



## “ALTERATION OF BETA 2 MICROGLOBULIN LEVEL IN ORAL SQUAMOUS CELL CARCINOMA AND POTENTIAL MALIGNANT DISORDER”

### Dental Science

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### ABSTRACT

**Background and Objective:** Tumor markers are substances, which change quantitatively in serum during tumor development. One such tumor marker is  $\beta 2$  microglobulin. It ( $\beta 2$  m) is a 11,800 kD protein of 100 amino acids associated with cell membrane of all nucleated cells as the small subunit of the MHC class I molecule.  $\beta 2$  m is present in small amounts in free form in the serum, cerebrospinal fluid and urine of normal people and to a much greater degree in patients with malignancies. The purpose of this study was to assess the serum levels of  $\beta 2$  microglobulin in oral squamous cell carcinoma and potential malignant disorder and to evaluate the possible role of  $\beta 2$  microglobulin as a biochemical parameter in the diagnosis of oral cancer.

**Method:** Serum  $\beta 2$  microglobulin levels were evaluated using ELISA in 50 patients with oral squamous cell carcinoma, 60 patients with potential malignant disorder (30 Oral Submucous Fibrosis and 30 Leukoplakia), and 50 age- & sex matched disease-free controls.

**Results:** It was observed that there was a significant increase in serum  $\beta 2$  microglobulin levels in oral squamous cell carcinoma patients as compared to potential malignant disorder and controls. Also it was found that significant increase in serum  $\beta 2$  microglobulin levels in oral squamous cell carcinoma patients as compared to Leukoplakia but not significant increase in serum  $\beta 2$  microglobulin levels in oral squamous cell carcinoma patients as compared to Oral Submucous Fibrosis. Significant increase in serum  $\beta 2$  microglobulin levels in Oral Submucous Fibrosis patients as compared to Leukoplakia. Although, serum  $\beta 2$  microglobulin levels were increased in oral leukoplakia and Oral Submucous Fibrosis compared to controls, it was found to be statistically insignificant.

**Conclusion:** From these results, it seems that evaluation of serum  $\beta 2$  microglobulin levels may be useful as one of the battery of tests in assessment of oral carcinoma and leukoplakia.

### KEYWORDS

Serum  $\beta 2$  microglobulin; Oral squamous cell carcinoma; leukoplakia; ELISA.

### INTRODUCTION:

Cancer constitutes a major health problem in developing countries, representing one of the leading causes of death. Although oral cancer represents 2–4% of the malignancies in the West, it accounts for almost 40% of all cancers in the Indian subcontinent.<sup>1</sup> Squamous cell carcinoma accounts for 92% of all malignancies of Head and Neck region.<sup>2</sup>

A significant proportion of oral squamous cell carcinomas (OSCC) develop from premalignant lesions such as leukoplakia and conditions such as oral submucous fibrosis.<sup>3</sup> Precancerous lesion is defined as “morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart”, e.g. leukoplakia, erythroplakia, actinic cheilitis etc. Precancerous condition is defined as a “generalized state associated with significantly increased risk of cancer”, e.g. Oral Submucous Fibrosis, Plummer Vinson syndrome, Lichen Planus, Xeroderma Pigmentosum, Dyskeratosis Congenita, etc.<sup>4</sup> The development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions and conditions. Leukoplakia and OSMF are the most common precancers representing 85% of such entities.

Tumor markers are substances that are produced either by the tumor itself or by the body in response to the presence of cancer or certain benign (noncancerous) conditions that can aid in the diagnosis of cancer and in the assessment of tumor burden. Tumor markers can often be detected in higher than normal amounts in the blood, urine, or body tissues of patients with certain types of cancer. Estimation of tumor marker level can be useful when used along with radiographs or other tests in the detection and diagnosis of certain types of cancer.<sup>5</sup>

In the carcinomas of oral cavity, various serum markers have been studied: these include oncofetal proteins (alpha-fetoprotein, CEA), B-

protein and enzymes (LDH) etc. In addition to the markers already studied, several tumor markers with clinical promise need further evaluation.<sup>5</sup> Two such tumor markers are beta 2 microglobulin and Sialic acid in serum.

