RESEARCH PAPER

Estimation of Serum Beta 2-Microglobulin Levels in Individuals Exposed to Carcinogens: Clinical Study in Indian Population

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Abstract

Aim The aim of the study is to evaluate the prognostic value of β 2-m as a biochemical parameter for the diagnosis and prognosis of oral squamous cell carcinoma (SCC).

Materials and Methods The study included 60 patients (15 oral SCC, 15 leukoplakia, 15 individuals exposed to various carcinogens and without oral cancerous or precancerous lesions, 15 healthy individuals). The levels of β 2-m were estimated using chemi-luminescent immunometric assay on Immulite fully automated machine. Statistical analysis of the data was done using Tuckey's test, ANOVA and independent sample *t* test.

Results Results showed that β 2-m was increased in individuals exposed to carcinogens without precancerous and cancerous lesion.

Conclusion Serum β 2-m can be used as a better indicator and can give an early indication of malignant change and therefore malignancy can be detected at an early and treatable stage.

Keywords Oral cancer · β 2-Microglobulin · Chemiluminescent immunometric assay · Tumor markers

Introduction

Oral cancer is the sixth most common cancer worldwide and is highly prevalent in Indian population [1]. Tobacco chewing habit is postulated to have a close co-relation with high incidence of oral precancer, cancer and a precancer is considered to hold an increased risk of cancer transformation [2]. Tumour markers are a major part of the secondary prevention (detection) efforts. Tumor markers are important for predicting the future behaviour of initiation, promotion, conversion, and progression in carcinogenesis process. Tumour markers are detected in higher than normal amounts in the serum. The alterations in the serum immunoglobulins, circulating immune complexes and complement factors have been reported earlier to show significant changes in oral cancer and precancerous conditions. Currently, the role of tumour markers in head and neck cancer has been introduced for the early detection of the lesion and is receiving increasing attention [3]. In oral carcinoma, the study of tumor markers have been limited. Several tumor markers (ferritin, nacetyl neuraminic acid, phosphohexose isomerase, CEA) with clinical promise need further evaluation. One such tumor marker is serum β 2-microglobulin (β 2-m). Crispian scully was the first to assess β 2-microglobulin as a marker in oral premalignant lesions. It was first described and isolated from the urine of patients with tubular proteinurias by Berggard and Bearn in 1968. β 2-Microglobulin is a low molecular weight, 11,600 Da 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 β 2-

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microglobulin is in the free form. It consists of a single polypeptide chain with one intrachain disulfide bridge. It does not contain carbohydrate. Increased β 2-microglobulin levels have been reported in patients with oral cancer as well, but there are only limited studies to correlate the levels of serum β 2-microglobulin with oral cancer and precancer [4]. Hence, the purpose of this study is to assess and evaluate the correlation between serum β 2-microglobulin levels in oral cancer, precancer, individuals exposed to carcinogens without precancerous or cancerous lesions and compared to healthy individuals.

Materials and Methods

The present study was conducted in the out patient department of our institute. Institutional ethical review board approval was obtained prior to the commencement of the study. Informed consent was obtained from all the individuals in the study. Inclusion criteria were individuals clinically and histopathologically diagnosed as oral squamous cell carcinoma, leukoplakia, individuals with history of exposure to various carcinogens with no oral lesions. Exclusion criteria were individuals suffering from any allergic, inflammatory or systemic diseases (renal disease, hepatobiliary disorders, systemic lupus erythematosus, acquired immunodeficiency syndrome, psoriasis, non-Hodgkins lymphoma, chronic myelogenous leukemias, lymphoproliferative malignancies, immunological disorders) and patients with any other malignancy involving sites other than the oral cavity. On the basis of inclusion and exclusion criterias, 60 individuals were divided into following four categories: Group I (15 individuals with oral squamous cell carcinoma), Group II (15 individuals with leukoplakia, Group III (15 individuals exposed to various carcinogens with no oral lesions), Group IV (15 age and gender matched healthy individuals). Under aseptic conditions, 5 ml of venous blood was collected from antecubital vein of 60 individuals included in the study. The blood was transferred to sterile, dry vacutainer containing clot activator by the laboratory and was stored at 2–8 °C and transported and analysed by chemiluminescent

