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ESTIMATION OF SERUM β2 MICROGLOBULIN IN ORAL CANCER AND POTENTIALLY MALIGNANT LESIONS OF ORAL CAVITY BY CHEMILUMINESCENCE IMMUNE ASSAY

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ABSTRACT

Introduction: Oral cancer is highly prevalent in Indian population. It accounts for approximately 30-40% of all cancers in India, tumor markers have been introduced for screening purpose, diagnosis, prognosis. **Aim**: To find out the correlation of the serum level of β2 microglobulin in patients with oral cancer and potentially malignant lesions of oral cavity and to evaluate its potential as a biomarker in early detection and prognosis. **Materialsand methods**: It was a case control study. The study comprised of three group of 60 subjects.Group I consisted of 20 subjects with clinically and histopathologicallydiagnosed cases of Oral cancer. Group II consisted of 20 subjects withoral potentially malignant lesion (PML) cases and group III consisted of control group with 20 healthy subjects without any deleterious habits. "Serum β2 microglobulin" was estimated using chemical luminiscenceimmuno assay kit. Biochemical parameter was compared between study group and control group. **Results:** There was increase in level of serum β2 microglobulin from stage I: 2072.5±316.0(ng/mL) to stage IV (3624.5±1591.7ng/ml.) of oral cancer and average serum β2 microglobulin level differed significantly between stage I and stage IV in oral cancer group. ($p<0.05$). Also the average serum β2 microglobulindiffered significantly across Moderately Differentiated and Well Differentiated groups (P-value<0.05). **Conclusion**: Study revealed that serum β2 microglobulinwas seen increased in oral cancer and a positive correlation of serum β2 microglobulin with the disease progression was noted. Therefore Serum β2 microglobulin can be used as a prognosticmarker for oral cancer.

KEYWORDS: biomarker, chemiluminiscenceimmunoassay, oral cancer, potential malignant lesions, serum β2 microglobulin.

INTRODUCTION

Cancer is not a new disease, it has been affecting the life since its evolved but interest in cancer has grown during the past century as infectious diseases have increasingly been controlled.Oral cancer is highly prevalent in Indian population. It accounts for approximately 30- 40% of all cancer in India in contrast to 3-5% incidence of oral cancer from other western countries, primary associated with various habits.[1]

Recently, there have been a number of scientific approaches to the problem of precancerous lesions, with aim to establish fundamental biochemical basis of understanding. The goal of such methods is to find a reliable indicator of the biological potential of precancerous and cancerous lesions. The quench for the early detection of the lesion has made it very important for the dental professionals to maintain high levels of diagnostic methods to assure the diagnosis.[2] Oral SCC can arise from pre-existing potentially malignant disorders including oral leukoplakia, erythroplakia, submucous fibrosis and lichenoid dysplastic lesions, or can arise de novo.[3]

Early diagnosis and treatment are the goals. Since the COE has undetermined sensitivity and specificity .There is a need for more accurate diagnostic tools that can detect early lesions and determine either the potentially malignant or the benign nature of lesions.Currently available and developing tools apart from biopsy and histopathological examination and Vital staining are Biomarkers, DNA ploidy(chromosomal polysomy), Brush biopsy, optical techniques.[2,4]

Various markers have been studied in oral cancer these include oncofoetal protein, (a- fetoprotein: CEA), other proteins B-Protein, β-2 microglobulin), and enzymes (LDH). One such marker is cell surface HLA associated

marker, β2microglobulin. CrispianScully (1980) was the first to assess the potential use of β2 microglobulin as a marker in oral premalignant lesions.[5,6]

Till now very few studies have been done regarding the of β2 microglobulin in human Oral SCC. So this study has been carried out to evaluate the role of β2 microglobulin as a biochemical parameter in oralpotentially malignant lesions and oral cancer.

