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Original Article

Pathology Section

Evaluation of Serum β2-Microglobulin Levels in Histologically Diagnosed Oral Squamous Cell Carcinoma Patients

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ABSTRACT

Introduction: Oral cancer presents challenging and unresolved problems for the human population, accounting for as much as 30-40% of all carcinomas in India. The current research focuses on the use of the tumour marker β2-microglobulin as a surrogate marker in patients with Oral Squamous Cell Carcinoma (OSCC) for early detection of cancer.

Aim: To evaluate the level of serum β2-microglobulin in histologically diagnosed OSCC patients and compare it with age- and sex-matched healthy controls.

Materials and Methods: This was a cross-sectional study conducted in the Department of Pathology at SN Medical College, Agra, over a period of one year and six months. The study included 50 histologically diagnosed OSCC cases and 40 age- and sex-matched healthy controls. Blood samples were taken from the healthy controls and OSCC patients, and the level of serum β2-microglobulin was measured using Enzyme Linked Immunosorbent Assay (ELISA). Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 11. Z test and ANOVA test were used to compare various parameters. A p-value of <0.05 was considered significant.

Results: In the 50 cases of OSCC, the mean±SD of serum β2-microglobulin was 2.99±0.85 µg/mL, while in the healthy controls, it was 1.30±0.10 µg/mL, with a p-value <0.001, which was statistically significant. The mean±SD of serum β2-microglobulin in cases of Well Differentiated Squamous Cell Carcinoma (WDSCC) was 2.40±1.59 µg/mL, whereas it was 3.09±1.52 µg/mL in Moderately Differentiated Squamous Cell Carcinoma (MDSCC) and 3.46±0.03 µg/mL in Poorly Differentiated Squamous Cell Carcinoma (PDSCC), with a p-value of <0.05, which was statistically significant. Increased levels of serum β2-microglobulin were observed among all cases of OSCC. Loss of differentiation in Squamous Cell Carcinoma (SCC) was associated with an increase in levels of serum β2-microglobulin.

Conclusion: Due to its minimally invasive nature and quick availability of results, serum β2-microglobulin can be used for diagnosis of OSCC. Therefore, it is recommended to monitor levels of serum β2-microglobulin in patients with OSCC.

Keywords: ELISA, Oral cancer, Surrogate marker for oral cancer

INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignant tumours found in humans. It is reported to be the sixth most common malignant lesion [1]. OSCC is more common in men between the sixth and eighth decades of life, accounting for 92% of all malignancies in the head and neck region [2,3]. Early diagnosis and monitoring of disease progression may potentially decrease the mortality and morbidity associated with oral cancer. β2-microglobulin was described and isolated from the urine of patients with tubular proteinuria by Berggard I and Bearn AG. β2-microglobulin is a low molecular mass protein present in the membranes of possibly all nucleated cells, where it appears to be in structural association with histocompatibility antigen [3]. Increased expression is significantly correlated with tumour stage, lymph node metastasis, and survival [1]. Increased β2-microglobulin levels have been reported in patients with oral cancers, but there have only been limited studies related to serum β2-microglobulin in oral cancer [1-3].

In this context, the level of the tumour marker β2-microglobulin was studied in cases of OSCC and healthy individuals, and the levels were compared with each other. The present study aimed to evaluate the level of serum β2-microglobulin in diagnosed cases of OSCC, as well as, in age- and sex-matched healthy individuals.

MATERIALS AND METHODS

It was a cross-sectional study carried out in the Department of Pathology at SN Medical College, Agra, over a period of one year and six months (January 2021-June 2022). The study included a total of 90 subjects, out of which 50 were histologically diagnosed with OSCC and 40 were age- and sex-matched healthy controls. Ethical clearance was obtained from the Institutional Ethical Committee (IEC/2021/39).

Inclusion criteria: All patients with a histopathologically confirmed diagnosis of SCC and for whom a serum sample was available for serum β2-microglobulin estimation were included in the study.