immunometric assay (CLIA) on Immulite fully automated machine at Golwilkar Metropolis pathology laboratory, Pune. The present study was conducted to assess and evaluate the correlation between the levels of serum β 2-microglobulin in oral cancer, leukoplakia, individuals exposed to carcinogens with no oral lesions and healthy individuals and also to evaluate the prognostic value of serum β 2-microglobulin as a biochemical parameter for the diagnosis, prognosis of oral squamous cell. Statistical analysis of the data was done using Tuckey's test for comparison of serum β 2-microglobulin levels in the groups, ANOVA for comparing the duration of exposure to carcinogens in the groups, independent sample *t* test for comparing serum β 2-m level with respect to clinical staging and histopathological grading in oral cancer and precancer groups. Values of $p < 0.05$ were considered significant.

Results

The age distribution of all individuals included in the study is shown in Table 1. The mean age in oral cancer patients (Group I) is 53.26 ± 9.53 , in leukoplakia patients (Group II) is 47.26 ± 11.16 , in individuals exposed to various carcinogens with no oral lesions (Group III) is 47.00 ± 7.28 and in healthy controls (Group IV) is 48.22 ± 9.04 . The gender-wise distribution among the various groups is shown in Table 2. Oral cancer was most commonly found in males (73 %) compared to females (26 %) and leukoplakia was also most commonly found in males (86 %) compared to females (13 %). The duration of exposure to carcinogens in Group I, Group II and Group III is shown in Fig. 1. The mean duration of exposure to carcinogens was more in Group I individuals compared to Group II individuals and Group III individuals. By using ANOVA, this difference was statistically significant ($p < 0.001$). The distribution of site of oral cancer in Group I and site of leukoplakia in Group II is shown in Fig. 2. The most common site in oral carcinoma patients was the mandibular alveolus and sulcus (53 %) followed by buccal mucosa (33 %) and the most common site in

Table 1 Age distribution

Age group	Group			
	Group I	Group II	Group III	Group IV
31–40	2 (13.33 %)	6(40.00 %)	2 (13.33 %)	2 (13.33 %)
41–50	2 (13.33 %)	5 (33.33 %)	11 (73.33 %)	8 (53.33 %)
51–60	10 (66.66 %)	4 (26.67 %)	2(13.33 %)	4 (13.33 %)
>60	1(6.67 %)	0 (0.00 %)	0 (0.00 %)	1 (6.67 %)
Total	15	15	15	15
Mean age	53.26 ± 9.53	47.26 ± 11.16	47.00 ± 7.28	48.22 ± 9.04

Table 2 Gender distribution

Group	Gender		Total
	Male	Female	
Group I	11 (73.33 %)	4 (26.66 %)	15
Group II	13 (86.67 %)	2 (13.33 %)	15
Group III	13 (86.66 %)	2 (13.33 %)	15
Group IV	10 (66.67 %)	5 (33.33 %)	15

Fig. 1 Mean level of exposure to carcinogens in Group I, Group II and Group III **Fig. 2** Distribution of site of oral cancer and precancer in Group I and Group II

leukoplakia patients (Group II) was buccal mucosa (66 %) followed by mandibular sulcus region (33 %). The mean serum β 2-m levels in oral cancer group (Group I), leukoplakia group (Group II), individuals exposed to various carcinogens with no oral lesions (Group III) and healthy controls (Group IV) is shown in Fig. 3. The comparison of β 2-microglobulin levels between the different groups was done using Tuckey's test and this difference is highly significant ($p < 0.001$). The mean β 2-microglobulin levels with respect to clinical staging of oral cancer in Group I is shown in Table 3. The β 2-microglobulin levels were

