ASSESSMENT OF SERUM TOTAL SIALIC ACID AND LIPID BOUND SIALIC ACID LEVELS IN PATIENTS WITH LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA-A CASE CONTROL STUDY

Dental Science

Dr Daneshwari Koshti*

MDS, Lecturer, Department of Oral medicine and radiology, K. L. E. V. K. Institute of Dental Sciences, KAHER, Belgaum *Corresponding Author

Dr Arvind Shetti

MDS, Professor, Department of Oral medicine and radiology, K. L. E. V. K. Institute of Dental Sciences, KAHER, Belgaum

Dr Anjana Bagewadi

MDS, Professor and Head, Department of Oral medicine and radiology, K. L. E. V. K. Institute of Dental Sciences, KAHER, Belgaum

Dr Anuradha Patil

Professor, Dept of Biochemistry, Jawaharlal Nehru Medical College, KAHER, Belgaum

ABSTRACT

Background and objectives: Oral cancer (OC) is the eleventh most common cancer worldwide. The reason for high prevalence of Oral cancer in India is primarily attributed to tobacco consumption in the form of gutka, quid, snuff or misri. The most common diagnostic method is biopsy followed by analysis of serum and saliva that are an effective approach for screening and monitoring the patients. Tumor markers are substances which change quantitatively in serum during tumor development. Various tumor markers are identified in oral cancer, one such tumor marker is sialic acid. The objective of the study was to evaluate clinical value of circulatory levels of total and lipid bound sialic acid for the early diagnosis and management of OC.

Method: A total of 90 patients were included in the study were further divided into three groups with 30 subjects each. Group 1 - leukoplakia. Group 2- oral squamous cell carcinoma(OSCC). Group 3- healthy age and sex matched individuals. All the three groups were subjected to determination of Serum total and lipid bound sialic acid. Statistical analysis was done using Student t- test, One way ANOVA was used to compare between the three groups. Multi variate analysis was performed to determine the alterations in TSA and LSA levels. Results Mean value of serum TSA in Group 1 - 53.18 ± 9.03 mg/dl, Group 2 - 91.58 ± 19.26 mg/dl Group 3 - 36.74 ± 5.94 mg/dl. Mean value of serum LSA in Group 1 - 19.50 ± 3.87 mg/dl, Group 2 - 33.64 ± 8.05 mg/dl, Group 3 - 14.10 ± 2.86 mg/dl. Mean value of serum TSA and LSA in OSCC were significantly higher than leukoplakia and control group ($P=0.0001$). When multivariate analysis was done it was observed that there is progressive rise in TSA and LSA

Conclusion: In the present study there were significant elevations in sialic acid levels in OSCC. This finding can be used as adjunct diagnostic marker in head and neck cancer

KEYWORDS

oral squamous cell carcinoma, oral leukoplakia, serum, total sialic acid, lipid bound sialic acid

INTRODUCTION

Cancer is the major threats to mankind worldwide. Oral cancer is eleventh most common cancer worldwide. Annually over 3, 00,000 cases of oral cancer are reported all over the world. It accounts for 3-10% of mortality rate. Oral cancers develop most commonly from potentially malignant lesions to name a few leukoplakia, erythroplakia and oral submucous fibrosis or it may be de novo.

The morbidity and mortality rate can be decreased by early detection and multimodal therapy. Hence a need for reliable and prognostic marker is required to identify high risk individuals in early stages.² Apart from biopsy there are other effective screening methods for early detection which include analysis of serum and saliva.³

Studies of malignant cells have revealed alteration in cell membranes and cell surfaces. N-acetylneuraminic acid (sialic acid) is a negatively charged nine carbon monosaccharide commonly attached by a glycosidic linkage to the non reducing residues of the carbohydrate chains of glycoproteins and glycolipids.³ Glycoprotein and glycolipids, the major constituents of the cells, and hence cell surface glycoconjugates are important in malignancy. They are released into circulation through increased turnover, secretion and shedding from malignant cell.⁴⁵

The present study was undertaken to assess the level of total and lipid bound sialic acid in premalignant and malignant lesions.

