ESTIMATION AND COMPARISION OF SERUM β2-MICROGLOBULIN IN ORAL SQUAMOUS CELL CARCINOMA AND ORAL LEUKOPLAKIA

Bhavana Agrawal[1], R. N. Mody[2], Arun Tadas[3]

1 Department of Oral Medicine and Radiology, Jodhpur Dental College General Hospital, Jodhpur, India
2 Department Of Oral Medicine and Radiology, Hitkarini Dental College, Jabalpur, India
3 Department of Biochemistry, Vasantrao Naik Govt. Medical College, Yavatmal, India

CORRESPONDING AUTHOR: *drbhavana_1@yahoo.com*

ABSTRACT

Aim: To estimate and compare serum β2-microglobulin levels in oral leukoplakia and oral squamous cell carcinoma patients with that of control group.

Material and Methods: The study was carried out on 70 subjects divided into three groups (20 oral leukoplakia patients, 30 oral squamous cell carcinoma patients and 20 controls). Serum β2-microglobulin was estimated by an enzyme linked immunosorbent assay (ELISA).

Results: A significant increase in serum levels of β2-microglobulin was observed in oral squamous cell carcinoma patients as compared to control group.

Conclusions: Results of this study suggest that estimation of serum β2-microglobulin can be useful in as biomarker for diagnosis of oral squamous cell carcinoma.

KEYWORDS: serum β2-microglobulin, oral squamous cell carcinoma, tumor markers

INTRODUCTION

Squamous cell carcinoma is the most common malignancy of oral cavity representing 90-95% of all oral malignancies in India[1]. It is a major health problem in India, forming about 10% of the new cancers that occurs in all parts of body each year[1]. The current research focuses on faster, specific and sensitive tests to detect cancer in early stages. In this context, many tumor markers have been studied[2]. In the oncogenic process, specific events occur at each step and these can be studied by assessing the associated biological markers[3].

The tumor markers are substances that are produced by body in response to cancerous growth or by the cancer tissue itself and released in blood and other body fluids. There are only a few well-established tumor markers that are being routinely used like prostate specific antigen (PSA), α-fetoprotein for hepatocellular carcinoma, cancer antigen-125 for ovarian cancer etc[4]. For the detection of oral malignancy various markers like oncofoetal protein, carcinoembryonic antigen (CEA), other proteins like B-Protein & β_2 microglobulin , and enzymes like lactate dehydrogenase (LDH)[5]. These markers have wide range of potential applications like screening, diagnosis, prognosis, and

monitoring the response to treatment.

The search for "ideal tumour marker" has become a major goal in research oncology[6].

β_2-microglobulin is a tumor marker, which has received considerable attention. It was described and isolated from the urine of patients with tubular proteinurias by Berggard and Bearn[7].

Scully[8] was the first to assess the potential use of β-2 micro globulin as a marker in oral premalignant lesions. β_2 microglobulin is low molecular mass protein and which is present in membranes of possibly all nucleated cells where it appears to be present in structural association with the histocompatibility antigen (HCA)[9,10].

Studies have reported increased β_2-microglobulin in various malignancies like myelomas, acute and chronic leukemia, Non-Hodgkin's lymphoma, melanoma, carcinomas of breast, lung, colon, stomach, cervix and uterus[11,12]. The increased β_2-microglobulin is reported in patients with oral malignancies as well[13-15].

Early detection of oral cancer is important to reduce morbidity and mortality. Plasma biomarkers are thought to have a great potential for assisting the early detection of oral cancer and monitoring cancer progression or recurrence[16]. The advantage of early detection, before lymph node involvement is shown by the 70 percent 5 year survival rate of patients who do not have nodal involvement. This is in marked contrast to the 30 percent 5 year survival rate of patients whose diagnosis is established after nodal disease exists. Very few studies have been carried out related to serum β_2-microglobulin in oral cancer and precancer. Considering the high prevalence of oral malignancy in India, the present study was carried out to estimate and compare serum β_2-microglobulin levels in patients with oral squamous cell carcinomas and in patients with oral leukoplakia with that of healthy controls.