Beta-2-microglobulin (B2M) is a protein of low molecular weight (11,800 daltons). It was first isolated from urine in patients with Wilson's disease in 1968.<sup>6</sup> It was found on the cell membrane of all nucleated cells and platelets and it forms the light chain moiety of the major histocompatibility antigens. Cell membrane turnover is the principle source of B2M in blood, plasma and body fluids.<sup>7</sup> Elevated serum levels has been found to be associated with increasing age,<sup>8</sup> relation to immune system<sup>6</sup> and a variety of malignancies and appears to be a reflection of tumor load in patients with myeloma<sup>9</sup>, bronchial carcinoma<sup>10</sup>, breast cancer<sup>11</sup>, nasopharyngeal carcinoma<sup>7</sup> and squamous cell carcinoma of the head and neck<sup>12</sup>.

The present study is an attempt to correlate the serum levels of beta 2 microglobulin in oral precancer and oral squamous cell carcinoma and to evaluate the role of the same as a biochemical parameter for screening purposes.

### MATERIAL AND METHOD:

**Patients and controls:** Serum was obtained from 50 untreated, clinically evident oral cancer patients, proved by clinical and histopathological evidence: 60 patients with potential malignant disorder (30 leukoplakia and 30 OSMF) but no evidence of invasion: and 50 age and sex matched controls.

Subjects who had taken treatment for any Precancerous lesions/ conditions or Oral cancer and Subject suffer from any systemic diseases like diabetes, cardiac diseases, renal diseases, liver diseases and other malignancies and those who are taking antioxidants/

Multivitamin preparations were excluded from the study.

Patient further divided into 3 groups Group A: 50 healthy individuals, 38 males and 12 females. Group B: 60 potential malignant disorder, 51 males and 9 females. Group B further divided into two groups, Group Ba: 30 patients with Leukoplakia and Group Bb: 30 patients having OSMF. Group C: 50 oral carcinoma patients, 40 males and 10 females.

#### METHODS OF SAMPLE COLLECTION

Ethical clearance was obtained from the institute and the hospital. All the patients fulfilling the above criteria were informed about the study being conducted and only those who agreed were enrolled in the study. All the enrolled subjects were then interviewed using clinical examination tools and recorded in a case history Performa.

After obtaining consent from the patient, 5 ml of venous blood samples were collected by venipuncture of the median cubital vein under aseptic precautions. The blood samples were allowed to clot for 1 hour and then centrifuged at 3000 rpm for 10 minutes to provide serum. This serum was preserved in a frozen state at -20 degree until the analysis.

**Beta 2 microglobulin** was estimated by using ELISA method for which Kit was obtained from DRG International, Inc., USA

#### PRINCIPLE OF THE ASSAY

The  $\beta$ -2-Microglobulin ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The concentration of  $\beta$ -2-Microglobulin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

Thus the observations obtained by these methods were tabulated and statistically analyzed By **STUDENT 't' TEST and Tukey's HSD test**.

#### RESULT:

The mean serum B2M levels in group A was  $2.57 \pm 0.10$   $\mu$ g/ml. It was elevated when compared to group C in which it was  $0.89 \pm 0.02$   $\mu$ g/ml. It is statistically significant (p value <0.0001). {Table 1, Graph 1} The mean serum B2M levels in Group B was  $2.23 \pm 0.33$   $\mu$ g/ml, it was significantly elevated when compared to Group C in which it was  $0.89 \pm 0.02$   $\mu$ g/ml. It was statistically significant (p value <0.0001). {Table 1, Graph 1} The mean serum B2M levels in Group A was  $2.57 \pm 0.10$   $\mu$ g/ml. It was significantly elevated when compared to Group B ( $2.23 \pm 0.33$   $\mu$ g/ml). It was statistically significant (<0.0001). {Table 1, Graph1}

The mean serum B2M levels in Group A was  $2.57 \pm 0.10$   $\mu$ g/ml. It was elevated when compared to Group Ba ( $1.89 \pm 0.03$   $\mu$ g/ml) which was statistically significant (p value <0.0001). {Table 2, Graph 2}

The mean serum B2M levels in group A was  $2.57 \pm 0.10$   $\mu$ g/ml. It was elevated when compared to Group Bb ( $2.56 \pm 0.04$   $\mu$ g/ml) which was not statistically significant. {Table 3, Graph 3}

The mean serum B2M levels in Group Ba was elevated ( $1.89 \pm 0.03$   $\mu$ g/ml) when compared to Group C ( $0.89 \pm 0.02$   $\mu$ g/ml) which was statistically significant (p value <0.0001). {Table 2, Graph 2}

The mean serum B2M levels in Group Bb was  $2.56 \pm 0.04$   $\mu$ g/ml. It was elevated when compared to Group C ( $0.89 \pm 0.02$   $\mu$ g/ml), which was statistically significant (p value <0.0001). {Table 3, Graph 3}

