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Estimation and Comparison of Serum Beta 2 Microglobulin Level Among Patients with Oral Leukoplakia, Oral Submucous Fibrosis, Oral Squamous Cell Carcinoma and Controls – An Observational Study

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Conflicts of Interest: Nil

Abstract

Background: The second most common cause of death in India is oral cancer, which develops from potentially malignant disorders like oral leukoplakia and oral submucous fibrosis. There is a need for early detection of potentially malignant disorders and tumor marker serum beta 2 microglobulin looks promising for oral leukoplakia.

Aim: The study was conducted with the aim to estimate the serum β_2 Microglobulin levels among patients with oral leukoplakia, oral submucous fibrosis, oral squamous cell carcinoma and compare with controls.

Materials and Method: A total of 100 subjects with 25 in four groups were selected. Group I comprised of clinically and histopathologically confirmed cases of oral leukoplakia. Group II comprised of clinically diagnosed cases of oral submucous fibrosis. Group III is clinically and histopathologically diagnosed cases of oral squamous cell carcinoma and group IV is control group. Serum β_2M levels were measured by chemiluminescent method using Immulite 1000 (Siemens). Kruskall-Wallis one way ANOVA was done to compare among 4 groups and Mann-Whitney U test was used to compare between groups. The p-value of less than 0.001 was considered statistically significant.

Results and conclusion: Mean serum β_2M level in oral leukoplakia group is 2597 ± 148.6 ng/mL, oral submucous fibrosis group is 2187.68 ± 678.6 ng/mL, oral squamous cell carcinoma group is 3166.04 ± 357.7 ng/mL and control is 1542.60 ± 337.7 ng/mL. Statistically significant increase in mean serum β_2M level was observed in oral leukoplakia and oral squamous carcinoma patients compared to controls. However, increase in serum β_2M levels was not statistically significant in oral submucous fibrosis. The present study confirmed that serum β_2M can be used as a promising tumor marker for oral leukoplakia and oral squamous cell carcinoma, as other condition causing elevation of serum beta 2 microglobulin were excluded.

Introduction

Oral cancer (OC) is the eleventh most common cancer worldwide. Annually over 300,000 new cases of oral cancer are diagnosed all over the world and makes an important public health concern. Highest incidence and mortality rates are reported in India and Sri Lanka. An increasing number of young people are being affected and almost 25% of the cases have no associated risk factors. Among all cancers in India, oral cancer is the commonest and 4 in 10 are oral cancer. Annually 130,000 people succumb to oral cancer in India which translates into approximately 14 deaths per hour. The reason for high prevalence of OC in India is primarily attributed to tobacco consumption in the form of gutkha, quid, snuff or misri.[1]

Oral leukoplakia (OL) is the most common potentially malignant disorder (PMD) of the oral cavity.[2,3] Estimates of the global prevalence of oral leukoplakia range from 0.5% to 3.46%, and the rate of carcinomatous transformation ranges from 0.7% to 2.9%.[4] It is more prevalent in India due to tobacco and areca nut chewing habit. Although 90% is due to tobacco use, remaining 10% of oral leukoplakia is idiopathic.[5] The reported prevalence of epithelial dysplasia (ED) in oral leukoplakia ranges from 5% to 25%.[6]

Oral submucous fibrosis (OSMF) is a chronic, potentially malignant disorder (PMD) of the oral mucosa. Worldwide, estimate of OSMF shows a confinement to Indians and Southeast Asians. The overall prevalence rate in India is about 0.2% to 0.5 % and prevalence varies from 0.2-2.3% in males and 1.2-4.57% in females.[7] OSMF is well known for its malignant transformation rate of 7.6% and is commonly attributed to the use of areca nut in various forms.[8]

Early detection would improve the cure rate, lower the cost and morbidity associated with the treatment. Recently, there have been a number of scientific approaches for early diagnosis of potentially malignant disorder. One such tumor marker is beta 2 microglobulin (β_2M). It is a low molecular weight protein found on the membranes of all nucleated cells where it appears to be present in structural association with the histocompatibility antigen (HCA).[9]

The existing literature reveals very little information regarding the level of serum β_2M in patients with OL, OSMF and oral squamous cell carcinoma (OSCC). Hence the present study was undertaken to estimate and compare the serum levels of β_2M in patients with OL, OSMF, OSCC and controls and to predict the role of β_2M as a biochemical parameter for the diagnosis of OL, OSMF and OSCC.

The aim of the study was to estimate serum β_2M level among patients with oral leukoplakia, oral submucous fibrosis, oral squamous cell carcinoma and compare with controls.

Materials And Method

Patients reporting to the Department of Oral Medicine and Radiology at K.L.E. Vishwanath Katti Institute of Dental Sciences, Belgaum, were included in the study after obtaining an informed consent. The study was approved by the Ethical and Research Committee of K.L.E. Vishwanath Katti Institute of Dental Sciences, Belgaum, in compliance with the Helsinki Declaration. A total of 100 patients were included in the study and divided into four groups.