Methodology

Patients reporting to the Department of Oral Medicine and Radiology at K.L.E. Vishwanath Katti Institute of Dental Sciences, Belgaum were included in the study after obtaining an informed consent. Patients between age group of 25-60 years of either sex with clinically and histopathologically confirmed leukoplakia, oral squamous cell carcinoma were included. The study was approved by the Ethical and Research Committee of K.L.E. Vishwanath Katti Institute of Dental Sciences, Belgaum.

Method of selection of data

A total of 90 patients were included in the study and divided into three groups. Group 1 - 30 subjects with clinically and histopathologically confirmed leukoplakia Group 2- 30 subjects with clinically and histopathologically confirmed OSCC Group 3- Control group consisting of 30 healthy age and sex matched individuals without leukoplakia and OSCC.

Inclusion criteria-Patients with Oral leukoplakia, Oral squamous cell carcinoma and healthy individuals age and sex matched with no tobacco history.

Exclusion criteria- Patients with known systemic disease like diabetes, hypertension, cardiovascular disease, liver disease and Pregnant and lactating women.

Aseptic measures were undertaken during blood collection. The serum was separated from blood and stored at -20°C in deep freezer

The TSA and LSA was measured according to the method described by Katopodis and co-workers.⁶

Statistical Analysis

Statistical analysis was done using SPSS . Student t- test, One way ANOVA was used to compare between the three groups. Multivariate analysis was performed to determine the alterations in TSA and LSA levels. The p-value of less than 0.001 was considered to be statistically significant.

RESULTS

The present study included 90 subjects, who were grouped as group 1-leukoplakia (30), group 2- Oral squamous cell carcinoma(30), and group 3- normal control group. Serum samples were collected from all these subjects and were analyzed for total and lipid bound sialic acid. The age distribution was between 20-60 years in all the 3 groups. The mean age was 47.43 ± 11.35 in the group 1, 52.73 ± 7.52 in group 2, and

34.07±8.66 in group 3. Male predominance was seen in all the three groups. Buccal mucosa showed higher predilection in both leukoplakia and OSCC groups.

The serum total sialic acid were estimated in all the three groups

Group I showed the mean of 53.18±9.03 mg/dl

Group II showed the mean of 91.58±19.26 mg/dl

Group III showed the mean of 36.74±5.94 mg/dl

The Serum LSA levels were estimated in all the three Groups and the mean along with standard deviation was calculated.

Group I showed the mean of 19.50±3.87 mg/dl

Group II showed the mean of 33.64±8.05mg/dl

Group III showed the mean of 14.10±2.86 mg/dl

The study showed the ascending order of Serum Total and lipid bound Sialic acid levels in Controls, Leukoplakia and OSCC.

The pair wise comparison of groups was by Newmann-Keuls multiple post hoc procedures. The results obtained were statistically significant (P<0.05).

DISCUSSION

Oral cancer is a serious and increasing problem worldwide, with oral and pharyngeal cancer together being the sixth most common cancer in the world. This high incidence of oral cancer is due to the extensive habits of tobacco chewing and smoking^{7,8,9}. Leukoplakia is the most common premalignant lesion of the oral mucosa^{10,8}. Smoking and areca nut use contribute to 70% to 90% of oral leukoplakia.

Early detection of oral cancer is the most effective way to improve survival. Screening method deals with the diagnosis of lesions that have already been detected by the clinician but a definitive diagnosis can only be made by a tissue biopsy.¹¹ It is required to develop tumor markers to identify high risk individuals, improve cancer detection in early stages, and predict disease outcome and response to therapy.¹²

Tumor markers are a major part of the secondary prevention but associated with major logistic and economic constraints. To overcome this a simple laboratory test could be devised that can indicate the presence of cancer with a high degree of specificity and sensitivity before there is metastasis.¹³

The present study included 30 subjects with leukoplakia, 30 subjects with OSCC and 30 controls. All the patients had a habit of using tobacco either in the form of smokeless tobacco or smoking. Smokeless tobacco use was the most common. In the present study, patients of Oral leukoplakia the mean age was 47.43 ± 11.35 years and patients of OSCC the mean age was 52.73 ± 7.52 years which was consistent with other studies by Rajpura et al,¹⁴ Joshi M et al,¹⁶ Taqi S¹⁵ Kaushik Patolia et al,¹⁷