The mean serum B2M levels in Group Bb  $2.56 \pm 0.04$   $\mu$ g/ml. It was elevated when compared to Group Ba ( $1.89 \pm 0.03$   $\mu$ g/ml), which was statistically significant (p value <0.0001). {Table 4, Graph 4}

#### DISCUSSION:

Various changes occur in the body in presence of any type of cancer because the tumor cells produce certain types of chemical mediators into blood like oncofetal proteins (alpha-fetoprotein, carcinoembryonic antigen), B-protein, enzymes (e.g. Lactate Dehydrogenase), B2M and sialic acid etc. In the presence of any kind of tumor, the levels of these substances will change. In the present study, the levels of B2M and sialic acid in oral cancer and precancer patients were estimated and compared with the levels of normal healthy, deleterious habit free individuals.

The level of B2M in oral cancer patients was increased when compared with controls which was in accordance with few other studies reported in the literature.<sup>7, 12, 13-17</sup> We also found the increased level of B2M in different stages (stage II & III) of oral cancer. The mechanism of increase in B2M levels in malignancies is not known but various possible hypotheses have been put forward. The B2M is a cell membrane constituent along with the HLA chain, so an accelerated membrane turnover or accelerated cell division could increase the shedding of B2M. The ability of the carcinoma cells to produce a higher concentration of B2M than the non-neoplastic cells may be due to either active synthesis or increased cell breakdown or both.<sup>14, 15</sup> In many neoplasms, especially those of epithelial origin, a decrease or lack of expression of HLA-I particles was reported. The weakened expression of HLA-I complex on the neoplastic cells may lead to increased level of B2M in the blood serum. 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 B2M.<sup>15</sup> Most frequently quoted hypothesis for high levels of B2M in neoplastic diseases explains this phenomenon with mono or polyclonal activation of lymphocytes, destruction of MHC I (Major histocompatibility complex) particles, and increased cellular transformation into neoplastic cells which could lead to higher concentration of protein B2M. 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.<sup>18</sup> The latest studies showed that a high concentration of free B2M may have a negative influence on the immunological system by decreasing the expression of MHC-I particles and indirectly by increasing the levels of cytokines: IL-6, IL-10, which accelerate the development of neoplasms.<sup>17,19</sup>

The B2M level is also significantly increased in Oral Precancer group when compared with controls. Similar findings were also observed in other studies.<sup>12,14</sup> In the present study the level of B2M in leukoplakia patients were significantly increased when compared with controls but it differed from one study conducted in Trivandrum which shows no change in the mean value of B2M in leukoplakia.<sup>14</sup> It could be attributed to geographic variations and/or selection criteria differences. In this study we also observed that the level of B2M was slightly elevated in speckled leukoplakia as compared to homogeneous leukoplakia but it was not statistically significant; the reason behind this could be that speckled leukoplakia had more malignant potential as compared to homogenous leukoplakia.<sup>4</sup> The B2M level was also significantly increased in OSMF patients when compared with controls. Similar finding was also observed in one study reported in the literature.<sup>14</sup> The level of B2M was also marginally elevated in different OSMF stages but it was not statistically significant.

Increased B2M levels were observed in Oral Leukoplakia, which was found to be significant. The increased levels of B2M in Oral Leukoplakia patient's serum may be due to increased production or impaired excretion.<sup>14</sup> The reason for increased level of B2M in OSMF is unknown but it has been suggested that cell mediated and humoral immune responses have been reported to be altered in patients with OSMF and OSMF also has high rate of malignant transformation. It has been proposed that OSMF may be an intermediary stage in malignant transformation.<sup>14</sup> This can be the plausible explanation for the reason behind the increased level of B2M in OSMF patient.

In present study the level of B2M in oral cancer patient was significantly increased when compared with leukoplakia patients which is comparable to a study reported in the literature.<sup>20</sup> The B2M level was not significantly increased in oral cancer patients when compared with OSMF patients. Similar finding was also found in a study reported in the literature.<sup>14</sup>

In present study, the level of B2M in OSMF patients were significantly increased when compared with leukoplakia patients which was comparable to a study reported in the literature.<sup>14</sup>

#### CONCLUSION:

Identification of reliable biological tumor markers or substance associated with neoplasia that can be used for the detection, staging and evaluation has been the goal of many investigators. On the basis of present study we concluded that the levels of  $\beta$  2 microglobulin was increased in oral cancer and precancer, however further comprehensive studies involving large sample size are required to

confirm the clinical usefulness of serum  $\beta$  2 microglobulin as a biochemical parameter.