MATERIALS AND METHODS

Based on inclusion and exclusion criteria 20 clinically &histopathologically diagnosed oral cancer patients and 20 patients with Oral potentially malignant lesions and 20 healthy patients without any tobacco habit were included in the study after obtaining permission from SAC and IEC committees. Patients who have undergone any treatment for oral cancer or potentially malignant lesions were excluded from the study. All the participants were explained the need and design of the study and the need for undergoing a thorough clinical examination, biopsy and blood investigations at the start of the study. Only those patients, who gave a signed informed consent on an institutionally approved document, participated in the study.

After taking thorough clinical history and confirmation of diagnosis by histopathological report, blood was collected from both oral cancer and PML group of patients,and healthy age and sex matched controls . About 4 ml venous blood was drawn from midcubitial / antecubital vein, under aseptic conditions. The blood sample was then collected in a plain test tube with a separator gel and allowed to clot completely to avoid hemolized, icyeric and lipemic specimenas presence of fibrin may erroneous result and then centrifuged for 15 minutes at 1000 rpm at 37^0C to get the serum separated. This serum sample was then used to measure the serum β2 Microglobulin by using commercial BMG kit.

METHOD OF MEASURING SERUM BETA2-MICROGLOBULIN

Measurement of serum β2 Microglobulin was done using chemiluminescence immune assay (CLIA).

Principle of CLIA method

CLIA is a method in which concentration of samples is determined according to the intensity of the luminescence that the chemical reaction emits.

The β2 Microglobulin CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labelled with horseradish peroxidase is used as the tracer. The β2 M molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody- enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by adding the substrate reagents and undergoing the chemiluminescent reactions. The intensity of the emitting light from the associated well is proportional to the amount of enzyme present and is directly related to the amount of β2 M antigen in the sample. By reference to a series of β2 M standards assayed in the same way, the concentration of β2 M in the unknown sample is quantified.

Method of CLIA

At first test samples (Serum: quantity required 5 µL) (serum diluted) was added into black opaque microplates which were coated with antibodies. Serum was prediluted by adding diluents supplied in kit. Then specific antibody conjugated with horseradish peroxidase is added, and then, the un-reacted ingredients are washed away. Finally,chemiluminescent substrate was added into the microwells, and then relative luminosity values (RLU) was scanned by photon counter reader.

RESULTS

The study included 60 subjects, 20 oral cancer patients, 20 PML patients and 20 patients in control group. All the three groups of patients were thoroughly examined clinically and blood samples were collected and samples were then sent to the laboratory for measuring quantitatively serum β2 Microglobulin level. Serum β2microglobulin estimation was done by using CLIA method.

Stastistical analysis: The intergroup comparison of serum β2 microglobulin across three study groups was done using one way analysis of variance (ANOVA) with Post-Hoc Bonferroni's. The comparison of serum β2 microglobulin across various stages of oral cancer was performed using one –way analysis of variance (ANOVA) with Post-hoc bonferroni's correction for multiple group comparison. The comparison of serum β2 microglobulin in histopathological variants of Oral cancer group was done using independent sample 't' test. The intergroup comparison for distribution of cases studied according to histopathological diagnosis between three study groups was done using Chi-square test.

In the present study the mean age of the Oral cancer group was 54.7 ± 13.2 with maximum of 76 and minimum of 32yrs of age and maximum patients were above the age of 45yrs (Table1 ,Graph 1) and the mean age among PML group was found to be 45.3 ± 13.3, with the maximum age of 65 and minimum age of 25 yr. Mean ±SD age range of patients in control group was found to be 44.8± 6.8 yrs .It was found that maximum patients (70%) were above 40 yrs. In our study out of 20 Oral cancer patients 15 were males (75%) and 5 were females (25%) (Table 2, Graph 2).In PML group out of 20 patients, 80% were males(16) and 20% were females(4) patients.The control group consisted of 15 males (75%) and 5 females (25%) patients.Number of males weremore than females in this study. The average age of cases studied did not differ significantly and the

gender distribution too did not differ significantly across three study groups (P-value=0.999>0.05 for all).
In oral cancer group 40% showed involvement of alveolar ridge and in PML group 80% showed involvement of buccal mucosa.In this study we found most common site to be buccalmucosa and alveolus was found as second most common site. The distribution of site involved differed across oral cancer and PML groups (P-value=0.005<0.01) and it was statistically significant (Table 3 & Graph 3).

