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Original Article

Estimation of serum β2-microglobulin in potentially malignant disorders and squamous cell carcinoma of the oral cavity: A clinicopathological study

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ABSTRACT

Background: Tumor markers are substances, which quantitatively changes in serum, during the tumor development, one such tumor marker is serum β2-microglobulin (β2-m). The aim of this study was to establish the role of β2-m as a biochemical parameter for diagnosis and prognosis of oral carcinoma by estimation of serum β2-m levels in potentially malignant lesions, conditions, and oral squamous cell carcinoma.

Materials and Methods: The study was carried out on 48 subjects (16 control, 8 oral submucous fibrosis, 8 oral leukoplakia, and 16 oral squamous cell carcinoma patients of different stages), conducted at department of Oral Medicine, Kothiwal Dental College, Moradabad, India. Under aseptic precautions, 5 ml venous blood was drawn and serum was separated. Estimation of β2-m level in serum was carried out by enzyme linked immunosorbent assay. The data were analyzed by using the statistical package for social sciences (SPSS 17.0) software. Cases and controls were tested for statistical significance with one-way ANOVA with post-hoc Tukey's HSD. Values of $P < 0.05$ were considered significant.

Results: The mean serum β2-m level in the control group was 1.173 ± 0.059, in potentially malignant lesions/conditions group was 1.688 ± 0.137 and in oral squamous cell carcinoma group was 2.835 ± 0.0313. This progressive increase in serum β2-m level was found to be highly significant (*P* value < 0.001). Results of Receiver operating characteristic analysis showed β2-m as a 100% sensitive and specific biomarker for oral squamous cell carcinoma.

Conclusion: The present study establishes β2-m as a specific biological tumor marker for diagnostic and prognostic evaluation of oral squamous cell carcinoma.

Key Words: β2-microglobulin, enzyme linked immunosorbent assay, squamous cell carcinoma, tumor markers

INTRODUCTION

Oral carcinoma is highly prevalent in Indian population and is primarily associated with various habits.[1] A close co-relation between tobacco habit and oral carcinoma is well-established. Oral carcinoma constitutes 30-40% of all carcinoma in India.[2] A high rate of malignant potential or oral submucous fibrosis (OSMF) is also well-established.[3]

Tumor markers are substances, which quantitatively changes in serum, during the tumor development. Recently, biological tumor markers have been introduced for early diagnosis of carcinoma. These markers have a wide range of potential application, for screening, diagnosis, prognosis, and follow-

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up monitoring.[1,4] Now-a-day, tumor markers in the head and neck carcinoma are receiving increase attention. Serum level of variety of substances shows a significant change in oral carcinoma and potentially malignant conditions. Alterations in serum globulins, circulating immune complexes, and complement factors have been reported earlier.[2]

One such tumor marker is serum β2-microglobulin (β2-m) and it was first described and isolated from the urine of patients with tubular proteinurias by Berggard and Bearn in 1968. β2-m is a low molecular weight, 11600 Dalton protein found on the surface of all cells except erythrocytes. It was also shown to occur in small quantities in normal human urine, plasma, and cerebrospinal fluid. This protein is the light or β-chain of the human leukocyte antigen (HLA). It exists in two main forms free and non-covalently linked to the HLA antigens, forming an invariant part of the HLA molecules. The serum β2-m is in the free form and it consists of a single polypeptide chain with one intrachain disulfide bridge and it does not contain carbohydrate.[5]

Oral squmous cell carcinoma (OSCC) causes a high degree of local invasiveness and a high rate of metastasis, which leads to its high incidence of mortality.[6] Early detection of oral cancer would greatly improve long-term survival rates. Plasma biomarkers are thought to have a great potential for assisting the early detection of oral cancer and monitoring cancer progression or recurrence.[7,8]

In the present study, an attempt was made to estimate the serum β2-m globulin level in potentially malignant lesions/conditions and OSCC, to predict the role of β2-m as a biochemical parameter, for diagnosis and prognosis of oral carcinomas.

MATERIALS AND METHODS

The present study was conducted to estimate the serum β2-m level in potentially malignant lesions/conditions and oral squamous cell carcinoma, to determine the role of β2-m as a biochemical parameter for diagnosis and prognosis of oral carcinoma in Department Of Oral Medicine, Kothiwal Dental College and Research Centre, Moradabad, Uttar Pradesh, India. An Ethical Committee approval by the institutional ethics committee and informed consent was obtained from all the subjects.

Subjects clinically and histopathologically diagnosed as leukoplakia, OSMF and OSCC, which had not received any treatment before and free from the conditions where the serum β2-m level may be elevated (acute and chronic leukemias, non-Hodgkin's lymphoma, myeloma, tumors of breast, lung, colon, stomach, cervix, uterus, hepatobiliary disorders, systemic lupus erythematosus) were included while the subjects having such conditions and treatment were excluded from the study. Biopsy was performed to confirm the diagnosis.