Group I: 25 clinically and histopathologically confirmed patients with oral leukoplakia.

Group II: 25 clinically confirmed patients with oral submucous fibrosis.

Group III: 25 clinically and histopathologically confirmed patients with oral squamous cell carcinoma.

Group IV: 25 controls.

Patients with oral leukoplakia, oral submucous fibrosis, oral squamous cell carcinoma and control subjects with no history of tobacco habits were included in the study. Patients with any other malignancy like multiple myeloma, lymphoma, leukemia and Hodgkin's disease, patients with any other systemic diseases like systemic lupus erythematosus, dilated cardiomyopathy and Bechet's syndrome, which will alter serum β_2M level and patient over 70 years of age, where serum β_2M is normally raised, were excluded from the study.

A detailed history and thorough clinical examination was recorded as per the case history proforma. 5 ml blood was collected and the serum separated by centrifugation. The serum was separated from blood and stored frozen at -20°C. Serum beta 2 microglobulin was measured chemiluminescent immuometric assay using Immulite 1000 (Siemens). Statistical analysis was done using SPSS software version 17. Kruskall-Wallis one way ANOVA was used to compare mean serum β_2M level between the four groups. Pair wise comparison of four groups with respect to serum β_2M levels was done by Mann-Whitney U test. The p-value of less than 0.001 was considered to be statistically significant.

Results and Discussion

Statistically significant difference in mean serum β_2M levels was observed among the four study groups (Table 1). Within group comparison using Mann-Whitney U test revealed statistically significant increase in mean serum beta 2 microglobulin in oral leukoplakia and oral squamous cell carcinoma when compared to controls. No statistically significant increase in serum β_2M level was observed in oral submucous fibrosis. (Table 2)

β_2M is a low molecular weight, 11800 Dalton protein found on the surface of all cells except erythrocyte. It occurs in small quantities in normal human urine, serum, plasma and cerebrospinal fluid. Elevated levels of β_2M have been observed in patients with malignancy.[10] Therefore, possibly β_2M estimation can be incorporated in a battery of tests for diagnosis and prognosis in oral cancers as an adjunct to other tumor markers.[11]

In the present study, 25 patients of OL were included in the age range of 27-68 years with 32% of them in their fifth decade. The mean age was 51.40 ± 12.05 years. A male preponderance was seen in OL in the ratio of 3:1. All the patients had a habit of using tobacco either in the form of smokeless tobacco or as bidis and cigarettes. In the present study, the site distribution of OL revealed 72% are present in buccal mucosa followed by buccal sulcus (24%), which is consistent with the study done by Warnakulasuriya S, Johnson NW. In the present study, 44% of OL had mild dysplasia, 24% had moderate dysplasia, 28% had hyperkeratosis and 4% had severe dysplasia and it is consistent with the study done by Warnakulasuriya S, Johnson NW.[12]

In the present study, the serum β_2M level of group I was 2597 ± 148.62 ng/mL, which is slightly higher and consistent with the study done by Diwan NN et al. There was statistically significant increase in serum β_2M level between group I and IV.[13] However, this is not consistent with the previous studies by Anil et al.(1995)[9] and Vaishali N, Tupkari (2005)[14]. Progressive increase in serum β_2M level was also observed from hyperkeratosis to severe dysplasia in group I patients which was similar to study done by Diwan NN et al (Graph 1).

In the present study, 25 patients of OSMF subjects were included in the age range of 19-50 years. The mean age in our study was 31.16 ± 8.102 years. Most of the patients were in the second and third decade of life with 52% in less than 30 years and 40% in the age group of 30- 40 years. The higher prevalence of OSMF patients in younger age group is explained by popularity of refined areca nut products, which are readily available and starting of betel

nut chewing habit at an early age. All the subjects in group II were males.

In the present study, the serum β_2M level group II was 2187 ± 678.61.62 ng/mL, which was within the normal reference range. There was no statistically significant increase in serum β_2M level between group II and IV. This is not consistent with the previous studies by Anil et al.(1995)[9] and Vaishali N, Tupkari (2005)[14]. According to present study, serum β_2M cannot be used as a TM for OSMF.

In the present study, 25 subjects with OSCC were included in the study out of which 21 (84%) were male subjects and 4 (16%) were female subjects. The age of the subjects ranged from 35 to 68 years with the mean age of 49.44 ± 8.491 years. 36% of the subjects were above 50 years of age which shows that the incidence of OSCC is more in elderly subjects.