The mean values of Serum β2 microglobulin in Cancer group was2580.9 ± 773.4 (ng/mL). In PML group mean values of Serum β2 microglobulinis2231.0 ± 509.9 (ng/mL). In control group mean value of serum β2 microglobulin was 2235.9 ± 623.7 (ng/mL). When Oral cancer group was compared with control itwas not statistically significant (p- value= 0.288>0.05).When Oral cancer group compared with PML group it was not statistically significant (p-value =0.275 >0.05).When PML group compared with control group it was not statistically significant (p-value =0.999>0.05). The average serum β2 microglobulin level did not differ significantly across three study groups.(p value >0.05). (Table 4 & Graph 4)

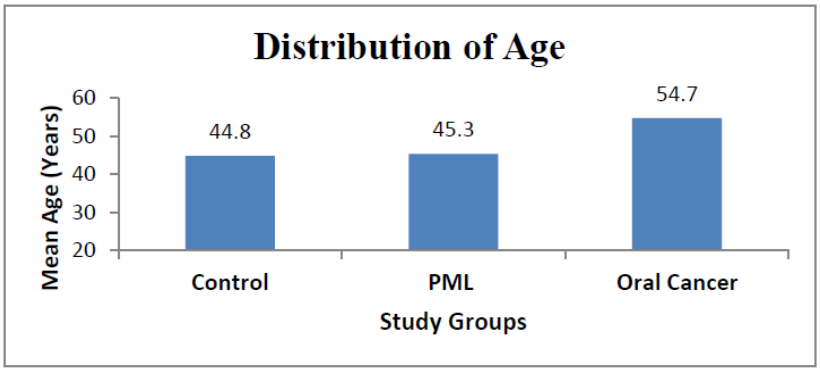
In the distribution of biochemical parameters between different stages of oral cancer, mean serum β2-Microglobulin in different stages of oral cancer were Stage I: 2072.5±316.0 (ng/mL), Stage II: 2143.3 ± 330.4, Stage III: 2920.3 ± 607.9, Stage IV: 3624.5±1591.7. When stage I compared with stage II it was not statistically significant. (p-value= 0.999 >0.05). When stage I was compared with stage III it was not statistically significant (p-value= 0.122>0.05). When stage I was compared with stage IV it was statically significant (p-value : 0.040<0.05). When stage II compared with stage III it was not significant (p-value= 0.323>0.05). When stage II compared withstage IV it was not statistically significant (p- value= 0.076>0.05). When stage III compared with stage IV it was not statistically significant (p-value=0.980>0.05). The average serumβ2 microglobulin differs significantly between Stage I and Stage IV oral cancer (P-value<0.05). The average serum β2 Microglobulin levels did not differ significantly across four study groups (P-value >0.05 for all). (Table 5 & Graph 5) In the comparison of serum microglobulin in Oral Cancer group according to Histopathological diagnosis, mean serum β2 microglobulin in moderately differentiated carcinoma was 2884.9 ± 796.1 (ng/mL). Mean serum β2microglobulinin well differentiated carcinoma group was 2016.6 ± 234.0 (ng/mL). When moderately differentiated carcinoma group was compared with well differentiated carcinoma it was statistically significant (P-value=0.012 <0.05). The average serum β2 microglobulindiffer significantly across moderately differentiated and well differentiated groups (P-value<0.05). (Table 6 & Graph 6)

Tables & Graphs

Table 1: The age distribution of the cases studied acrossthree study groups.