On the basis of exclusion and inclusion criteria, 32 subjects and 16 healthy controls (total 48 subjects) were selected and divided as group 1, control (16 subjects), group 2, potentially malignant lesions/conditions (16 subjects), subdivided in group 2A, OSMF (8 subjects, stage I-IV) and group 2B, oral leukoplakia (8 subjects, Mild to Moderate dysplasia located on the commissure, buccal mucosa, tongue, and floor of the mouth) and group 3, oral squamous cell carcinoma (16 subjects, located on the tongue, alveolar ridges, retromolar areas, and lower lip).

All subjects were screened clinically, biochemically, and biophysically to exclude any infections, renal, hepatobiliary disorders, systemic lupus erythematosus, lymphoproliferative disorders as well as other malignancies, previous history of allergy or autoimmune disease. None of the included patients had received any treatment before the study.

Under aseptic precautions, 5 ml venous blood was drawn and serum was separated out. The samples were frozen at 20°C until assay. The serum β2-m level was analyzed by enzyme linked immunosorbent assay using commercially available kit (β2-mEIA kit, Orgentec, Germany).

Statistical analysis

The data were analyzed by using the statistical package for social sciences (SPSS 17.0) software. Cases and controls were tested for statistical significance with one-way ANOVA with post-hoc Tukey's HSD. Values of $P < 0.05$ were considered significant.

For the sensitivity and specificity, receiver operating characteristic (ROC) analysis was used, a value of 1.36 and 2.095 were taken as cut-off value for differentiating cases from controls and Group 2 from Group 3 respectively.

RESULTS

The age and sex distribution of all the subjects included in the study are shown in Table 1. The mean

serum β2-m levels in the Group 1 (control) was 1.173 ± 0.059, in Group 2 (potentially malignant lesions/conditions) was 1.688 ± 0.137 and in Group 3 (oral squamous cell carcinoma) was 2.835 ± 0.313 [Table 2].

A progressive increase in the serum β2-m levels was observed in patients with both OSMF and OSCC [Table 3].

The increase in serum β2-m values of OSMF, oral leukoplakia, and oral squamous cell carcinoma was found to be highly significant ($P < 0.001$) in comparison with healthy controls [Table 4].

In ROC analysis, a value of 1.36 and 2.095 were taken as the cut-off value for differentiating cases from controls and Group 3 from Group 2 respectively. After analysis following values were seen:

Cut-off for differentiating case from control-1.28 (100% sensitive and 99.94% specific).

Cut-off for differentiating group 2A from control-1.28 (100% sensitive and 99.94% specific).

Cut-off for differentiating group 2B from control-1.28 (100% sensitive and 99.94% specific).

Cut-off for differentiating group 3 from control-1.28 (100% sensitive and 99.94% specific).

Cut-off for differentiating group 3 from group 2 (2A+2B)-1.875 (100% sensitive and 99.94% specific).

Due to having similar values of β2-m, no cut-off value was derived for differentiating groups 2A from 2B.

DISCUSSION

Oral cancer is one of the most common neoplasm of all human malignancies and OSCC constitutes approximately 90% of oral cancer.[9] Multistep theory of carcinogenesis describes that, oral cancer develops from premalignant mucosal lesions to invasive malignant changes, which causes serial histological and clinical changes.[10] Common premalignant lesions present clinically as leukoplakia or erythroplakia, but these lesions may have histologically diverse manifestations such as hyperkeratosis, dysplasia or even carcinoma. While OSMF is a well-established precancerous condition and has been proposed that it may be an intermediary stage in malignant transformation.[2,3]

In this study, total 48 subjects (32 subjects and 16 healthy controls) were selected and divided in three groups as group 1, group 2 (2A and 2B) and group 3.

Table 1: Distribution of age and sex

Group	Male		Female	
	n	Age (years) mean ± SD	*n*	Age (years) mean ± SD
1 (control)	10	42.50±11.15	6	42.00±10.70
2A (oral submucous fibrosis)	8	30.50±3.16	–	–
2B (oral leukoplakia)	8	52.75±7.46	–	–
3 (oral squamous cell carcinoma)	11	60.00±4.27	5	61.80±4.09

n: Number of subjects

Table 2: Mean serum levels of β2-m in different groups

Group	*n*	β2-m (mg/L) (Range)	β2-m (mg/L) (Mean ± SD)
1	16	1.09-1.32	1.173±0.059
2A	8	1.48-1.89	1.710±0.139
2B	8	1.40-1.86	1.668±0.140
2 (2A+2B)	16	1.40-1.89	1.688±0.137
3	16	2.30-3.30	2.835±0.313

n: number of subjects; 1: Control; 2A: Oral submucous fibrosis; 2B: Oral leukoplakia; 3: Oral squmous cell carcinoma