Fig. 3 Comparison of β 2-microglobulin levels in all groups**Table 3** Comparison of β 2M level with respect to clinical staging of cancer in Group I

Stage	Number of cases	β 2M (μ g/ml, mean $\pm$ SD)	<i>p</i> value
III	9 (60 %)	2652.224 $\pm$ 411.07	0.238
IV	6 (40 %)	2937.67 $\pm$ 445.61	

increased in Stage IV oral cancer patients as compared to Stage III oral cancer patients. By using independent sample *t* test, this difference was statistically non-significant ($p > 0.05$). The mean serum β 2-microglobulin level with respect to histopathological grading in oral cancer group is shown in Table 4. The levels were increased in moderately differentiated squamous cell carcinoma as compared to well differentiated squamous cell carcinoma. By using ANOVA test, this increase was statistically non-significant ($p > 0.05$). The mean serum β 2-microglobulin level with respect to histopathological grading in leukoplakia group is shown in Table 5. The serum β 2-microglobulin level progressively increased from mild dysplasia to moderate dysplasia to severe dysplasia. By using ANOVA test, this increase was statistically highly significant ($p < 0.001$).

Discussion

Oral cancer is a disease of increasing age, 95 % of cases occur in people older than 40 years with an average age at diagnosis of around 60 years [5]. The lifetime ratio of males to females receiving an oral cancer diagnosis is 2:1, although advancing age changes that ratio to nearly 1:1. In the present study the most affected age group in oral cancer patients was between 51 and 60 years and maximum number of patients with oral cancer were males (73 %) as

Table 4 Comparison of $\beta 2$ -microglobulin level with respect to histopathological grading in oral cancer patients (Group I)

Histopathological grading	Percentage	$\beta 2\text{M}$ ($\mu\text{g/ml}$, mean $\pm$ SD)	<i>p</i> value
Well differentiated squamous cell carcinoma (n = 9)	60.00	2709.78 $\pm$ 394.48	0.581
Moderately differentiated squamous cell carcinoma (n = 6)	40.00	2851.33 $\pm$ 514.00	

Table 5 Comparison of $\beta 2\text{M}$ level with respect to histopathological grading in Leukoplakia patients (Group II)

Histopathological grading (grading of dysplasia, n = 15)	Percentage	$\beta 2\text{M}$ ($\mu\text{g/ml}$, mean $\pm$ SD)	<i>p</i> value
Hyperkeratosis with mild dysplasia (n = 5)	33.33	1800.60 $\pm$ 93.3	<0.001
Hyperkeratosis with moderate dysplasia (n = 7)	46.66	2045.00 $\pm$ 59.960	
Hyperkeratosis with severe dysplasia (n = 3)	20.00	2215.30 $\pm$ 158.00	

compared to females (26 %). These findings were consistent with literature. The incidence rate of leukoplakia was usually seen with increasing age and males were more commonly affected than females [6–9]. In the present study, the maximum number of leukoplakia patients were males (86.67 %) as compared to females (13.33 %) and most affected age group in leukoplakia patients was between 31 and 40 years [10]. The literature has reported that significant number of oral cancers and leukoplakia in smokeless tobacco users develop at the site of tobacco placement [11]. The most common site of oral cancer and leukoplakia was the buccal mucosa (63.75 %), followed by retromolar area (15 %), floor of mouth (11.25 %), lateral border of tongue (3.75 %), labial mucosa (3.75 %) and palate (2.5 %) [12]. However in the present study, oral cancer and leukoplakia was found in the mandibular alveolus and sulcus region, which was consistent with the literature. Various studies have reported that tobacco in any form contains a number of hydrocarbons i.e. several potent nitrosamines that are powerful carcinogens and these chemicals are all DNA toxic carcinogens that play a key role in the initiation and promotion of precancer and cancer which increases the risk factor (8–15 times) with a strong dose response relationship [13]. In the study, there was an observed positive dose–response relationship and this was consistent with the previous studies. The researchers have suggested that the level of serum $\beta 2$ -microglobulin increased in patients with oral cancer, leukoplakia and this increase could be due to the ability of the carcinoma cells to produce a higher concentration of $\beta 2$ -microglobulin than the non-neoplastic cells either due to active synthesis and increased cellular activity or increased cell breakdown or both [14, 15]. Consistent with the literature, in the present study there was significant increase in $\beta 2$ -microglobulin levels in individuals with oral cancer and leukoplakia. In addition to oral cancer and leukoplakia, we also observed increased level of serum $\beta 2$ -microglobulin in patients exposed to various carcinogens with no oral lesions (Group