MATERIAL AND METHODS

For the present study, 70 patients were selected at random from Department of Oral Medicine and Radiology, Govt. Dental College, Nagpur, Maharashtra, India. The informed consent was obtained from all patients and ethical clearance was obtained from institutional ethical committee. The patients were divided into three groups: G1: control group consisted of 20 age and sex matched individuals who gave no history of any habit nor presented with any signs of systemic disease or pathological oral lesions; G2: consisted of 20 patients in age range of 21 to 69 years, with clinically and histopathologically confirmed leukoplakia; G3: consisted of 30 patients in age range of 25 to 70 years, with clinically staged and histopathologically confirmed squamous cell carcinoma of oral cavity.

None of the patients had received any treatment before study and were free from conditions where β_2-microglobulin level may be elevated (acute and chronic leukemia, non hodgkins lymphoma, multiple myeloma, tumors of breast, lung, colon, cervix, uterus, hepatobiliary disorders & systemic lupus erythematosus). To avoid false positive results, care was taken to exclude subjects with other malignancies or with history of systemic diseases.

Under aseptic conditions, five ml of blood was collected from anticubital vein and allowed to clot at room temperature for two hours and then serum was separated by centrifuging at 3000 rpm for 10 minutes.

The serum was stored in Laxbro storage vials at –70°C until assayed β_2-microglobulin was estimated by an indirect solid phase Enzyme Linked Immunosorbent Assay (ELISA), which was designed for the quantitative measurement of β_2-microglobulin in human serum. Quantitative estimation of serum β_2-microglobulin was done using Immunometric Enzyme Immunoassay kit manufactured by Orgentec Diagnostika GmbH, Mainz, Germany.

The data was analyzed by using statistical package for social sciences (SPSS) software. Cases and controls were tested for statistical significance by Students' unpaired t-test. Values of $p<0.05$ were considered significant. Analysis of variance (ANOVA) was used to compare β_2-microglobulin in various age groups of control group.

RESULTS

Mean serum β_2-microglobulin level in control group was1.88 µg/ml with standard deviation of 0.82, it was 2.23 ± 0.84 µg/ml in oral leukoplakia group and 3.23± 0.96 µg/ml in oral squamous cell carcinoma group (Table 1). The increase in serum β_2-microglobulin level in oral carcinoma group compared with control group was statistically highly significant ($p<0.001$). Statistically significant increase was also found when oral squamous cell carcinoma group was compared with oral leukoplakia group ($p<0.05$). Though, increased β_2-microglobulin levels were observed in oral leukoplakia, it was not found to be significant when it was compared with control group ($P>0.05$) (Table 2). Serum β_2-Microglobulin levels were also found to be increasing with advancing age in

control group. This increase was statistically highly significant (Table 3).

In control group the mean serum β_2-microglobulin level in females was 1.7 ± 0.50 µg/ml and in males it was 2.07 ±1.04 µg/ml which was higher than in females, but found to be statistically non-significant (Table 4).

Table 1. Serum levels of β2-microglobulin in various groups.

Sr. No.	Groups	β_2-microglobulin (µg/ml) Mean +SD
1	Group I [Controls (n= 20)]	1.88µg/ml ±0.82
2	Group II [Oral Leukoplakia (n=20)]	2.23 µg/ml ±0.84
3	Group III [Oral squamous cell carcinoma (n=30)]	3.23µg/ml ±0.96

Table 2. Comparison of serum levels of β2-microglobulin between various groups.

	Parameter	T-value	P-value	Significance of P-value
1	Control versus oral squamous cell carcinoma	5.35	p< 0.001**	Highly significant
2	Oral leukoplakia versus oral squamous cell carcinoma	3.84	p<0.05*	Significant
3	Control versus oral leukoplakia	1.34	p>0.05	Non- Significant

*Stands for p < 0.05, **stands for p < 0.001.

Table 3. Mean serum β2-microglobulin level in various age groups in control group.