Group	Mean Age (Years)	SD Age (Years)
Control (n=20)	44.8	6.8
PML (n=20)	45.3	13.3
Oral Cancer (n=20)	54.7	13.2
Inter Group Comparisons		
Control v/s PML	0.999 (NS)	
Control v/s Oral Cancer	0.025 (S)	
PML v/s Oral Cancer	0.038 (S)	

Inter Group comparison is performed using one-way analysis of variance (ANOVA) with Post- Hoc Bonferroni's correction for multiple group comparisons, after confirming the underlying normality assumption. P-value<0.05 is considered to be statistically significant. S: Statistically Significant, NS: Statistically Non-Significant.

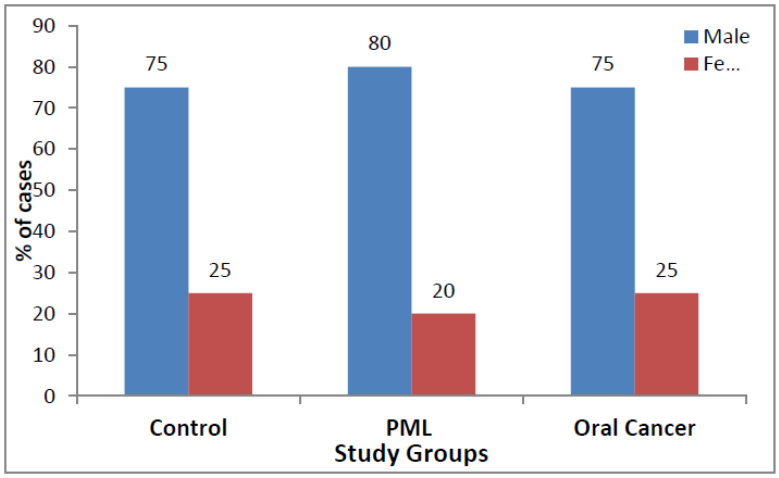


Graph 1: The age distribution of the cases studied acrossthree study groups.

Table 2 :The sex distribution of the cases studied across three study groups.

Group	Male	Female
Control (n=20)	15 (75.0)	5 (25.0)
PML (n=20)	16 (80.0)	4 (20.0)
Oral Cancer (n=20)	15 (75.0)	5 (25.0)
Inter Group Comparisons		
Control v/s PML	0.999 (NS)	
Control v/s Oral Cancer	0.999 (NS)	
PML v/s Oral Cancer	0.999 (NS)	

Values are n (% of cases). Inter Group comparison is performed using Chi-square test. P- value<0.05 is considered to be statistically significant. S: Statistically Significant, NS: Statistically Non-Significant.

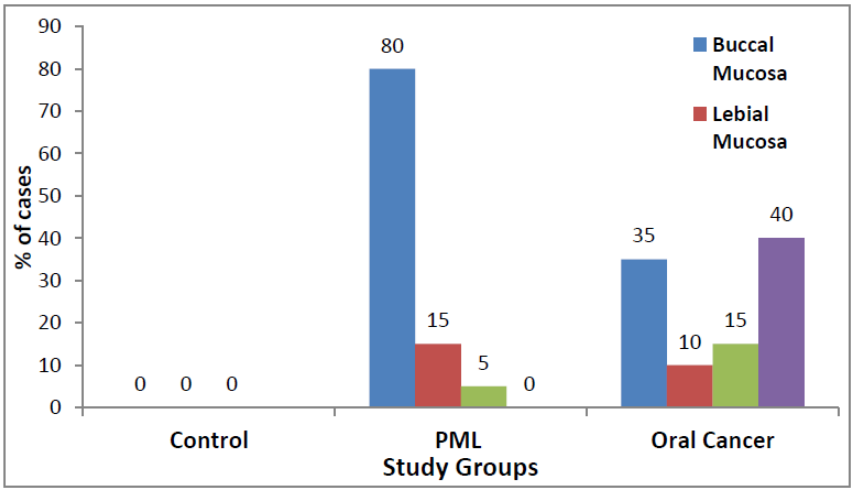


Graph2: The sex distribution of the cases studied across three study groups.

Table 3: The distribution of site involved across three study groups.

