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Original Research Article

Quantitative Estimation of Salivary & Serum C-Reactive Protein & Alkaline Phosphatase Levels in Different Grades of Oral Squamous Cell Carcinoma

Madhurya Namana[1], Ayyagari Kameswara Rao[2], Kuna Rajani[3], Bolem Padmavathi[4], Parameswar Peela[5], Naga Hima Bindu Vennamaneni[6]

[1]Dental Practitioner, Hyderabad, Telangana, India.

[2]Assistant Professor, Department of Dental Surgery, Government Medical College, Vizianagaram, Andhra Pradesh, India.

[3]Associate Professor, Department of Pathology, Government Medical College, Vizianagaram, Andhra Pradesh, India.

[4]Assistant Professor, Department of General Medicine, Government Medical College, Vizianagaram, Andhra Pradesh, India.

[5]Associate Professor, Department of Oral Pathology, Anil Neerukonda Institute of Dental Sciences, Tagarapuvalasa, Visakhapatnam, Andhra Pradesh, India.

[6] Dental Practitioner, Hyderabad, Telangana, India.

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Corresponding author: Dr. Bolem Padmavathi
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Abstract

Background: Elevated Serum C-Reactive Protein (CRP) and Alkaline Phosphatase (ALP) levels can be considered as a prognostic indicator in Oral Squamous Cell Carcinoma (OSCC). They can act as indices of proliferation, metastasis and survival rate. However previous studies on these biomarkers in combination were rarely correlated. The study aimed to compare salivary & serum CRP and ALP levels of adults with OSCC patients using quantitative estimation and relate them with Histological grading, and TNM staging.

Materials and Methods: A total of 50 individuals of which 10 healthy individuals and 40 individuals with clinically histopathologically confirmed OSCC with deleterious habits were considered for the study. Incisional biopsy was performed from the lesion proper, haematoxylin and eosin staining was performed. In the consequent visit saliva and blood samples were collected from the confirmed patients and controls. The values of CRP and ALP were calculated using immunoturbidity method and Colorimetric method respectively. Statistical analysis was performed using Kruskal-Wallis test, ANOVA and chi - square test.

Results: There was statistically significant correlation between serum and salivary CRP, ALP levels in OSCC and also with TNM staging as compared to controls, with increase of histopathological grading- CRP/ ALP levels increased.

Conclusion: To conclude, for assessment of survival rate the present study showed positive results. Further elaborated studies including larger cross section of population, pre and post treatment recurrence and survival period should be included, so that these biomarker values can be emphasized as one of the prognostic biomarkers of individual with OSCC.

Keywords: C-Reactive Protein, Alkaline Phosphatase, TNM staging, Oral Squamous Cell Carcinoma.

Introduction

Oral Squamous Cell Carcinoma (OSCC) is the common most malignancy in the head and neck region, annually it accounts for 300,400 new cases and 145,400 deaths from oral cavity cancer including lip cancer. [1] Worldwide, it is eleventh most common cancer, with an especially high incidence reported in Indian sub-continent. Approximately 94% of all oral cancers are OSCCs. [2] Oral cancer, in the Indian subcontinent ranks the top among three types of cancers. [3] The high risk in Indian population is due to lifestyle-related habits such as smoking with or without alcohol consumption, chewing of areca nut and it's related products. In India, 57% of all men and 11% of women between 15 and 49 years of age use one or the other form of tobacco. [4] C- Reactive Protein (CRP) is an acute-phase inflammatory protein, discovered by Tillet and Francis while investigating the sera of patients suffering from the acute stage of *Pneumococcus* infection and was named for its reaction with the capsular (C)-polysaccharide of *Pneumococcus*. It is mainly produced in hepatocytes of the liver in response to increased levels of inflammatory cytokines. [5] CRP levels also act as a marker of chronic inflammation in the tumor microenvironment, with chronic inflammation itself acting as an inhibitor of apoptosis, stimulus for neo angiogenesis and cell proliferation. Elevated serum CRP levels can be considered as a prognostic indicator in cancers. Alkaline Phosphatase (ALP) is an enzyme that catalyse the hydrolysis of phosphate esters generating an organic radical and inorganic phosphate. ALP is mainly derived from liver and bone, increased activity of this enzyme is associated with increased osteoblastic activity and hepatic diseases. Serum ALP levels are raised in patients with primary and metastatic tumours of liver and bone.