Sr. No.	Age Groups in years	Mean Serum β_2-microglobulin level (µg/ml)
1	21-30 years	1.17 ± 0.35
2	31-40 years	1.55 ± 0.23
3	41-50 years	1.55 ± 0.31
4	51-60 years	2.32 ± 0.43
5	61-70 years	2.82 ± 1.18

Table 4. Mean serum beta-2 microglobulin level in males and females in control group.

Mean Serum β_2-microglobulin in females of Control group with SD	Mean Serum β_2-microglobulin in males of Control group with SD	p-value
1.7 ± 0.50	2.07 ± 1.04	p>0.05

DISCUSSION

In present study, increased mean serum β_2-microglobulin level in oral squamous cell carcinoma patients as compared to control group was found. This is in accordance with studies by- Scully[13], Lin[17], Vinzez et al.[2], Manzar et al.[14], Anil et al.[18], Delphine Silva et al.[19] and Singh[20]. However, Wennerberg et al.[15] reported that only 12% of patients with oral squamous cell carcinoma had elevated serum β_2-microglobulin. Teasdale et al.[21] reported that serum β_2-microglobulin more than 3 mg/l are frequently associated with advanced and persistent malignant disease even after allowing for the effects of age and non-specific illness. No correlation between various stages of oral squamous cell carcinoma and serum β_2-microglobulin level could be established. This is in accordance with Vinzez et al.[2] and Manzar et al.[14]. However, Delphine Silva[19] reported that progressively higher values were obtained as the oral squamous cell carcinoma advanced clinically. There was increase in mean serum β_2-microglobulin levels in oral leukoplakia group as compared with control group. This is in accordance with Scully[13] and Anil et al[18].

When serum β_2-microglobulin level was compared between various age groups of control, it was observed that values increased with the advancing age and this difference was statistically highly significant, however no co-relation between age and serum β_2-microglobulin could be established in oral squamous cell carcinoma group. These finding were consistent with study of Teasdale et al.[21] and Parildar[22]. In control group, mean serum β_2-microglobulin level in male patients was higher compared to female patients of same group however statistically it was not significant. This finding is in contrast with Teasdale et

al.[21], who found mean serum β_2-microglobulin levels consistently higher in females than males. The increased β_2-microglobulin levels reflect a heightened level of immune activation as reported by Wanchu et al. [23] in a study of HIV/TB co-infection. Also increased serum β_2-microglobulin levels are reported in old age which again reflects decreased host immunity. These findings prompt us to think that malignancies either develop in immunocompromised host or there is immune deficiency secondary to malignant process.

In the present study, following findings were also noted which were not the objectives of study. These are (oral carcinoma group had 30 patients) 20 males and 10 females and in oral leukoplakia group, out of 20 patients, only 1 was female. From this, it can be concluded that oral pre-cancer and cancer are more common in males which can be attributed to relatively higher indulgence of males in tobacco and alcohol habits in India. Out of 30 oral carcinoma patients, only two patients had stage I, 4 patients had stage II and 24 patients had stage III and stage IV oral squamous cell carcinoma. The most common habit was tobacco plus lime chewing in both oral squamous cell carcinoma and oral leukoplakia group. In both oral leukoplakia and oral squamous cell carcinoma group elderly patients were affected most. The mean age in oral leukoplakia group was 46.45 ± 14.77 years and in oral carcinoma group mean age was 46.47 ± 9.96 years.

CONCLUSIONS

Decreased mortality and morbidity can be achieved in oral cancer and pre-cancer by identification of tumor bio-markers which assist in early diagnosis and monitoring of progression of disease. This study confirms the results of other investigators, that β_2-microglobulin levels are increased with progression from precancer to oral cancer. From the results presented it can be concluded that β_2-microglobulin can be used as an adjunct to clinic-pathogical diagnosis or in combination assay along with other relevant tumor markers in diagnosis of oral squamous cell carcinoma. Further studies are necessary to find out whether serum β_2-microglobulin would be of help as an individual tumor marker in clinical diagnosis.

