Serum beta-2 microglobulin analysis in patients with oral squamous cell carcinoma

ABSTRACT

Aim: This study aims to compare the level of serum beta-2 microglobulin (β2-M) in normal healthy individuals and patient with squamous cell carcinoma (SCC).

Methodology: This study has been conducted in patients attending the Department of Oral and Maxillofacial Surgery, Yenepoya Dental College, Deralakatte, Mangalore. Sample comprises of 25 cases of clinically and histologically diagnosed oral cancer and 25 normal healthy individuals as control group. The serum was analyzed for β2-M by enzyme-linked immunosorbent assay.

Results: It was observed that there was a significant increase in serum β2-M levels in oral SCC patients as compared to controls. Circulating levels in serum β2-M were also elevated significantly among different clinical stages with progressive rise from stage I to stage IV of the disease.

Conclusion: The evaluation of these markers would be useful in assessing malignant change, increasing accuracy of clinical diagnosis and also in assessing the spread and invasiveness of the cancer of the oral cavity.

Keywords: Enzyme-linked immunosorbent assay, nodal status, oral squamous carcinoma, serum β2 microglobulin, tumor size

INTRODUCTION

Cancer of the head and neck region is the sixth most common malignancy worldwide. Head and neck squamous cell carcinoma (SCC) and oral SCC (OSCC) show variations in clinical progression and prognosis based on the subsite affected. OSCC is one of the most common malignancy found in the head and neck region, accounting for more than 90% of all oral cancers.[1-3] It is generally accepted that oral carcinogenesis is a multi-step process of accumulated genetic damage leading to cell dysregulation with disruption in cell signaling, DNA-repair, and cell cycle which are fundamental to homeostasis. The main etiological factors in Western countries include alcohol consumption, tobacco use, and poor oral hygiene. In the Asiatic world, chewing of betel quid with tobacco products represents another important risk factor.

Despite the attainments already achieved concerning OSCC diagnosis and therapy, mortality and morbidity rates are still exceedingly high, challenging the available methods of prognosis assessment and encouraging the search for new and better markers, namely, molecular markers that relate comprehensively with known alterations of tumor progression. A vast number of molecular markers have been correlated with OSCC outcome, events leading to carcinogenesis and cancer progression.[4,5]

In the presence of abnormal stimuli, the body as well as the neoplastic cells synthesize several abnormal cellular products.

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The presence of these substances can be easily found in different body fluids and on the surface of neoplastic cells. These products that are detected and measured are known as “tumor markers.” These tumor markers can be effectively made use of for early screening and detection of cancer. Diagnosis can be aided by the use of these and clinical staging can be better applied in the light of the revelations by these markers. The prognostic evaluation can be noted. These tumor markers can facilitate early detection, prognosis, or recurrences if any.\[^{[6-8]}\]

Serum beta-2 microglobulin (β2-M) – 176 (132-m), first described by Berggaard and Bearn in 1968, is a low molecular weight, 11600 Dalton protein which is the light or β-chain of the human leucocyte antigen (HLA), found on all cells except the erythrocytes. It is a tumor marker, which was first isolated from the urine of patients with tubular proteinuria and was later shown to be present in normal human urine, plasma, and cerebrospinal fluid. It mainly exists in two forms – free and noncovalently. Serum β2-M is in the free form. It consists of a single polypeptide chain with one intrachain disulfide bridge. The HLA– chain, along with the serum β2-M forms a constituent of the cell membrane, thus, it is understood that an increase in the membrane turnover or an accelerated cell division which is a hallmark of cancer, could increase its shedding over time.\[^{[9]}\]

In the present study, we are attempting to correlate the serum levels of β2-M in OSCC and to evaluate its role as a biochemical parameter in OSCC for screening purposes.

**Aim and objectives**

1. To estimate serum β2-M in clinically and histologically diagnosed OSCC cases
2. To estimate serum β2-M in healthy controls
3. To compare level of serum β2-M of healthy controls with those of SCC cases
4. To compare serum β2-M levels among different staging of OSCC
5. To compare serum β2-M levels among the different tumor size of OSCC
6. To compare serum β2-M levels among the different nodal status of OSCC
7. To compare serum β2-M levels among the different growth of OSCC
8. To compare staging among different tumor size and nodal status.

