

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 (β 2-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 β 2-M by enzyme-linked immunosorbent assay.

Results: It was observed that there was a significant increase in serum β 2-M levels in oral SCC patients as compared to controls. Circulating levels in serum β 2-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 β 2 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.

JOYCE SEQUEIRA, SHREYA SENGUPTA, BHUPENDRA MHATRE

Department of Oral and Maxillofacial Surgery, Yenepoya Dental College, Mangalore, Karnataka, India


Address for correspondence: Dr. Joyce Sequeira, Department of Oral and Maxillofacial Surgery, Yenepoya Dental College Mangalore - 575 018, Karnataka, India.
E-mail: joycemathias26@yahoo.com

Received: 11 January 2020, **Revised:** 15 December 2020, **Accepted:** 01 February 2021, **Published:** 15 July 2021

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Sequeira J, Sengupta S, Mhatre B. Serum beta-2 microglobulin analysis in patients with oral squamous cell carcinoma. *Natl J Maxillofac Surg* 2021;12:227-32.

Access this article online	
Website: www.njms.in	Quick Response Code 
DOI: 10.4103/njms.NJMS_242_20	

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 (β 2-M)– 176 (132-m), first described by Berggard 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 β 2-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 β 2-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 β 2-M in OSCC and to evaluate its role as a biochemical parameter in OSCC for screening purposes.

Aim and objectives

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

To prepare for the test, all patient samples were diluted to 1:100 with sample buffer. 10 μ l of sample was combined with 990 μ l 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 μ l 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 μ l of wash solution. 100 μ l 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 μ l of TMB substrate solution was incubated for 30 min at room temperature, after dispensing into each well and 100 μ l 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 β 2-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 $\beta 2$ -M was estimated in all the two groups and the mean, median, standard deviation, and 95% confidence in $\beta 2$ -M levels were calculated. The range value of serum $\beta 2$ -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 $\beta 2$ -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 $\beta 2$ -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 mg/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 $\beta 2$ -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 $\beta 2$ -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

	n	Concentration		Percentiles			Sum of ranks	Mann-Whitney U	Wilcoxon W	Z	Asymptotic Significant (two-tailed)
		Minimum	Maximum	25	Median	75					
Group A cases	25	0.02	13.41	1.98	2.95	5.39	845	105.000	430.000	4.027	<0.001
Group B Cases	25	1.23	2.04	1.525	1.68	1.845	430				
Group	Beta-2 microglobulin in mg/l										
	Mean \pm SD		95% CI for mean								
Group A	3.696 \pm 2.06		2.55-4.83								
Group B	1.676 \pm 0.215		1.58-1.76								

SD: Standard deviation, CI: Confidence interval

range of serum β 2-M levels in Group B was 1.52–1.85 mg/L with a mean of 1.68 and a standard deviation of \pm 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 β 2-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 β 2-M consists of a single polypeptide chain with one intrachain disulfide bridge.

Various possibilities for increased serum level of β 2-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. β 2-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 β 2-M in serum. This mechanism is consistent with the fact that β 2-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 \pm SD
Stage I	4	1.3250 \pm 0.62303
Stage II	3	2.0400 \pm 0.34771
Stage IB	6	3.5967 \pm 2.30598
HSTAGE TV	12	4.9517 \pm 3.10656
Total	25	

SD: Standard deviation

Table 3: Serum beta-2 microglobulin level in mg/l according to tumor size

Tumor size	Number of cases	Mean \pm SD
T1	6	1.9717 \pm 2.33421
T2	5	2.8700 \pm 1.53403
T3	7	3.7700 \pm 1.36065
T4	7	5.6929 \pm 3.80525
Total	25	

Table 4: Serum beta-2 microglobulin level in mg/l according to nodal status

Nodal status	Number of cases	Mean \pm SD
NO	7	1.6314 \pm 0.61680
N1	11	3.8318 \pm 1.90636
N2a/b	7	5.5500 \pm 1.89699
Total	25	

SD: Standard deviation

Table 5: Serum beta-2 microglobulin level in mg/l according to growth of oral squamous cell carcinoma

Growth of OSCC	Number of cases	Mean \pm SD
Exophytic	8	3.0525 \pm 2.48178
Endophytic	10	3.6450 \pm 1.91600
Exophytic+endophytic		4.5071 \pm 1.07731
Total	25	

OSCC: Oral squamous cell carcinoma, SD: Standard deviation

Table 6: Serum beta-2 microglobulin level in mg/l according to histopathological diagnosis of oral squamous cell carcinoma

Histopathological diagnosis	Number of cases	Mean \pm SD
Well differentiated	10	4.7330 \pm 2.73901
Moderately differentiated	8	2.8700 \pm 1.57345
Poorly differentiated	7	3.1614 \pm 1.93566
Total	25	

SD: Standard deviation

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

The comparison of serum β 2-M in patients with OSCC (Group A) versus controls (Group B) demonstrated that the elevation of serum β 2-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 β 2-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 β 2-M were concerned in contrast to the studies that highlighted a positive correlation between the two.^[21]

