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Serum β2-Microglobulin and Total Sialic Acid levels in Oral Squamous Cell Carcinoma patients: A South Indian Study

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the most common malignant tumor worldwide. The main risk factors are tobacco in the form of chewing, smoking and alcohol consumption. The incidence is rated as one among the highest in the world and the use of tobacco in various forms is increasingly associated with the cause of OSCC. In oral cancer the study of tumor markers have been limited and several tumor makers with clinical promise needs further evaluation.

Methods: Present study was conducted in 30 histopathologically confirmed OSCC patients and 30 age matched controls form Coimbatore city, South India. Levels of β2-Microglobulin and Total Sialic Acid (TSA) in serum of the subjects were analyzed by ELISA and spectrophotometric method.

Results: The levels of β2-microglobulin (3.81±0.09) and TSA (72.81±2.31) were found to be elevated in OSCC patients when compared with the controls (2.07±0.15; 64.17±1.86). Mean values were compared between the smokers and non smokers, which showed a significant increase in the level of β2-microglobulin and TSA ($p < 0.05$).

Conclusion: In this study, the levels of β2-microglobulin and TSA were higher in OSCC patients with smoking habit than in non smokers. The study hypothesizes that this imbalance may be one of the major factors responsible for the progression of oral cancer.

Keywords: oral cancer, β2-microglobulin, total sialic acid, smokers.

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Introduction

Oral cancer is the most common form of cancer and of cancer related death in India. Its high risk in the Indian subcontinent is related to the popularity of tobacco products.[1] Globally 'oral cancer' is the sixth most common cause of cancer-related death.[2] Oral cancer accounts for approximately 30-40% of all cancers in India.[3] Higher prevalence of oral cancer has been associated with chewing tobacco, in addition to smoking and alcohol consumption.[4]

Despite the recent advances in tumor surgery and multimodal treatment regimes, the prognosis of oral squamous cell carcinoma is still relatively poor. This may be because of symptoms that indicate the presence of the carcinoma often appearing when the tumor is in an advanced stage.[5]

In the light of such problems, it would be very useful to find biochemical markers that allow suspecting the presence of the carcinoma at early stages. During the course of tumor development, quantitative changes have been shown to occur in a variety of substances in serum. These substances are collectively referred to as biochemical markers or tumor markers.[5, 6] One such tumor marker is serum β2 –microglobulin, which is a non-glycosylated peptide and is the invariant chain of the Major Histocompatibility Class (MHC) I molecules on the cell surface of all nucleated cells.[5, 7] It is called serum β2-microglobulin because it was initially discovered as a serum protein before its role as part of the Class I antigen was elucidated.

Analysis of serum or plasma glycoproteins have been used as valuable index in establishing diagnosis, staging of disease, detecting metastases, identifying patients at high risk for recurrence and evaluating therapeutic response.[8] Sialic acids, a group of sugars including neuraminic acid and its derivatives, are involved in the regulation of many biological and physiological processes including the regulation of cell surface phenomenon.[9] They are released into circulation through increased turnover, secretion, and/or shedding from malignant cells.[10, 8] Increased levels of β2-microglobulin and sialic acid have been reported in serum of patients of oral cancer.[11, 12]

Due to the higher incidence of oral cancer, the present study was undertaken to validate the level of serum β2-microglobulin and total sialic acid in OSCC cases and to correlate with the habit of tobacco smoking.

Materials and Methods

A total of 30 subjects (22 males and 8 females) in the age range of 55-75 were selected for the study. All the 30 subjects included are histopathologically confirmed for Oral Squamous cell Carcinoma. According to TNM staging system of the UICC, 13 out of 30 oral cancer patients studied, 11, 8 and 11 were of stages I, II and III respectively.

As controls, 30 (18 males and 12 females) age matched individuals with no systemic diseases were included in the study. Written informed consent was obtained from each case or control subjects before the data collection procedures were conducted. The work was carried out in accordance with the ethical standards laid down in 1964 Declaration of Helsinki.

Under aseptic precautions venous blood was drawn and serum was separated. The samples were frozen at -70° C until assay.

β2- Microglobulin assay

The serum was analyzed by Enzyme linked immunosorbent assay (β2–microglobulin EIA kit, Sigma Aldrich, India). 2.4mg/L was used as the upper limit, when 97% of normal values are below this cut off value.

Measurement of total sialic acid (TSA)

Serum total sialic acid level was determined by the method of Plucinsky et al.14. 20µl of serum was diluted with 980 µl distilled water. After treatment with resorcinol reagent the blue chromophore was extracted by butyl acetate/n-butanol (85:15) (v/v) and determined spectrophotometrically (Shimadzu) at 580nm and sialic acid was determined by the use of standard curve of N-acetyl neuraminic acid.

Statistical analysis

The data were analyzed by using statistical package for social sciences (SPSS) software. Cases and controls were tested for statistical significance with student's't' test. Values of $p<0.05$ were considered significant.