Exclusion criteria:

- Patients with a histopathological diagnosis of benign and premalignant lesions of the oral cavity.
- Patients with a histopathological diagnosis of SCC who have received any form of treatment.
- Patients with malignancy at any other site along with OSCC.

The commercially available Calbiotech, Inc (CBI) β2-microglobulin ELISA Kit was used for the quantitative determination of serum β2-microglobulin levels. The normal range for blood serum β2-microglobulin levels was reported to be 1.5-3 mg/L [4,5], based on the principle of a solid-phase ELISA.

Selection of Subjects

Group-1 (control group): Comprised of 40 age- and sex-matched healthy individuals, preferably from the same family.

Group-2 (case group): Comprised of 50 patients who were histopathologically diagnosed with OSCC of different grades.

Assay procedure for serum [Table/Fig-1a-c]: Samples of patient's serum and controls serum were diluted before use. A series of small tubes (such as 1.5mL microcentrifuge tubes) and mixed 10 µL of serum with 1.0 mL of sample diluent (101-fold dilution) were prepared. Then, 20 µL of standards, diluted specimens, and diluted controls were dispensed into appropriate wells. Next, 200 µL of sample diluent was added to each well and thoroughly mixed for 30 seconds. The plate was incubated at 37°C for 30 minutes. After incubation, the mixture was removed by flicking the plate contents into a waste container, and all liquid was removed from the wells. The wells were washed three times with 300 µL of 1X wash buffer and blotted on absorbance paper. Subsequently, 200 µL of the enzyme conjugate reagent was dispensed into each well and mixed for 10 seconds. The plate was incubated again at 37°C for 30 minutes. All the contents were removed, and the plate was washed as described in steps 6 and 7. Then, 100 µL of 3,3', 5,5'-tetramethylbenzidine Reagent was dispensed into each well, mixed for 10 seconds, and incubated at room temperature in the dark for 20 minutes. The reaction was stopped by adding 100 µL of stop solution to each well, mixed for 10 seconds to ensure that all the blue colour changed to yellow completely. Absorbance was measured at 450 nm using a microtiter well reader within 15 minutes.

[Table/Fig-1]: a) Showing diluted specimens and diluted controls were dispensed into appropriate wells; b) Showing enzyme conjugate reagent was dispensed into each well; c) Absorbance was noted at 450 nm with a microtiter well reader.

Calculation of serum β2-microglobulin [4]: The mean absorbance value (A450) for each set of reference standards, controls, and patient samples was calculated. A standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentration in µg/mL on graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Mean absorbance values were calculated for each specimen to determine the corresponding concentration of β2-microglobulin in µg/mL from the standard curve.

STATISTICAL ANALYSIS

The level of serum β2-microglobulin between cases and controls and various grades of differentiation of OSCC was statistically analysed using the SPSS software version 11. The Z test and ANOVA test were used to compare various parameters. A p-value of <0.05 was considered significant.

RESULTS

In the present study, serum from 90 individuals was analysed, of which 50 were histopathologically diagnosed cases of OSCC and 40 were age- and sex-matched healthy controls. The majority of patients in our study were in the age group of 51-60 years (17, 34%), followed by 41-50 years (14, 28%). The youngest patient in the study was a 20-year-old male, and the eldest was a 79-year-old female. The mean age±SD for OSCC was 53.24±2.12 years. The mean age±SD for Well-Differentiated Squamous Cell Carcinoma (WDSCC), Moderately Differentiated Squamous Cell Carcinoma (MDSCC), and Poorly Differentiated Squamous Cell Carcinoma (PDSCC) was 50.07±2.82 years, 51.69±7.77 years, and 61.70±1.41 years, respectively. Hence, it was observed that the majority of patients with PDSCC were in the 6th to 7th decade of life [Table/Fig-2].