	Site Involved			
Group	**Buccal Mucosa**	**Lebial Mucosa**	**Tongue**	**Alvelous**
Control (n=20)	0	0	0	0
PML (n=20)	16 (80.0)	3 (15.0)	1 (5.0)	0
Oral Cancer (n=20)	7 (35.0)	2 (10.0)	3 (15.0)	8 (40.0)
Inter Group Comparisons				
PML v/s Cancer	0.005 (S)			

Values are n (% of cases). Inter Group comparison is performed using Chi-square test. P- value<0.05 is considered to be statistically significant. NS: Statistically Non-Significant.

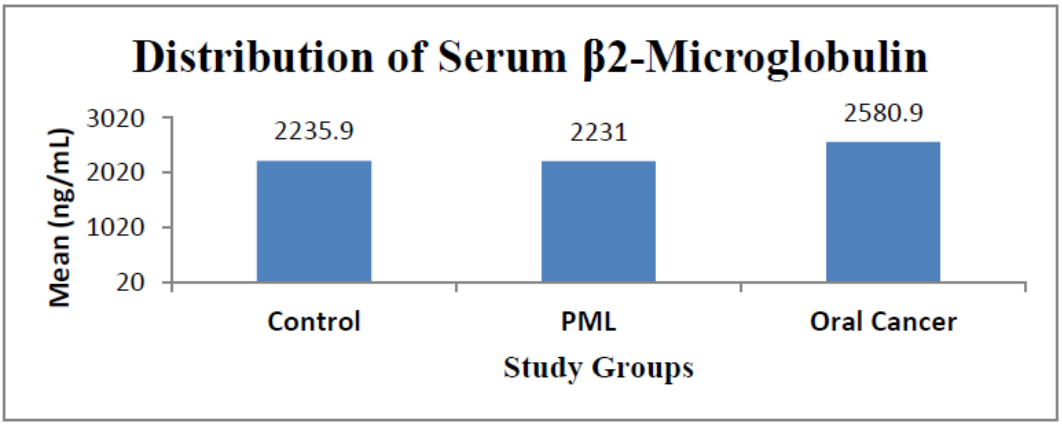


Graph3: The distribution of site involved across three study groups.

Table 4.The comparison of serum β2-microglobulin across three study groups.

Study Group	Serumβ2 Microglobulin (ng/mL)	
	Mean (ng/mL)	SD (ng/mL)
Control (n=20)	2235.9	623.7
PML (n=20)	2231.0	509.9
Oral Cancer (n=20)	2580.9	773.4
Inter Group Comparisons		
Control v/s PML	0.999 (NS)	
Control v/s Cancer	0.288 (NS)	
PML v/s Cancer	0.275 (NS)	

Inter Group comparison was performed using one-way analysis of variance (ANOVA) with Post- Hoc Bonferroni's correction for multiple group comparisons, after confirming the underlying normality assumption. P-value<0.05 was considered to be statistically significant. S: Statistically Significant.

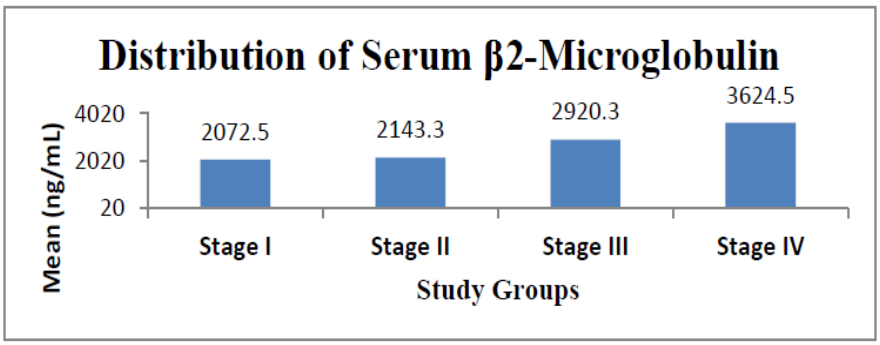


Graph 4: The comparison of serum β2-microglobulin across three study groups.

Table 5: The comparison of serum β2-microglobulin across various clinical staging in OC group.