**METHODOLOGY**

This study was carried out in the Department of Oral and Maxillofacial surgery, after obtaining clearance from the Ethical Committee of the college. With a power of 80%, the sample comprised of 25 clinically and histologically confirmed OSCC patients and 25 controls. Patients diagnosed with clinically and histologically confirmed cases of OSCC above 18 years of age were included while patients with history of treatment of malignancy were excluded. All the patients included in the study signed a consent form.

To estimate serum β2-M, blood specimens were collected in a 5 ml syringe from ante cubital vein. Blood was then allowed to clot and the serum was separated by centrifugation. Following which, specimens were refrigerated at 2°C–8°C for up to 5 days or stored at −20°C until use. The serum was analyzed for levels of β2-M by enzyme-linked immunosorbent assay.

To prepare for the test, all patient samples were diluted to 1:100 with sample buffer. 10 µl of sample was combined with 990 µl of sample buffer in a polystyrene tube and mixed well. The procedure required sufficient number of microplate modules to accommodate controls and patient samples. Once that was established, 100 µl of controls and patient samples (duplicate) were pipetted into the wells. It was then decided to incubate for 30 min at room temperature (20°C–28°C) and discard the contents of the microwells and wash 3 times with 300 µl of wash solution. 100 µl of enzyme conjugate was dispensed into each well and again incubated for 15 min at room temperature. The contents of the microwells were discarded and washed 3 times with solution. 100 µl of TMB substrate solution was incubated for 30 min at room temperature, after dispensing into each well and 100 µl stop solution was added to each well of the modules and incubated for 5 min at room temperature. The optical density was read at 450 nm to calculate the results. Bi-chromatic measurement with a reference at 600–690 nm was recommended. The developed color was stable for at least 30 min. Optical densities were to be read during this time.

**RESULTS**

In the present comparative study, the serum β2-M levels were estimated in two groups consisting of 25 cases each with OSCC designated as Group A and 25 controls designated as Group B.

In Group A of 25 cases with OSCC, 1 case was in age group of <30 years (4%), 4 cases were in age group of 31–40 years (16%), 6 cases were in age group of 41–50 years (24%), 8 cases were in age group of 51–60 years (32%), 3 cases were in age group of 61–70 years (12%), and 3 cases were in age group of >70 years (12%).
The OSCC cases selected include males (72%) than females (28%). Most of the patients with OSCC had deleterious habit of tobacco chewing (36%) in 9 cases and associated with alcohol consumption in 3 cases (12%), smoking in 8 cases (32%), and betel nut chewing habit in 5 cases (20%).

The lesions were distributed at different sites in the oral cavity with maximum cases showing lesions in buccal mucosa (11 cases - 44%). Other sites included alveolus (4 cases - 16%), tongue (5 cases - 20%), floor of mouth (1 case - 4%), lip (2 cases - 8%), and retromolar trigone (2 cases - 8%).

TNM staging of the 25 Group A cases revealed that one half of cases of OSCC selected, i.e., 7 cases (28%) had a tumor size of T4, 7 cases (28%) reported with a tumor size of T3, 5 cases (20%) were diagnosed to have a tumor size of T2, while 6 cases (24%) were assessed to have a tumor size of T1.

The nodal status of Group A showed 11 cases in N1 stage accounting for 44% of the total cases selected in Group A; 2 cases in N2a staging; 5 cases in N2b and 7 cases with no nodes (N0). Metastasis was not noted in any of the cases. Clinical staging, accordingly, showed 12 cases in Stage IV, 6 cases in Stage III, 3 cases in Stage II, and 4 cases in Stage I. Thus, most of the cases were in Stage IV.

Histopathological grading suggested 10 cases were well-differentiated SCCs, 8 cases were moderately differentiated SCCs, and 7 cases were poorly differentiated SCCs. The pattern of growth suggested that 10 cases were endophytic; 8 cases were exophytic; and 7 cases were both endophytic and exophytic.

The second group of controls (Group B) included 25 subjects of whom 5 were of ≤30 years, 7 were in the age group of 31–40 years, 8 were in age group of 41–50 years, 3 were in age group of 51–60 years and 2 were in the age group of 61–70 years. The control group of subjects was matched for age and sex with the Group A and Group B cases. The controls selected including 9 males and 6 females, with no history of any deleterious habits.

Serum β2-M was estimated in all the two groups and the mean, median, standard deviation, and 95% confidence in β2-M levels were calculated. The range value of serum β2-M levels in Group A was 1.98–5.39 mg/L. Mean was 3.69 mg/L with standard deviation of ± 2.06 and 95% confidence interval of 2.55–4.83 [Table 1].