Age groups (years)	WDSCC	MDSCC	PDSCC
0-10 years	00 (0%)	00 (0%)	00 (0%)
11-20 years	01 (6.25%)	00 (0%)	00 (0%)
21-30 years	00 (0%)	00 (0%)	00 (0%)
31-40 years	01 (6.25%)	05 (20.83%)	01 (10%)
41-50 years	06 (37.5%)	06 (25%)	02 (20%)
51-60 years	04 (25%)	09 (37.5%)	04 (40%)
61-70 years	03 (18.75%)	02 (8.33%)	02 (20%)
71-80 years	01 (6.25%)	02 (8.33%)	01 (10%)
Total	(100%)	(100%)	(100%)

[Table/Fig-2]: Showing correlation of age of patients with various grades of Oral Squamous Cell Carcinoma (OSCC).

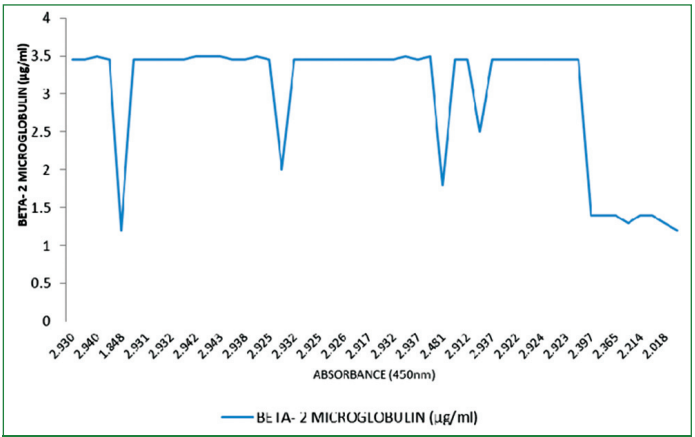
Out of the 50 cases, 24 cases (48%) were MDSCC, followed by 16 cases (32%) of WDSCC and 10 cases (20%) of PDSCC. MDSCC was the most common grade of OSCC in the present study.

Out of the 50 cases, 44 cases (88%) were males and six cases (12%) were females. The male-to-female ratio in our study was 7.3:1. In males, the most common lesion was MDSCC with 21 cases (52.4%), followed by 14 cases (27.2%) of WDSCC and 9 cases (20.4%) of PDSCC. In females, the most common type was also MDSCC, with three cases (50%), followed by two cases (33.3%) of WDSCC and one case (16.7%) of PDSCC, respectively.

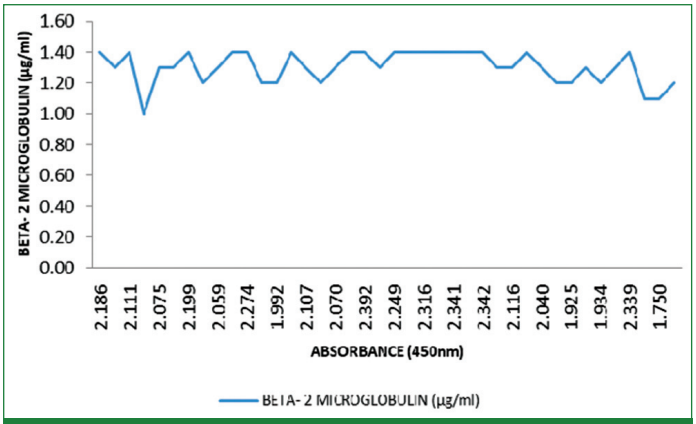
Buccal mucosa was the most common site involved, with 24 cases (48%), followed by the tongue with 18 cases (36%). Five patients (10%) had lesions in the tonsillar area. Three patients (6%) had lesions at different sites in the oral cavity, such as the soft palate, alveolus, and floor of the mouth, respectively. MDSCC was the most common carcinoma encountered in the buccal mucosa and tongue, with 13 cases (54.2%) and eight cases (44.4%), respectively. The tonsillar area had two cases each of MDSCC and PDSCC and one case of WDSCC.

