serum was analyzed for soluble E-cadherin levels/concentration by ELISA method (using Human E-cadherin ELISA kit) and recorded. The

study was carried out after approval from the Institutional Ethical Committee of Peshawar Medical College, Peshawar.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) Version 23. Means and standard deviation were calculated for continuous variables while frequencies were calculated for categorical data. To find out the relationship between continuous and categorical data Independent Samples T-test was used while Chi square test was used to find whether there is any association between categorical variables. Probability value of less than or equal to 0.05 ($P=0.05$) was considered statistically significant.

RESULTS

Site Distribution in OPMDs

Buccal Mucosa was the main site affected by OPMDs (50%) followed by Labial Mucosa (12.5%), Gingival Mucosa (10%), Tongue (10%), Alveolar Ridge (5%), Maxillary Sulcus (5%), Buccal Sulcus (5%) and Lower Lip (2.5%) as given in Pie chart.

There were 29(72.5%) tobacco users, and 11 (27.5%) non-users in OPMDs. While in healthy controls 7(17.5%) were tobacco users and

33(82.5%) were non-users as given in the bar chart below.

Independent Samples T-Test and P-Value For OPMDs and Controls

Independent Samples T-test was applied to find out whether there is any association between mean serum concentration of E-cadherin level in OPMDs and control groups.

Table 1 shows there was difference between OPMDs group and healthy controls regarding E-cadherin concentration but it was statistically insignificant (T-test = 1.50, P = 0.14).

Table 1. E-cadherin Concentration in OPMDs (n=40) and Controls (n=40) With Independent T-test and P-value

Group	Mean serum E-cadherin with Standard Deviation ng/mL	Standard Error Mean	T-Test value	P-value	Mean difference	95% Confidence Interval	
						Lower	Upper
A OPMDs	25.94 ± 4.65	0.735	1.50	0.14	1.84	-0.06	4.28
B Healthy controls	24.10 ± 6.21	0.982					

Chi square test was used to show the relation of OPMDs and controls in tobacco users and non-users, and both gender.

A statistical comparison was made between OPMDs and healthy controls for tobacco use and males revealed significance difference with a (P=0.00) and (P=0.002) respectively as given in table 2.

Table 2. Using Chi-square Test for Tobacco Users and Non-users and Gender

Characteristics	Overall N=80	OPMDs N=40 (%)	Controls N=40 (%)	P-value
Tobacco users	36	29 (80.6%)	7 (19.4%)	0.00
Non-users	44	11 (25.0%)	33 (75.0%)	
Males	55	34 (61.8%)	21 (38.2%)	0.002
Females	25	6 (24.0%)	19 (76.0%)	

Risk Estimate (Odds Ratio)

Odds ratio was calculated for OPMDs group and healthy controls with a value of 12.43 having confidence interval of 4.26-36.27 as given in

table 3. This means that those who are tobacco users are 12.43 times more at risk to develop OPMDs than that of non-users.

Table 3. Risk Estimate (Odds Ratio)

	Odds Ratio	95% Confidence Interval	
Category		Lower	Upper
Tobacco users	12.43	4.26	36.27

DISCUSSION

The novelty of the present study can be best assessed from the fact that, to our knowledge, this is the first study conducted on the serum samples of cases of OPMDs for the assessment of soluble E-cadherin levels. There are two studies conducted on the E-cadherin levels in salivary samples of OSCC and the serum of head and neck squamous cell carcinoma (HNSCC) patients^{11,12}. Al Kassam et al., found high serum levels of soluble E-cadherin among 39 cases of HNSCC, to be significantly different from those in the control group, comprising of 10 subjects. Lopez-Verdin et al found increase in salivary E-cadherin levels among 26 cases of OSCC compared to 10 individuals without oral carcinoma, its in accordance with our study.

When we look at the site distribution in our observations, the commonest site was buccal

mucosa followed by labial and gingival mucosa and tongue. Kumar et al studies in 2019 observation were, the commonest site was buccal mucosa followed by tongue and palate. Other studies on OPMDs have identical observations, i.e., buccal mucosa followed by alveolar sulcus and tongue (76%, 19% and 5% respectively) included findings of Raina et al¹³⁻¹⁵.

The study results confirmed the observation that in healthy controls the soluble serum E-cadherin concentration was less than the patients with OPMDs. The mean value of soluble serum E-cadherin concentration was 25.94 ± 4.65 ng/mL in OPMDs group and 24.10 ± 6.21 ng/ml in healthy controls and the rapid turn-over of the tumor cells might be responsible for the soluble E-cadherin origination. Thus, increase concentration of the soluble E-cadherin means, the higher is the dysplasia. As shown in study whereby the expression of E-cadherin levels in tissues decreases proportionally to the degree of

dysplasia in tissue. Thus, it may be inferred that serum E-cadherin levels increases in sera as observed in our study¹⁶.

In this study tobacco users and non-users, males and females showed highly significant association with OPMDs group as compared to healthy controls with a ($P=0.00$) and ($P=0.002$) respectively. Nicotine, the major addictive component of tobacco smoke, has been proven to induce invasion and epithelial-mesenchymal transition by increasing serum E-cadherin levels in cancers of the oral cavity, lungs, breast, pancreas and colon^{17,18}.

Odds Ratio of 12.43 with 95% confidence interval of 4.26 ? 36.27 in the present study was calculated for tobacco users. Which indicates that tobacco users are 12.43 times more at risk to develop OPMDs than non-users. Recently published data also stated that increase in serum E-cadherin levels indicate phenotypic changes in early stages of oral carcinogenesis¹⁹⁻²³.

The main shortcomings of our methodology included small sample size, lack of follow up of the patients. Despite the limitation of our study, the main strengths are multicenter study, novel study, no need for special training of collection of samples, easy and minimally invasive sample collection and no need of anesthesia.

CONCLUSION

The study concluded that the concentration of serum soluble E-cadherin were higher in OPMDs cases when compared with healthy controls but it was found statistically insignificant.

Statistically significant relation has been established in our study between tobacco users and OPMDs. Risk estimate (odds ratios) showed that tobacco users were 12.43 times more at risk to develop OPMDs than non-users.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Saboor A, Muhammad K: Concept and design of study, Collection of data, statistical analysis

Rehman I, Amanullah A: Writing of manuscript, critical review of manuscript

Khan AS: Analysis and interpretation of data, statistical analysis

Khan MM: Data collection, bibliography

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.