**TABLE: 1 BETA 2 MICROGLOBULIN LEVELS IN STUDY AND CONTROLS GROUPS**

PARAMETERS	GROUP	N	Mean $\pm$ SD	p-Value	Result
B2M (mg/ml)	Group C	50	0.89 $\pm$ 0.02	<0.0001	Significant
	Group A	50	2.57 $\pm$ 0.11		
	Group B	60	2.23 $\pm$ 0.34		

**TABLE: 2 BETA 2 MICROGLOBULIN LEVELS IN GROUP A, GROUP C AND LEUKOPLAKIA**

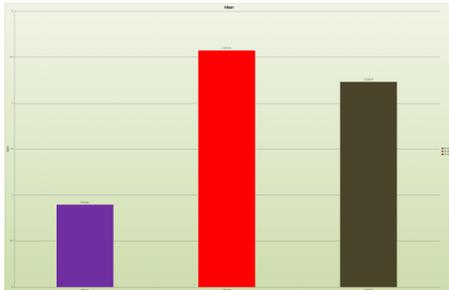
PARAMETERS	GROUP	N	Mean $\pm$ SD	p-Value	Result
B2M (mg/ml)	Group C	50	0.89 $\pm$ 0.02	<0.0001	Significant
	Group A	50	2.57 $\pm$ 0.11		
	LEUKOPLAKIA	30	1.89 $\pm$ 0.03		

**TABLE: 3 BETA 2 MICROGLOBULIN LEVELS IN GROUP A, GROUP C AND OSMF**

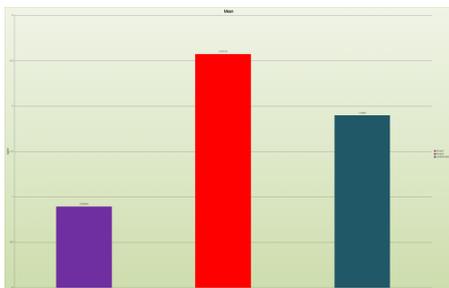
PARAMETERS	GROUP	N	Mean $\pm$ SD	p-Value	Result
B2M (mg/ml)	Group C	50	0.89 $\pm$ 0.02	<0.0001	Significant
	Group A	50	2.57 $\pm$ 0.11		
	OSMF	30	2.57 $\pm$ 0.04		

**TABLE: 4 BETA 2 MICROGLOBULIN LEVELS IN OSMF AND LEUKOPLAKIA**

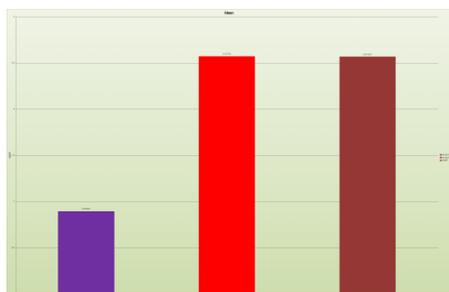
PARAMETERS	GROUP	Mean $\pm$ SD	n	p-Value	Result
B2M (mg/ml)	OSMF	2.57 $\pm$ 0.04	30	<0.0001	Significant
	LEUKOPLAKIA	1.89 $\pm$ 0.03	30		



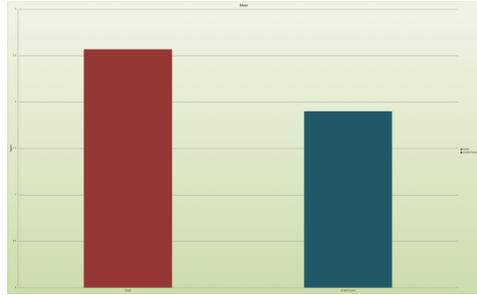
**GRAPH: 1 MEAN VALUE OF BETA 2 MICRIGLOBULIN IN STUDY AND CONTROLS GROUPS**



**GRAPH: 2 MEAN VALUE OF BETA 2 MICROGLOBULIN IN GROUP A, GROUP C AND LEUKOPLAKIA**



**GRAPH: 3 MEAN VALUE OF BETA 2 MICROGLOBULIN IN GROUP A, GROUP C AND OSMF**



**GRAPH: 4 MEAN VALUE OF BETA 2 MICROGLOBULIN IN OSMF AND LEUKOPLAKIA**

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