Tobacco chewing was observed in the majority of patients, 21 cases (42%), followed by tobacco smoking in 18 cases (36%). Eight cases (16%) had a habit of both chewing and smoking tobacco, while 6 cases (6%) had no addiction history.

The mean±SD of serum β2-microglobulin levels among cases of OSCC was 2.99±0.85 µg/mL, while in healthy controls, it was 1.30±0.10 µg/mL, with a p-value of ≤0.001, which was statistically significant using the Z test [Table/Fig-3,4]. The mean±SD of serum β2-microglobulin levels in cases of WDSCC was 2.40±1.59 µg/mL,



[Table/Fig-3a]: Serum Beta-2 microglobulin concentration (µg/mL) of cases of Oral Squamous Cell Carcinoma (OSCC) patients against its absorbance at 450 nm.



[Table/Fig-3b]: Serum β2 microglobulin concentration (µg/mL) in healthy controls against its absorbance at 450 nm.

Subjects	Number of cases	β2-microglobulin (Mean±SD) (µg/mL)
Cases	50	2.99±0.85
Controls	40	1.30±0.10

[Table/Fig-4]: Comparison of serum β2-microglobulin in cases and controls.

while it was 3.09±1.52 µg/mL in MDSCC and 3.46±0.03 µg/mL in PDSCC [Table/Fig-5]. Increased serum β2-microglobulin levels were associated with loss of differentiation, with a p-value <0.05, which was statistically significant using the Z test and ANOVA test.

	WDSCC	MDSCC	PDSCC
β2 microglobulin (Mean±SD) (µg/mL)	2.40±1.59	3.09±1.52	3.46±0.03

[Table/Fig-5]: Serum β2-microglobulin level in different grade of Oral Squamous Cell Carcinoma (OSCC).
p-value <0.05, statistically significant

DISCUSSION

Diagnostic and prognostic biomarkers are quantifiable traits that help clinical oncologists in their initial interaction with suspected patients. These biomarkers play a crucial role in: (i) identifying individuals at risk; (ii) diagnosing at an early stage; (iii) selecting the most suitable treatment approach; and (iv) monitoring treatment response [5].

β2-microglobulin was an 11.7-kDa polypeptide expressed on the surface of almost all cells in the body. It forms complexes with Major Histocompatibility Complex (MHC) class I molecules, which are believed to play a role in antigen presentation to cytotoxic (CD8+) T lymphocytes. Under normal physiological conditions, β2-microglobulin is present as a soluble protein at low levels in the serum, urine, and other bodily fluids. However, its level is elevated in patients with kidney failure and certain malignancies, including solid and liquid tumours. The mechanism responsible for the increase in β2-microglobulin expression during cancer progression remains unclear. One interpretation is that the level increases as a result of increased cell turnover in the tumour and an enhanced immune response to the malignant process. Another possibility is that tumours contain three β2-microglobulin alleles instead of one. Consequently, the elevated expression of β2-microglobulin may be associated with increased resistance to apoptosis [1].

β2-microglobulin modulates cellular proliferation, as well as tumour cell migration and invasion. Inhibition of β2-microglobulin expression by siRNA was sufficient to reduce cellular migration and invasion in vitro. Consequently, these findings have the following clinical implications:

(a) β2-microglobulin likely plays a more significant role than just being a housekeeping gene or involved in the stabilisation and presentation of MHC Class-I molecules in cells.

(b) β2-microglobulin may act as an effective growth-promoting factor, facilitating tumour progression, invasion, and migration in OSCC.

(c) The increased synthesis and/or release of β2-microglobulin, resulting in elevated serum or urine β2-microglobulin concentrations, may become an important prognostic factor and predictor of survival in OSCC [1].