Disease progression in cancer is dependent on the complex interaction between the tumor and the host inflammatory response. This systemic inflammatory response is evidenced by elevated levels of CRP. [5] Higher CRP levels are associated with staging of tumour, locoregional invasiveness in OSCC and so can be used to predict tumour invasion, lymph node metastasis, staging and survival. Although CRP is considered as indirect marker of stages of inflammation in neoplastic cells, estimated range had not been standardised to reflect the progressive potentiality of malignancy in most of the studies. In cells ALP is necessary for proliferative activity associated with tumour invasion, important marker for tumour cell differentiation. Elevation in neoplasia may be contributed from hepatic metastasis, bone metastasis or direct contribution of neoplastic cells. [6,7] As saliva is a by-product of serum, assessment of biomarkers done through saliva has several advantages such as easier execution, faster results and less discomfort to the patients. Salivary analysis holds promise as a non-invasive approach to identify biomarkers for oral malignancy.

Materials and Methods

The aim of the study is to quantitatively estimate serum and salivary CRP and ALP in normal individuals and in individuals with different grades of squamous cell carcinoma.

Design and Settings:

A total of 50 individuals of which 10 healthy individuals and 40 individuals with clinically histopathologically confirmed OSCC with deleterious habits were considered for the study. Incisional biopsy was performed from the lesion proper,

haematoxylin and eosin staining was performed. In the consequent visit saliva and blood samples were collected from the confirmed patients and controls.

Characteristics of Participants:

A total of 50 subjects were considered for the study. 40 patients of clinically and histopathologically diagnosed Oral squamous cell carcinoma were included in study population and 10 healthy individuals without any deleterious habits were selected as control group. The AJCC/UICC,(American Joint Cancer Committee/ International Union Against Cancer) TNM staging system for OSCC is based on primary tumor size (T); quantification of nodal metastases according to size, number and distribution (N); and the presence of distant metastases (M). TNM Classification was used for clinical staging of OSCC. Biopsy was done to confirm the diagnosis. For histopathological grading each case was graded according to Broder's classification. The saliva and blood samples were collected and evaluated for CRP and ALP. Under all aseptic precautions about 5 ml fasting venous blood was collected from antecubital vein of study and control group into plain sterile bulb. The sample was allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 min and serum was separated. Immediately it was used for estimation of CRP and ALP. Whole unstimulated saliva was collected by method described by Dawes & Weatherell. Estimation of CRP and ALP was done using Immunoturbidimetric and Calorimetric method respectively. Correlation was done between the CRP and ALP Levels of cases with histopathological grade of the primary tumor and TNM stage of the tumor using Kruskal-Wallis test, ANOVA and chi –square test.

Results

The present study showed a maximum number of OSCC cases in 6th decade. In this study, 82.5% of the patients were in age group range of 40 - 69 years indicating a trend towards an increase in the incidence of the malignancy in older age group. The present study observed the occurrence of OSCC predominantly in males (60%), compared to females (40%). The increased occurrence of OSCC in men could be attributed to the high consumption of tobacco. Regarding the distribution of site in this study, the highest number of cases were observed in the tongue (22.5%), followed by buccal mucosa (20%), palate (17.5%), alveolar ridge(15%), oropharynx (15%), floor of the Mouth (10%) . Based on the clinical staging of OSCC (TNM Staging), the highest number of cases were observed in stage 4 (50%), followed by stage 3 (45%) and stage 2 constituted 5%. In this study population, well differentiated SCC, moderately differentiated SCC and poorly differentiated SCC accounted for 35%, 42.5% and 22.5% respectively.

In the present study, cases showed serum CRP values in the range of 0.1-12.6mg/dl and in saliva 0-0.6mg/dl. ALP values were in serum in a range of 68-373IU/L, in saliva 32-203 IU/L. The CRP and ALP showed a progressive change in relation to the TMN staging and grade of the tumor (Table-1, Table-2, Table-3).

Table 1: Comparison of histological grading with CRP & ALP (saliva & serum) in OSCC cases

Histological grading	Frequency	CRP range		ALP range	
		Serum	Saliva	Serum	Saliva
Well differentiated	14	0.4-2.9	0-0.3	43-181	10-181
Moderately differentiated	18	0.4-12.6	0-0.6	56-183	12-65
Poorly differentiated	8	0.4-2.6	0-0.4	61-373	12-203
Total	40				

Table 2: Comparison of TNM staging with CRP & ALP (saliva & serum) in OSCC cases

Histological grading	Frequency	CRP range		ALP range	
		Serum	Saliva	Serum	Saliva
Stage 1	**0**	0	0	0	0
Stage 2	**2**	0.4-0.8	0-0.3	68-94	32-86
Stage 3	**18**	0.4-2.6	0-0.4	43-218	181-203
Stage 4	**20**	0.4-12.6	0-0.6	56-373	10-181
Total	**40**				