On the basis of nodal status, an important inference in our study was that there was a linear progressive increase in serum β 2-M level in accordance with nodal status. This feature has contributed to the uniqueness of our study as it suggests that serum β 2-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 β 2-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 to that of Kadam^[22] and Wilma Dclphine.^[9] Depending on the pattern of growth of OSCC, combined endophytic and exophytic growth showed a higher serum β 2-microglobulin level followed by endophytic and then exophytic growth. Elevated serum levels of β -2M, 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 β -2M 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 β 2-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 β 2-microglobulin levels were found to be significantly increased in OSCC compared to controls, serum β 2-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 β 2-microglobulin as a biochemical parameter. However, a more elaborate study with larger number of clinical cases is very much essential to be more conclusive.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bagan J, Sarrion G, Jimenez Y. Oral cancer: Clinical features. *Oral Oncol* 2010;46:414-7.
2. Bello IO, Soini Y, Salo T. Prognostic evaluation of oral tongue cancer: Means, markers and perspectives. *Oral Oncol* 2010;46:630-5.
3. Bettendorf O, Piffkò J, Bãnkfalvi A. Prognostic and predictive factors in oral squamous cell cancer: Important tools for planning individual therapy? *Oral Oncol* 2004;40:110-9.
4. Massano J. Oral squamous cell carcinoma: Review of prognostic and predictive factors. *Oral Surg Oral Med Oral Pathol Oral Radio Endo* 2006;102:67-76.
5. Scully C, Bagan J. Oral squamous cell carcinoma overview. *Oral Oncol* 2009;45:301-8.
6. Nayak AG, Chatra L. Tumor markers: An overview. *J Indian Acad Oral Med Radiol* 2010;22:147-50.
7. Bhargava D., Bhargava K. Tumor markers: An overview. *J Innov Dent* 2011;1:213-6.
8. Choontharu MM. Role of tumor markers in oral squamous cell carcinoma: Review of literature and future consideration. *SRM J Res Dent Sci* 2012;3:455-60.
9. Wilma Dclphine S. Alteration of serum beta2 microglobulin in oral carcinoma. *Indian J Clin Biochem* 2002;17:104-7.
10. Bhatt AN. Cancer biomarkers – Current perspectives. *Indian J Med Res* 2010;132:129-49.
11. Reddy S. Tumour markers in oral neoplasia. *IJDA* 2010;2:318-22.
12. Kesheng LI. Serum β 2 – Microglobulin levels in patients with various solid cancer. *J Mol Biomark Diagn* 2012;S: 2.
13. Koo BS. Recurrence and salvage treatment of squamous cell carcinoma of the oral cavity. *Oral Oncol* 2006;12:789-94.
14. Mathur PT. Correlation of clinic of oral squamous cell carcinoma with age, site, sex and habits. *J Indian Acad Oral Med Radiol* 2011;23:81-5.
15. Sankaranarayanan R. Oral cancer in India: An epidemiologic and clinical review. *Oral Surg Oral Med Oral Pathol* 1990;69:325-30.
16. Kiran G. Demographics and histopathological patterns of oral squamous cell carcinoma at a tertiary level referral hospital in Hyderabad, India: A 5- year retrospective study. *J Orofac Res* 2012;2:198-201.
17. Agrawal KH, Rajderkar SS. Clinicoepidemiological proof of oral cancer: A hospital based study. *Indian J Community Health* 2012;24:80-5.
18. Iype EM. Oral cancer among patients under the age of 35 years. *J Postgrad Med* 2001;47:171-6.
19. Anil S, Beena VT, Nair RG, Vijayakumar T. Evaluation of serum β 2microglobulin in premalignant and malignant lesions of the oral cavity. *Oral Surg Oral Med Oral Pathol Oral Radio Endo* 1995;79:750-2.
20. Yoneda K, Hirota J, Yamamoto T, Ueta E, Osaki T. Clinical evaluation of tumor markers in head and neck squamous cell carcinomas. *Gan No Rinsho* 1990;36:458-64.
21. Vaishali N, Iupkari JV. An estimation of serum 2-microglobulinlevel in premalignant lesions/conditions and oral squamous cell carcinoma: A clinicopathological study. *J Oral Pathol* 2005;9:16-9.
22. Kadam CV. Biochemical markers in oral cancer. *Biomed Res* 2011;22:76-80.
23. Bataille R, Durie BG, Grenier J. Serum beta2 microglobulin and survival

- duration in multiple myeloma: A simple reliable marker for staging. *Br J Haematol* 1983;55:439-47.
24. Melillo L, Musto P, Tomasi P, Cascavilla N, Bodenizza C, Ladogana S, *et al.* Serum beta 2-microglobulin in malignant lymphoproliferative disorder. *Tumori* 1988;74:129-35.
 25. Swan F Jr., Velasquez WS, Tucker S, Redman JR, Rodriguez MA, McLaughlin P, *et al.* A new serologic staging system for large-cell lymphomas based on initial B2-microglobulin and lactate dehydrogenase levels. *J Clin Oncol* 1989;7:1518-27.
 26. Litam P, Swan F, Cabanillas F, Tucker SL, McLaughlin P, Hagemester FB, *et al.* Prognostic value of serum beta-2 microglobulin in low-grade lymphoma. *Ann Intern Med* 1991;114:855-60.
 27. Hagberg H, Killander A, Simonsson B. Serum beta 2-microglobulin in malignant lymphoma. *Cancer* 1983;51:2220-5.
 28. Constantinides IP, Pathouli C, Karvountzis G, Papadopoulos P, Varvoutsis-Constantinides M, Eliakis P, *et al.* Serum beta 2 microglobulin in malignant lymphoproliferative disorders. *Cancer* 1985;55:2384-9.
 29. Chronowski GM. An elevated serum beta-2-microglobulin level is an adverse prognostic factor for overall survival in patients with early-stage Hodgkin disease. *Cancer* 2002;95:2534-8.