Increased serum β2-microglobulin levels can be attributed to increased cellular activity or its presence as a constituent of HLA molecules. Additionally, increased cell membrane turnover or cell division could also contribute to its shedding [5-7].

Serum was collected from 90 individuals, including 50 cases of histopathologically diagnosed OSCC and 40 healthy controls. The highest number of OSCC cases was in the age group of 51-60 years (34%), followed by 41-50 years (28%). This finding is consistent with the studies conducted by Sequeira J et al., and Saddiwal R et al., which reported 32% and 66.6% of cases in the age group of 51-60 years, respectively [6,8]. In the present study, the mean age±SD for OSCC was 53.24±2.12 years, and there was a progressive increase in mean age with loss of differentiation (WDSCC=50.07±2.82 years, MDSCC=51.69±7.77 years, and PDSCC=61.70±1.41 years). None of the authors observed the mean age in relation to the degree of differentiation.

The majority of patients in our study were males (44 out of 50 cases, 88%). Similarly, studies conducted by Sequeira J et al., Saddiwal R et al., and Agrawal B et al., also found a higher proportion of males affected by OSCC, with percentages of 72%, 73.3%, and 78%, respectively [6,8,9]. Buccal mucosa was the most commonly affected site (48%), followed by the tongue (36%) in the study. This finding was also observed in the study conducted by Sequeira J et al., where buccal mucosa accounted for 44% of cases, while Saddiwal R et al., and Narayanan MS et al., found that the mandibular alveolus and sulcus and the tongue were the most frequently involved sites, with percentages of 53% and 47.6%, respectively [6,8,10].

Tobacco chewing was the most common habit among OSCC cases (42%) in our study, followed by tobacco smoking (16%). In contrast, Narayanan MS et al., observed tobacco smoking as the most common addictive habit in OSCC, accounting for 71.4% of cases [10].

In the present study, the mean±SD value of β2-microglobulin levels in OSCC individuals was 2.99±0.85 µg/mL, while it was 1.30±0.10 µg/mL in the healthy control group (p-value=<0.001). This finding is consistent with several other studies as mentioned in [Table/Fig-6]. The mean±SD of serum β2-microglobulin levels in cases of WDSCC was 2.40±1.59 µg/mL, while it was 3.09±1.52 µg/mL in MDSCC and 3.46±0.03 µg/mL in PDSCC [Table/Fig-5]. Increased serum β2-microglobulin levels were correlated with loss of differentiation, with a p-value <0.05, which was statistically significant. This is in accordance with the results observed by Agarwal B et al., with a p-value of <0.001, Singh AP with a p-value of <0.001, Silvia CR et al., with a p-value of <0.05, and Vaishali N and Tupkari JV with a p-value of <0.005, respectively [9,11-13].

Name of the study	Serum β2 microglobulin level in cases of OSCC (µg/mL)	Serum β2 microglobulin levels in healthy controls (µg/mL)	p-value
Silvia CR et al., [12] Manipal, 2002	2.69±0.11	1.58±0.32	<0.05
Vaishali N and Tupkari JV [13] Gujrat, 2005	2.2	1.17	<0.005
Singh AP et al., [11] Moradabad, India, 2014	2.835±0.0313	1.173±0.054	<0.001
Agrawal B et al., [9] Jodhpur, 2016	3.23±0.92	1.88±0.82	<0.001
Sequeira J et al., [6] Manglore, 2021	3.69±2.06	1.676±0.215	-
Present study Agra	2.99±0.85	1.30±0.10	<0.001

[Table/Fig-6]: Comparison of serum beta 2-microglobulin level with other studies.

Limitation(s)

One of the limitations of the present study was the small sample size. Further studies with a larger sample size can be conducted in future.

CONCLUSION(S)

Increased levels of serum β2-microglobulin were observed in all cases of OSCC. Loss of differentiation in SCC is associated with an increase in serum β2-microglobulin levels. Hence, we conclude that measuring serum β2-microglobulin aids in the early detection of OSCC.

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