Stages	Serumβ2 Microglobulin (ng/mL)	
	Mean (ng/mL)	SD (ng/mL)
Stage I (n=6)	2072.5	316.0
Stage II (n=4)	2143.3	330.4
Stage III (n=8)	2920.3	607.9
Stage IV (n=2)	3624.5	1591.7
Inter Group Comparisons		
Stage I v/s Stage II	0.999 (NS)	
Stage I v/s Stage III	0.122 (NS)	
Stage I v/s Stage IV	0.040 (S)	
Stage II v/s Stage III	0.323 (NS)	
Stage II v/s Stage IV	0.076 (NS)	
Stage III v/s Stage IV	0.980 (NS)	

Inter Group comparison is performed using one-way analysis of variance (ANOVA) with Post- Hoc Bonferroni's correction for multiple group comparisons, after confirming the underlying normality assumption. P-value<0.05 is considered to be statistically significant. S: Statistically Significant, NS: Statistically Non-Significant.

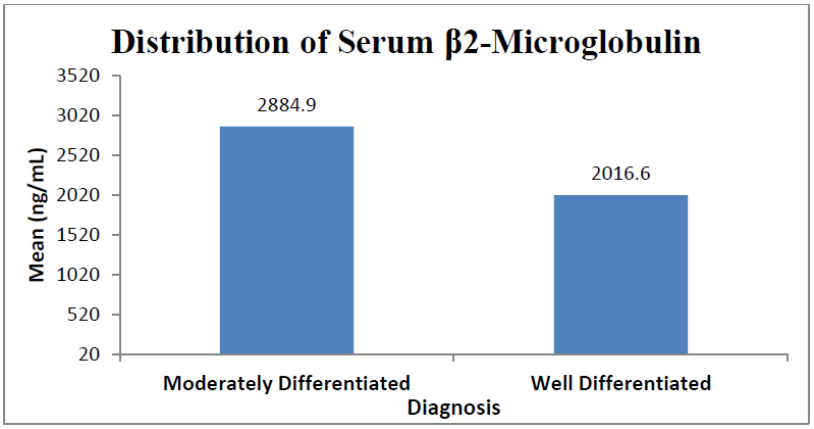


Graph 5:The comparison of serumβ2-microglobulin across various clinical staging in OC group.

Table 6: The comparison of serumβ2microglobulin in OC group according to Histopathological diagnosis.

Diagnosis	Serumβ2 Microglobulin (ng/mL)	
	Mean (ng/mL)	SD (ng/mL)
Moderately Differentiated (n=13)	2884.9	796.1
Well Differentiated (n=7)	2016.6	234.0
Inter Group Comparisons		
Moderately v/s Well Differentiated	0.012 (S)	

Inter Group comparison is performed using independent sample 't' test, after confirming the underlying normality assumption. P-value<0.05 is considered to be statistically significant. S: Statistically Significant.



Graph 6: The comparison of serum β2-microglobulin in OC group according to Histopathological diagnosis.

DISCUSSION

Oral cancer has always been a challenge to medical sciences. The global burden of Oral cancer continues to increase because of aging and growth of world population and also due to increased trends in adoption of Oral cancer causing habits. Oral cancer usually arises from pre- existing lesions called precancerous lesions. According World Health Organization (WHO) Workshop, 2005,The term ''potentially malignant" was preferred above ''premalignant" or ''precancerous".[8] Majority of oral cancerare usually preceded by any of potentially malignant lesions.Leukoplakia accounts for about 0.2-5.2% in oral cavity in Indian population[7] and OSMF has a prevalence of 0.2-0.5%.[8,9] In recent era Biomarkers are in trends for earlier detection of Oral cancer and potentially malignant lesions to prevent them to develop into malignancy.

One such marker is epithelial cell surface antigen associated with Human leukocyte antigen, β2 microglobulin. This study was carried out to find correlation of serum β2 microglobulin in Oral cancer and potentially malignant lesions of oral cavity (OSMF and Leukoplakia) and to evaluate serum β2 Microglobulin as a biochemical marker for early detection of oral cancer.