In the present study, the site of occurrence of carcinoma was highest in buccal mucosa (40 %), followed by alveolus (24%) and tongue (16%). This is consistent with the study conducted by R Sankaranarayanan 1990. [15]

In the present study, 52% of the subjects had well differentiated OSCC, 28% had poorly differentiated OSCC, 12% had early invasive OSCC and 2% had poorly differentiated OSCC. Progressive increase in serum β_2M level was observed from well differentiated OSCC to poorly differentiated OSCC (Graph 2). This is consistent with study by Vaishali N and Tupkari JV (2005)[13] and Anand Pratap Singh et al.[16]

The increase in serum β_2M level in OL and OSCC patients is a true phenomenon as other non neoplastic conditions that increase serum β_2M level were excluded from the study. The mechanism of increase in serum β_2M level was not known but various possible hypothesis has been put forward. As β_2M is a cell membrane constituent along with HLA chain, an accelerated membrane turnover or accelerated cell division could increase the shedding of β_2M. The ability of carcinoma cells to produce a higher concentration of β_2M may be due to either active synthesis or increased cell breakdown or both.

The present study confirms that serum β_2M levels are significantly increased in patients with OL and OSCC, though there was no statistically significant increase in serum β_2M level in OSMF patients. In overall assessment of serum β_2M level, it has been found that serum β_2M can be used as a reliable TM for OL and OSCC. There is a paucity of studies in regard to the evaluation of serum β_2M level in PMD and OSCC. Hence more studies have to be carried out with increased sample size.

Conclusion

The present study confirms that serum β_2M levels are significantly increased in patients with OL and OSCC, though there was no statistically significant increase in serum β_2M level in OSMF patients. Hence serum β2M can be used as a reliable TM for OL and OSCC patients. Further clinical research with larger sample size is required as there is paucity of data regarding the serum β2M level in PMD and OSCC.

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Table 1: Comparison of mean serum β2M values among study groups using Kruskall-Wallis ANOVA.

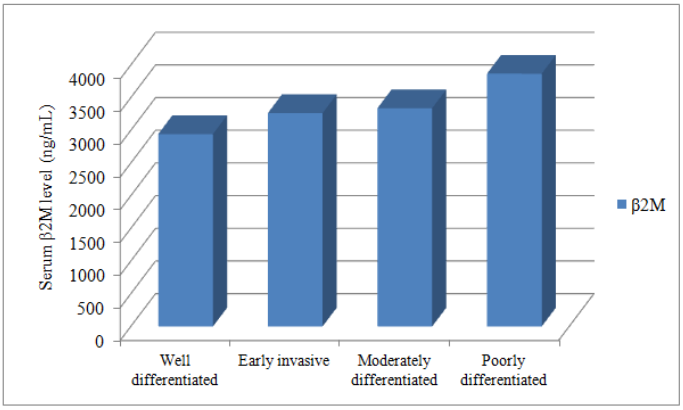
Groups	n	Mean β2M values (ng/mL)	Std. Deviation	Chi-square value	p-value
Group I	25	2597.00	148.620	65.896	<0.001*
Group II	25	2187.68	678.610		
Group III	25	3166.04	357.766		
Group IV	25	1542.60	337.740		

* Statistically significant

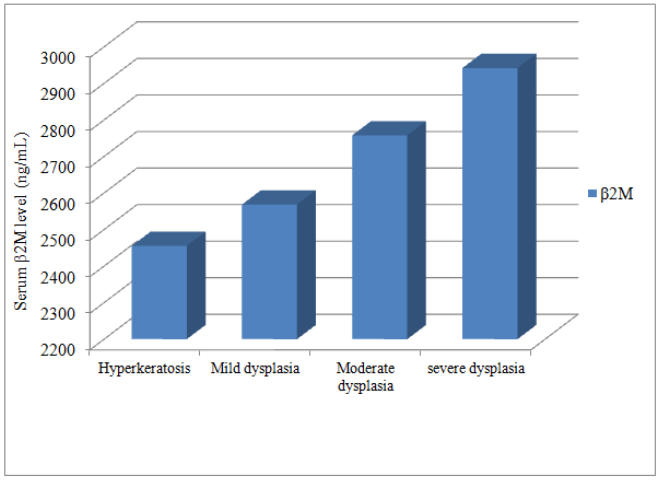
Table 2: Group wise comparison of β2M levels using Mann-Whitney U test.

Groups	n	Mean ranks	Sum of ranks	U-value	p-value
Group I vs Group IV	25	13.00	950.00	1.000	<0.001*
	25	38.00	326.00		
Group II vs Group IV	25	19.04	476.00	151.000	0.002
		31.96	799.00		
Group III vs Group IV	25	13.04	326.00	1.000	<0.001*
		37.96	949.00		
Group I vs Group II	25	21.02	525.50	200.500	.030
		29.98	749.50		
Group I vs Group III	25	14.32	358.00	33.000	<0.001*
		36.68	917.00		
Group II vs Group III	25	15.48	387.00	62.000	<0.001*
		35.52	888.00		

*Statistically significant.



Graph 1: Histopathological grading in oral leukoplakia (group I) and mean serum β2 levels



Graph 2: Histopathological grading of oral squamous cell carcinoma (group III) and mean serum β2M levels.