Results

General characteristics of study group were shown in **Table 1**. Study subjects were composed of 22 male and 08 female individuals. In general, the mean age was 65.5 years. Subjects are classified into tobacco smokers (16 -53%) and non-smokers (14 -46.6%). Moreover, OSCC patients are categorized based on the tumor stages, Stage I (11-36.6%), II (08-26.6%) and III (11-36.6%).

Levels of β2 microglobulin and total sialic acid were analyzed in OSCC patients and controls. The mean values of β2 microglobulin and TSA was found to be significantly elevated in OSCC patients when compared to controls (**Table 2**).

The mean, standard deviation and p value of β 2 Microglobulin and TSA levels in the oral cancer patients and controls were calculated and compared. There was a significantly elevated level of mean serum β 2 microglobulin and TSA was found in all groups of oral cancer patients as compared to the controls ($p<0.05$). Based on the smoking habits, the values were compared among smokers and non smokers in the OSCC and control group (**Table 3**). The levels of β 2 microglobulin and TSA was higher in tobacco smokers than non smokers ($p<0.05$).

Discussion

The β2- microglobulin is synthesized and secreted by lymphocytes as well as most other nucleated cells, is an intrinsic part of histocompatibility antigen. It has a low molecular weight and rapid turnover.[15] Elevated levels of β2-microglobulin have been observed in a variety of patients mostly with advanced malignancy and other disease states.[16, 17, 18] In the present study the level of β2 -microglobulin was found to be higher in OSCC patients than controls. We also found that serum β2-microglobulin levels were significantly higher in tobacco smokers than non smokers. The elevated level of β2-microglobulin in the serum of oral carcinoma patients is in concurrence with earlier reports12, 19. The mechanism of raise in β2-microglobulin levels in cancer is not known however, a variety of potential hypotheses for the augmented serum levels have been put further.

Smoking has currently been established to be a cancer risk factor.[20, 21] Tobacco use has been estimated to account for 30% of the worldwide cancer burden.[22] In addition, smoking was reported to be a risk factor for oral cavity and esophageal cancers.[23] The evidence so far accumulated demonstrates that tobacco habits increase en-

Table 1. General characteristics of the study subjects

Characteristics		Controls (N=30)	Cases (N=30)
Age	Mean (yrs)	64.86±6.59	65.40±5.67
	Range (yrs)	55-75	55-75
Sex	Male	18 (60%)	22 (73.3%)
	Female	12 (40%)	08 (26.6%)
Smoking habit	Smokers	13 (43.3%)	16 (53.3%)
	Non Smokers	17 (56.6%)	14 (46.6%)
Tumor Grading	Stage I	-	11 (36.6%)
	Stage II	-	08 (26.6%)
	Stage III	-	11 (36.6%)

Table 2. Level of β2 microglobulin and total sialic acid in controls and OSCC patients

Category	β 2 Microglobulin (mg/L)	TSA (mg/dl)
Controls	2.30±1.42	64.51±2.78
OSCC Patients	3.9±1.17*	74.63±7.72*

*Significance in comparison to controls ($p<0.05$).

Table 3: Comparison of β2-microglobulin, total sialic acid (TSA) levels based on Tobacco Smoking and Chewing

Parameters	Controls (N=30)		OSCC Patients (30)	
	Smokers (N=13)	Non-smokers (N=17)	Smokers (N=16)	Non-smokers (N=14)
β2 Microglobulin (mg/L)	2.6 ±1.65	2.05±1.19	3.8±1.10*	3.2±0.99
TSA (mg/dl)	66.0 ± 2.27	65.2 ± 2.47	77.8 ± 5.75*	75.2±7.99

*Significance in comparison to controls ($p<0.05$).

dogenous N-nitroso compounds formation, thus adding to the burden of exposure by preformed carcinogens in tobacco products.[24] On the other hand, serum TSA has also been used as a tumor marker for different types of cancers which includes colorectal, prostate, and breast cancers.[25, 14]

Cell surfaces and membrane components play a major role in neoplastic activities. Neoplasms often have an increased concentration of TSA on the tumor cell surface, and sialoglycoproteins are shed or secreted by some of these cells, which increases the concentration in blood or saliva.[25] Furthermore, cancer cells have been related with an increased activity of sialytransferase, leading to a higher amount of TSA on the cell surface, thus increasing the plasma or salivary concentration.[26, 27]

TSA concentrations have been reported to be related not only to diagnosis, but also to staging, prognosis, and detection of early recurrence.[25] It has been suggested that evaluations of the serum glycoconjugate levels may be useful in early detection and staging of oral precancerous conditions and oral cancer[28] which are often associated with smoking. In the present study we reported that serum TSA levels were significantly higher in tobacco smokers than non smokers.

Raised serum TSA concentration has been proposed to be a strong predictor of various diseases.[29, 30] Tobacco smoke contains approximately 4000 constituents. Nicotine is one of the most pharmacologically active tobacco components with a wide range of toxic effects.[31] Thus, our results suggest that the concentrations of β2-microglobulin and total sialic acid were significantly increased in smokers than non smokers among the oral cancer patients and correlates well with the disease progression. Consequently, these could be used as important parameters in the patients at risk for cancer and also draws attention as potential public health hazard.

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