Table 3: Comparison of serum and salivary CRP and ALP in OSCC cases and controls

Parameter	Group	Mean	SD	t-Value	P-value	Inference
Serum ALP	Case	104.83	55.09	1.600	0.11	NS
	Control	76.10	23.78			
Serum CRP	Case	1.26	2.05	1.560	0.12	NS
	Control	0.24	0.05			
Saliva ALP	Case	48.30	40.27	2.250	<0.05	S
	Control	19.20	10.03			
Saliva CRP	Case	0.20	0.11	4.870	<0.001	HS
	Control	0.02	0.04			

Discussion

Prognosis of OSCC is still difficult to predict, despite the diagnosis and therapeutic progress in the field of oral oncology. Currently, almost half of the patients affected die within the first two years of diagnosis. There are various factors which influence prognosis such as patient factors (age and gender) and tumor factors (size, site, histopathological grade and metastasis). Biological characters of cancer greatly affect the clinical outcomes of patients with the disease. If such biological characteristics of cancer could be predicted before treatment, it would be possible to select more effective and suitable treatment. In such patients, a combined assessment of histopathological grading, clinical staging along with a prognostic marker might serve as a more precise measure, to understand the tumor biology and for predicting the outcome of OSCCs.[8] The development of OSCC is strongly influenced by the immune system of the host. In the 1950s, Burnet and Thomas proposed the concept of immune surveillance of cancer. [9] According to Larsen SR et al, [10] Kademani D et al, [11] it is a widely held view that poorly differentiated or anaplastic carcinomas are more aggressive, i.e. they infiltrate more rapidly, more widely and metastasize earlier than well-differentiated neoplasms. It implicates that poorly differentiated SCCs have poor prognosis than well differentiated OSCCs with consequences on patient's decreased survival time. In contrast, Weijers et al [12] reported that there was no significant correlation between histopathological grade and prognosis. OSCCs are categorized in different stages to reflect prognosis and to determine the most adequate standard course of therapy. Tumor staging looks into the characteristics of the primary tumor along with lymph node and systemic metastases. The AJCC/UICC (American Joint Cancer Committee/ International Union Against Cancer), Tumor Node Metastasis (TNM) staging system for OSCC is based on primary tumor classification (T); quantification of nodal metastases according to size, number and distribution (N); and the presence of distant metastases (M). TNM staging includes stages I, II, III and IV. Neck lymph nodes

are usually the first site affected by regional metastases in patients with OSCC. Presence of lymph node metastasis is one of the most relevant prognostic factors for patients with OSCC. TNM staging has been widely used for treatment planning and estimate response to therapy. TNM clinical stages I and II were most observed in disease-free patients whereas in stages III and IV, individuals with ongoing or recurring disease or who had died were the most prevalent. [13] TNM clinical staging is one of the best prognostic indicators of OSCC, however, there are many patients who die despite the fact that their neoplasms were clinically Stage I or II. Hence the current clinical TNM staging is inadequate to accurately classify patients in terms of prognosis. The TNM classification system cannot predict the biological features of tumour cells and therefore, is unable to individualize the prognosis. In addition to the staging comparative evaluation of serum biomarkers will always be beneficial to patients.

The present study was conducted to evaluate, compare salivary & serum C-reactive protein levels & serum alkaline phosphatase levels of adults with OSCC patients using quantitative estimation.

In the present study CRP values showed a gradual increase in correlation with the tumor size in accordance with the study done by Khandavilli SD et al, [14] Chen IH et al, [15], Gockel I et al, [16] Wang CS et al. [17] In studies done by Chen IH et al, [15] Huang et al [18] there was positive correlation of patients with higher CRP, differentiation of tumour cells in accordance with the present study where there was increase of CRP values with the histological grade of the tumor. CRP levels in oral fluids are strongly and positively associated with levels in the circulation. Preoperative CRP was significantly associated with disease progression (tumor size, depth of wall invasion, lymph node metastasis, and distant metastasis) and pathological stages of patients. [17] Out et al [19] observed moderate associations between CRP measured in saliva and plasma In the present study salivary CRP values were highly significant. In the present study, Serum and salivary ALP showed positive correlation with tumour size, stage and differentiation in accordance with the studies done by Guerra et al, [20] Banseria N et al, [21] Saif et al, [22] George MJ et al. [23]

Conclusions:

Inspite of limited sample size and inadequate time period for assessment of survival rate, the present study showed positive results. However, further elaborated studies with larger cross-section of population and post-treatment survival period should be included, so that, in near future CRP and ALP values can be emphasized as one of the prognostic biomarkers of individual with OSCC.

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