Cases of Group A in clinical stage I showed a mean of 1.325 mg/L and standard deviation of ± 0.623. The cases in clinical stage II revealed a mean of 2.04 mg/L and a standard deviation of ± 0.347. The cases which were in clinical stage III depicted a mean of 3.596 mg/L and a standard deviation of ± 2.3. The cases of Group A which were noted to be in stage IV depicted a mean of 4.95 mg/L and a standard deviation of ± 3.10 [Table 2].

Serum β2-M levels varied with the tumor size in Group A cases with the mean being 1.97 mg/L and standard deviation of ± 2.3 in T1, the mean being 2.87 mg/L and standard deviation of ± 1.53 mg/L in T2, the mean being 3.7 mg/L and standard deviation of ± 1.3 in T3, and the mean being 5.69 mg/L and standard deviation of ± 3.8 in T4 [Table 3]. Serum β2-M levels differed among the different nodal status with the mean being 1.63 mg/L and a standard deviation of ± 0.61 in N0, mean being 3.83 ng/L and a standard deviation of ± 1.90 in N1, the mean being 5.55 mg/L and a standard deviation of ± 1.89 in N2 [Table 4]. Serum β2-M levels differed among the different growth of OSCC with the mean being 3.05 mg/L and a standard deviation of ± 2.48 in exophytic growth of OSCC, the mean being 3.64 mg/L and a standard deviation of ± 1.9 in endophytic growth of OSCC, the mean being 4.5 mg/L and a standard deviation of ± 1.07 in combine lesion, i.e., both exophytic and endophytic growth of OSCC [Table 5]. Serum β2-M levels differed among the different histopathological diagnosis with the mean being 4.73 mg/L and a standard deviation of ± 2.73 in well differentiated OSCC, the mean being 2.87 mg/L and a standard deviation of ± 1.5 in moderately differentiated OSCC, the mean being 3.16 mg/L and a standard deviation of ± 1.93 in poorly differentiated OSCC [Table 6]. The

### Table 1: Comparison of serum beta-2 microglobulin levels in mg/l between the groups using Mann-Whitney U-Test

| Group          | n  | Concentration | Percentiles | Sum of ranks | Mann-Whitney U | Wilcoxon W | Z   | Asymptotic Significant (two-tailed) |
|----------------|----|---------------|-------------|--------------|----------------|-------------|-----|------------------------------------|
|                |    | Minimum       | 25          | Median       |                |             |     |                                    |
| Group A cases  | 25 | 0.02          | 13.41       | 1.98         | 2.95           | 5.39        | 845 | 105.000                            |
| Group B Cases  | 25 | 1.23          | 2.04        | 1.525        | 1.68           | 1.845       | 430 |                                    |

**Beta-2 microglobulin in mg/l**

| Group          | Mean ± SD | 95% CI for mean |
|----------------|-----------|-----------------|
| Group A        | 3.69±0.06 | 2.55-4.83       |
| Group B        | 1.676±0.215 | 1.58-1.76     |

SD: Standard deviation, CI: Confidence interval
range of serum β2-M levels in Group B was 1.52–1.85 mg/L with a mean of 1.68 and a standard deviation of ± 0.215 and a 95% confidence interval of 1.58–1.76 [Table 1].

DISCUSSION

Neoplastic/cancer cells display a broad spectrum of alterations at the genetic level that includes gene rearrangements, amplifications, and point mutations, leading to disturbances in molecular pathways regulating cell growth survival, and metastasis. When such changes manifest in majority of patients with specific type of tumor, these can be used as biomarkers for detection and developing targeted therapies.[10,11]

Diagnostic and prognostic biomarkers are quantifiable traits that help clinical oncologists at the first interaction with the suspected patients. These particularly aid in (i) identifying who is at risk, (ii) diagnosing at an early stage, (iii) selecting the best treatment modality, and (iv) monitoring response to treatment.

In oral carcinoma, however, the studies of tumor markers have been limited and several tumor markers with clinical promise need further evaluation. One such tumor marker is serum β2-M. It is a low molecular weight protein consisting of a single chain of 100 amino acids which is a part of the HLA antigen molecule, where it represents the variant light chain. It is expressed on the membrane of almost all nucleated cells, and is detectable in all body fluids as a shedding product of cell membrane. The free form of serum β2-M consists of a single polypeptide chain with one intrachain disulfide bridge.