Table 3: A progressive increase in the serum β2-m levels in Group 2A and 3

Stage	Group 2A		Group 3	
	n	β2-m (mg/L) (Mean ± SD)	*n*	β2-m (mg/L) (Mean ± SD)
I	1	1.480	3	2.507±0.192
II	1	1.590	4	2.635±0.088
III	3	1.690±0.056	3	2.777±0.246
IV	3	1.847±0.051	6	3.162±0.154

n: Number of subjects; 2A:Oral submucous fibrosis; 3: Oral squmous cell carcinoma

Table 4: Comparison of serum β2-m between control and experimental groups

Comparison	Mean difference	*P* value
Group 1 versus 2A	0.537	<0.001*
Group 1 versus 2B	0.495	<0.001*
Group 1 versus 2	0.516	<0.001*
Group 2A versus 2B	0.042	0.975 NS
Group 2A versus 3	1.125	<0.001*
Group 2B versus 3	1.168	<0.001*
Group 2 versus 3	1.146	<0.001*
Group 1 versus 3	1.663	<0.001*

$P > 0.05$= Not significant; $P < 0.001$: Highly significant; 1: Control, 2A: Oral submucous fibrosis; 2B: Oral leukoplakia; 3: Oral squamous cell carcinoma

In group 1, total 16 subjects (10 male with a mean age of 42.50 ± 11.15 years and 6 female with a mean age of 42.00 ± 10.70 years) were selected as control.

Group 2A was consisted of eight male OSMF patients with a mean age of 30.50 ± 3.16 years, in which one patient was with stage I, one patient was with stage II and three patients were selected in each group of stage III and IV. In group 2B, eight male patients of oral leukoplakia with mild to moderate dysplasia located on the commissure, buccal mucosa, tongue, and floor of the mouth with a mean age of 52.75 ± 7.46 years were included.

Group 3 was consisted of sixteen OSCC patients (11 male with a mean age of 60.00 ± 4.27 years and 5 female with a mean age of 61.80 ± 4.09 years). In this group, 3 male patients of stage I, 3 male and 1 female with stage II, 1 male and 2 female with stage III, 4 male and 2 female patients with stage IV OSCC located on the tongue, alveolar ridges, retro molar areas, and lower lip were included. All subjects were untreated before the study.

Biological markers (biomarkers) have been defined by as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids."[11] Recently, biological tumor markers have been introduced into the clinical diagnosis of malignant lesions.

In the past few years, β2-m has received considerable attention, largely as a result of the demonstration of its close structural resemblance to certain domains of immunoglobulin G molecules[12] and of its association on cell membranes with HLA[1] serologically defined antigens. Although the biological function of this protein is still unknown,[1,2,5] a variety of malignancies have been shown to be associated with high serum concentrations of β2-m.

An elevated serum β2-m has been reported in patients with acute and chronic leukemias, non-Hodgkin's lymphoma, myeloma, and in tumors of breast, lung, colon, stomach, cervix, and uterus.[2]

The mechanism of altered β2-m level is not yet clearly understood.[1,2,5] Various suggested possibilities for increased serum level of β2-m are an increased cellular activity[1] and cell membrane turnover or cell division in malignancy.[12]

β2-m in oral premalignant lesions and malignancy was first assessed by Crispian Scully in 1981.[13] Wennerberg *et al*.[14] reported only 12% elevated β2-m levels in the study group of OSCC, whereas Vinzenz *et al*.[15] and Manzar *et al*.[16] observed significantly higher levels of β2-m in OSCC patients.

In the present study, serum β2-m levels showed a progressive increase with stage advancement in OSMF and OSCC groups. This result is in agreement with other studies conducted by Anil *et al*.,[2] Silvia *et al*.,[5] and by Vaishali and Tupkari.[1]

The result of the present study establishes serum β2-m as a specific and sensitive test for diagnostic and prognostic biomarker in squamous cell carcinoma of head and neck.

CONCLUSION

Identification of tumor biomarkers to assist early diagnosis and monitoring of disease progression may potentially decrease the mortality and morbidity associated with oral cancer.

β2-m level lacks specificity for oral carcinoma as an individual marker, because it can be elevated in other diseases also. However, the results of the present study strongly supports that estimation of β2-m level is a specific and sensitive test for diagnostic and prognostic evaluation of oral squamous cell carcinoma.

Hence, further studies are necessary to justify whether β2-m levels would be helpful in clinical diagnosis.

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