REFERENCES

1. Mehta FS, Hamner JE III. Tobacco-related oral mucosal lesions and conditions in India. Tata Institute of Fundamental Research: Mumbai, 1993.

2. Vinzenz K, Schonthal E, Zekert F, et al. Diagnosis of head and neck carcinomas by means of immunological tumor markers. J Cranio-Max Fac Surg 1987;15:270-7.

3. Bates SE, Longo DL. Use of serum tumor markers in cancer diagnosis and management. Semin Oncol 1987;14:102-38.

4. Sacher RA. Tumor markers. In: Widmann's Clinical Interpretation of Laboratory tests. Jaypee Brothers: New Delhi, 1995.

5. Vaishali N, Tupkari JV. An estimation of serum β-2 microglobulin level in premalignant lesions / conditions and oral squamous cell carcinoma: a clinico pathological study. J Oral Maxillofac Pathol 2005;9:16-19.

6. Burkhwdt SGA. Tissue markers of potentially malignant human oral epithelial lesions. J Oral Pathol Med 1993;22:246-56.

7. Berggard I, Bearn AG. Isolation and properties of a low molecular weight β2-globulin occurring in human biological fluids. J Biol Chem 1968;243:4095-103.

8. Rassekh CH, Johnson JT, Eibling DE. Circulating markers in squamous cell carcinoma of head and neck: a review- oral oncology. Eur J Cancer 1994;308:23-8.

9. Kubo RT, ColonSM, McColom TR, et al. Some biological properties of beta-2 microglobulins and its antibody. Fed Proc 1976;35:1183-7.

10. Peterson PA, Rask L,Ostberg L. Beta-2 microglobulin and the major histocompatibility complex. Adv Cancer Res 1977;24:115-63.

11. Ervin PE, Wibell L. Serum β2-microglobulin in various disorders. Clinic Chim Acta 1973;43:183-7.

12. Shuster J, Gold P, Poulik MD. β2-microglobulin levels in cancerous and other disease states. Clinic Chim Acta 1976;67:307-13.

13. Scully C. Serum β2-microglobulin in oral malignancy and pre malignancy. J Oral Path 1981;10:354-7.

14. Manzar W, Raghavan MRV, Aroor AR, et al. Evaluation of serum β2- microglobulin in oral cancer. Aust Dent J 1992;37:39-42.

15. Wennerberg J, Alm P, Logdberg L, et al. β2-microglobulin in squamous cell carcinomas of the head and neck and in tumors heterotransplanted into nude athymic mice. Acta Otolaryngol 1984;98:335-42.

16. Chen CK. Biomarkers in diagnostic head and neck tumor pathology. J Chin Oncol Soc 2009;25:89-101.

17. Lin SC. Serum levels of β2-microglobulin in oral tumor patients. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi 1986;19:183-8.

18. Anil S, Beena VT, Nair RG, et al. Evaluation of serum β2-microglobulin in premalignant and malignant lesions of the oral cavity. Oral Surg Oral Med Oral Patho Oral Radio Endo 1995;79:750-2.

19. Delphine Silvia CRW, Vasudevan DM, Prabhu KS. Alteration of serum β2-microglobulin in oral carcinoma. Ind J Clin Biochem 2002;17:104-7.

20. Singh AP, Kumar N, Raju MS, et al. Estimation of serum β2-microglobulin in potentially malignant disorders and squamous cell carcinoma of oral cavity: a clinicopathologic study. Dent Res J 2014;11:109-13.

21. Teasdale C, Mander AM, Fifield R, et al. Serum β2-microglobulin in controls and cancer patients. Clinic Chim Acta 1977;78:135-43.

22. Parildar Z, Gulter C, Habif S, et al. Age and Gender associated changes in Cystatin C and β2-microglobulin. Turk J Med Sci 2002;32:317-21.

23. Wanchu A, Arora S, Bhatnagar A, et al. Decline in β2-microglobulin levels after anti-tubercular therapy in tubercular patients with HIV infection. Indian J Chest Dis Allied Sci 2001;43:211-5.