In the present study the mean age of the Oral cancer group was 54.7 ± 13.2 with maximum of 76 and minimum of 32yrs of age and maximum patients were above the age of 45yrs**.**The mean age among PML group was found to be 45.3 ± 13.3, with the maximum age of 65 and minimum age of 25 yr. It was found that maximum patients (70%) were above 40 yrs. The age difference between Oral cancer and PML group was statistically significant with (P<0.05).The age difference between Oral cancer and control was also statistically significant (P<0.05). The observations regarding the age in the groups suggests that most cases of Oral cancer and PML were seen above 45 yrs. This could be attributed to the increased duration of Oral cancer related habits thus

increased exposure to the carcinogens. Most of them from the study group showed habits ranging from 5 years to 25 years duration. Also in our study males were more than females. All reported a higher incidence among males compared to females associated with PML lesions this could be co-related to prevalent habit of tobacco chewing and smoking in the males .The sex distribution did not differ significantly between three groups(P>0.05)

In this study we found most common site of involvement to be buccal mucosa and alveolus was found as second most common site. .In this study the site distribution differs significantly across PML and Oral cancer(P<0.01).

In this study mean serum β2 microglobulin was 2235.9 ±623.7 (ng/ml) in the control group.The mean value of Serum β2 microglobulin in Oral cancer group was 2580.9 ± 773.4 (ng/mL). We found increase in serum β2 M in Oral cancer group compared to control, this was also reported by Scully C[5], Kadam C et al[12] and Singh AP et al.[13] Difference in levels between Oral cancer and control group was not statistically significant.In PML group mean serum β2 M was 2231.0 ± 509.9 (ng/mL), which was almost similar to the levels of control group and was not statistically significant .similar findings were reported by Anil S et al[14] and Wilma C R et al.[15] The average serum β2 microglobulin did not differ significantly across three study groups(p>0.05). The rise in levels found in oral cancer may be either due to increased cell turn over, orescape from cell recognition phenomenon, or could be due to disturbance in the cell surface.[16] More significant results have been reported by various studies and predictive results in other type of malignancies.

In this study we found 13(65%) patients with well differentiated carcinoma and 7(35%) patients with moderately differentiated carcinoma. The similar finding were reported by Khandekar SP et al,[17] Krishna A et al.[18] This could be due to lack of symptoms early in disease leading to late diagnosis.In contrast to this Sherin N et al.[19] reported most patients with moderately differentiated carcinoma in their study .When moderately differentiated carcinoma group was compared with well differentiated carcinoma it was statistically significant (P-value =0.012 <0.05). The average serum β2 microglobulin differ significantly across moderately differentiated and well differentiated groups (P-value<0.05). (Table 6 & Graph 6)We found increase in serum β2 M elevated with increase in clinical staging and also found significant increasefrom well differentiated carcinoma tomoderately differentiated carcinoma. .so it can be considered as progression marker.

CONCLUSION

However, as serum β2 M lacks specificity for oral carcinoma as an individual marker because it is elevated in other diseases also. Hence further studies with larger samples may be necessary to find out whether serum β2 microglobulin could be used as a biomarker in early detection and diagnosis of oral cancer.

This study showed no significantly elevated serum β2 microglobulin level in potentially malignant lesions so we can conclude its not a specific marker for potentially malignant disorder of oral cavity. But as per few authors there was significant increase in serum β2microglobulin, this variation could be due to methods which was used for evaluation of serum β2 microglobulin and CLIA method was used in this study and very few studies have been done with this method and CLIA is considered sensitive.[20] Compared to other methods .so further studies need to be carried out with larger samples and CLIA method, to standardize the levels of serum β2 microglobulin. We also found increase in serum β2 M elevated with increase in clinical staging and also found significant increasefrom well differentiated carcinoma to moderately differentiated carcinoma, so we can use it as a progression marker for Oral cancer and can be useful for planning management of oral cancer.

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