A male preponderance was evident in both groups which was similar to other study.^{14,16,18} It was also seen that the site distribution of Leukoplakia revealed that 66.6% were present in buccal mucosa followed by buccal sulcus (20%), which is consistent with the study done by Warnakulasuriya S, Johnson NW¹⁹ and R Sankaranarayanan.²⁰ Kaushik Patolia et al¹⁷

The site of occurrence of carcinoma was highest in buccal mucosa that is 66.67%. which is consistent with the study done by R Sankaranarayanan²⁰ which may be due to the habit of keeping the betel-quid and tobacco in contact with the cheek for a long time which is similar to our study.²¹

In the present study, mean serum total and lipid bound sialic acid was compared with clinical staging of OSCC. It was noticed that the levels of TSA and LSA increased from stage 1 to stage 4, indicating that levels are directly proportional to the tumor burden and the increased capability of these tumors to secrete more quantity of glycoproteins. The results correlated with the other studies done by Rajpura,¹⁴ Joshi M¹⁶ Taqi S,¹⁵ M C Shashikant²³ Baxi et al.²⁴ Majority of patients were in stage 3 and 4, this may be due to negligence and lack of awareness of the disease.

In the present study Serum TSA and LSA was measured and compared in all 3 groups leukoplakia, OSCC and the controls. It was seen that the levels of serum TSA and LSA was higher in OSCC group TSA-

(91.58±19.26), LSA-(33.64±8.05) as when compared to the leukoplakia TSA-(53.18±9.03), LSA-(19.50±3.87) and the control group TSA-(36.74±5.94), LSA-(14.10±2.86) which is consistent with the studies done by Achalli, et al²²

The levels of TSA and LSA in OSCC were statistically significant compared with the subjects with leukoplakia and the controls (P=0.0001). The levels of TSA and LSA were also significant in subjects with leukoplakia when compared with the controls (P=0.0001), (P=0.0003), which is consistent with the previous studies carried out by Rajpura,¹⁴ Joshi M,¹⁶ Taqi S,¹⁵ M C Shashikant,²³ Baxi et al.²⁴ Karthik Krishnan and Sivapathasundharam Balasundaram²⁵

This shows that malignant cells have altered surface carbohydrate leading to aberrant cell to cell recognition resulting in breakdown of intercellular control mechanism.^{15,24} In the present study it was noticed that there was progressive increase in the serum TSA and LSA levels in OSCC with stage of malignant disease. Thus it showed that there is positive correlation between serum levels of TSA and LSA forms of sialic acid with the extent of the disease.¹⁴

Serum sialic acid can be used for initial diagnosis and monitoring precancerous and cancerous lesions.^{4,14} As the sialic acid is increased in various diseases and also due to medications it cannot be considered as a specific tumor markers. Further studies with increased sample size are required to include Sialic acid that would be helpful as a specific tumor marker in the initial diagnosis of Leukoplakia and Oral Squamous cell carcinoma.

Table: Comparison of three groups with respect to TSA mg/dl by one way ANOVA test

Groups	Mean	SD	SE
Leukoplakia group(Group1)	53.18	9.03	1.65
OSCC group(Group2)	91.58	19.26	3.52
Control group(Group3)	36.74	5.94	1.08
F-value	146.239		
P-value	0.00001*		
Pair wise comparison of groups by Newman - Keuls multiple post hoc procedures			
Leukoplakia vs OSCC	P=0.0001*		
Leukoplakia vs Control	P=0.0001*		
OSCC vs Control	P=0.0001*		

*p<0.05

Table: Comparison of three groups with respect to LSA mg/dl by one way ANOVA test

Groups	Mean	SD	SE
Leukoplakia group(Group1)	19.50	3.87	0.71
OSCC group(Group2)	33.64	8.05	1.47
Control group(Group3)	14.10	2.86	0.52
F-value	104.1315		
P-value	0.00001*		
Pair wise comparison of groups by Newmann-Keuls multiple post hoc procedures			
Leukoplakia vs OSCC	P=0.0001*		
Leukoplakia vs Control	P=0.0003*		
OSCC vs Control	P=0.0001*		

*p<0.05

The pair wise comparison of groups was by Newmann-Keuls multiple post hoc procedures. The results obtained were statistically significant (P<0.05)

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