Various possibilities for increased serum level of β2-M suggested are an increased cellular activity in malignancy being responsible for its increased release or being a constituent of HLA molecules, the cell membrane turnover or cell division could increase its shedding. β2-M and HLA molecules are variably expressed on the surface of tumor cells. Malignant cells with modified HLA expression may escape from the normal host immune response and proliferate uncontrollably. A structural defect of the HLA complex may also result in changes in epitope expression and increased release of β2-M in serum. This mechanism is consistent with the fact that β2-M levels reflect tumor burden and cell turnover.[12-14]

The present study involved 25 patients with OSCC were in the range of 30–71 years with maximum incidence in the age group of 41–60 years and more commonly occurring in male

| Table 2: Serum beta-2 microglobulin level in mg/l according to stage of oral squamous cell carcinoma |
|-----------------------------------------------|
| Staging               | Number of cases | Mean±SD         |
|-----------------------|-----------------|-----------------|
| Stage I               | 4               | 1.3250±0.62303  |
| Stage II              | 3               | 2.0400±0.34771  |
| Stage IB              | 6               | 3.5967±2.30598  |
| HSTAGE TV             | 12              | 4.9517±3.10656  |
| Total                 | 25              | 5.6929±3.80525  |
| SD: Standard deviation|

| Table 3: Serum beta-2 microglobulin level in mg/l according to histopathological diagnosis of oral squamous cell carcinoma |
|-----------------------------------------------|
| Histopathological diagnosis                 | Number of cases | Mean±SD         |
|----------------------------------------------|-----------------|-----------------|
| Well differentiated                          | 10              | 4.7330±2.73901  |
| Moderately differentiated                    | 8               | 2.8070±1.57345  |
| Poorly differentiated                        | 7               | 3.1614±1.93566  |
| Total                                        | 25              | 5.6929±3.80525  |
| SD: Standard deviation                      |

which is in accordance to various epidemiological studies of oral cancer done in India.[15-18]

The comparison of serum β2-M in patients with OSCC (Group A) versus controls (Group B) demonstrated that the elevation of serum β2-M in OSCC was highly significant compared to controls. This finding was reported previously by Wilma Dclphine[9] in 2002, Anil et al.[19] in 1995 and by Yoneda et al.,[20]
in 1990. Studies have shown that with the advancement of the T-stage, an elevation in both the original SCC-related antigen and the β2-M levels was present. In our study too, a similar relationship was observed. In our study, 10 (40%) cases were well-differentiated SCCs, 8 (32%) cases were moderately differentiated, and 7 (28%) cases were poorly differentiated. No significant correlation could be drawn as far as the histopathological grade and serum β2-M were concerned in contrast to the studies that highlighted a positive correlation between the two.[23]

On the basis of nodal status, an important inference in our study was that there was a linear progressive increase in serum β2-M level in accordance with nodal status. This feature has contributed to the uniqueness of our study as it suggests that serum β2-M could be a valuable tool in predicting nodal status, which is vital for determining the prognosis and postsurgical morbidity in carcinoma. Since a large number (44%) of cases of OSCC were in Stage IV, we found that as the clinical staging of OSCC increased, the serum β2-M too increased notably and in a progressive manner in all the stages, through stage I to stage IV of oral cancer patients (group A). Our findings were similar at that of Kadam[23] and Wilma Delphine.[9]

Depending on the pattern of growth of OSCC, combined endophytic and exophytic growth showed a higher serum β2-microglobulin level followed by endophytic and then exophytic growth. Elevated serum levels of β2-M, have been associated in patients with several lymphoid malignancies, including multiple myeloma, B-cell non-Hodgkin’s lymphoma, and chronic lymphocytic leukemia.[23-26] A number of groups have also reported that serum β2-M levels correlate with Ann Arbor stage and the presence of symptoms in patients with Hodgkin’s disease and that elevated levels of this polypeptide predict a less favorable prognosis.[27-29]

CONCLUSION

From the observations, we gathered in our present study on the evaluation of serum β2-microglobulin levels in OSCC and controls; we have arrived at the following conclusions: Highest incidence was observed in 5th–6th decade of life and male patient were outnumbered in OSCC, serum β2-microglobulin levels were found to be significantly increased in OSCC compared to controls, serum β2-microglobulin levels showed a progressive increase corresponding to clinical staging, tumor size, and notably in relation to nodal status of OSCC.

Further studies are required to confirm the clinical usefulness of serum β2-microglobulin as a biochemical parameter. However, a more elaborate study with larger number of clinical cases is very much essential to be more conclusive.

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Conflicts of interest
There are no conflicts of interest.

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