III). There are no studies carried out to estimate the $\beta 2$ -microglobulin levels in these individuals so far, but there are studies carried out to evaluate the lymphocyte DNA damage in individuals exposed to carcinogens which suggested that tobacco can induce lymphocyte DNA damage and there is a synergistic effect of carcinogens on DNA damage [16–18]. During the course of tumor development, quantitative changes have been shown to occur in a variety of substances in serum, such as various biochemical or tumor markers. One such biochemical marker is the $\beta 2$ -microglobulin which is a protein that is present on the surface of nucleated cells, abundantly on lymphocytes and tumor cells and hence these individuals are at increased risk to develop precancerous and cancerous lesions in near future. So, this could be suggested as a possible correlation of these individuals to have raised levels of $\beta 2$ -microglobulin. In the present study, there was a progressive increase in the levels of serum $\beta 2$ -microglobulin with respect to the staging and histopathological grading of oral cancer and leukoplakia. These findings were supported by previous studies and the increased levels of $\beta 2$ -microglobulin were correlated well with increased cellular activity and tumor burden [4, 8]. This progressively increasing serum $\beta 2$ -microglobulin level positively correlates with the degree of cellular atypia suggesting that $\beta 2$ -microglobulin level can serve as biochemical tool in assessing the malignant potential of premalignant lesions [4]. $\beta 2$ -Microglobulin is released especially by malignant cell lines [19, 20]. Elevated levels of $\beta 2$ -microglobulin has been observed in a variety of patients with advanced malignancy, other disease states like renal diseases, acquired immunodeficiency syndrome, psoriasis, non-Hodgkins lymphoma, chronic myelogenous leukemias, tumors of breast, colon, stomach, cervix, uterus, inflammatory conditions and oral carcinoma [20–22]. The mechanism of increase in $\beta 2$ -microglobulin levels in malignancies is not known but various hypothesis for increased serum levels have been put forward [8, 23].

(a) β 2-Microglobulin has a rapid turnover with a fractionation catabolism of about 1.4 per hour in healthy patients and in cancer patients [2, 8]. (b) The β 2-microglobulin is a cell membrane constituent along with the HL-A chain, so an accelerated membrane turnover or accelerated cell division could increase the shedding of β 2-microglobulin [8, 23]. (c) An alternative explanation is that the changes in serum β 2-microglobulin levels might be related to the immunological phenomenon associated with oral carcinoma since there is frequently a mononuclear cell infiltrate [20]. (d) Recent studies explained this phenomenon as due to an imbalance of light chain and heavy chain of HLA-I complex and relative over-production of β 2-microglobulin [23]. (e) Most frequently quoted hypothesis for high levels of β 2-microglobulin in neoplastic diseases explains this phenomenon with mono or polyclonal activation of lymphocytes, destruction of MHC I particles, and increased cellular transformation into neoplastic cells which could lead to higher concentration of protein β 2-microglobulin. Certain studies proposed that the systemic immunosuppression observed in oral cancer patients is due to the decreased functional activity of peripheral blood monocytes which is reflected by way of decreased phagocytic process [23, 24].

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