Can Serum Beta 2 Microglobulin be a Potential Biomarker for Oral Squamous Cell Carcinoma and Potentially Malignant Lesions - A Diagnostic Perspective

Sana Fatema [1], Hanan Ghazi Shanab [2], Farheena Ustad [2], Ghufran Alayfan [2] , Hoda Lotfy Abouzeid [3] , Abdulrhman Mohammed Alyousef [4] , Noura Alobaid [5] , Somaiah AlSaab [5] , Anand Marya [6] , Rithvitou Horn [7] , Shaeesta Khaleelahmed Bhavikatti,[8,*]

[1] M A Rangoonwala Dental College, Pune, India.
[2] Department of Maxillofacial Surgery and Diagnostic Sciences, College of Dentistry, Majmaah University. Al-Majmaah University, 11952,Saudi Arabia.
[3] Lecturer of Prosthetic dentistry, Department of Prosthetic Dentistry ,College of Dentistry, King Khalid University, Abha, Saudi Arabia.
[4] BDS, Majmaah University, Majmaah, Saudi Arabia.
[5] Department of Clinical Dental Sciences, College of Dentistry, Majmaah University. Al-Majmaah University, 11952,Saudi Arabia.
[6] Department of Orthodontics, Faculty of Dentistry, University of Puthisastra, Phnom Penh, Cambodia
[7] Faculty of Dentistry, University of Puthisastra, Phnom Penh, Cambodia.
[8] Department of Periodontics, School of Dental Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia.

Corresponding and reprints:
Dr. Shaeesta Khaleelahmed Bhavikatti
B.D.S., M.D.S. (Perio), (PhD Scholar)
Department of Periodontics,
School of Dental Sciences,
Universiti Sains Malaysia, Health Campus,
Kubang Kerian, Kelantan,
Malaysia.
Email: drshaeesta@gmail.com

Abstract

Early detection can help to reduce mutilating defects and the rising fatality rate in oral squamous cell carcinoma (OSCC). Tumor biomarkers are being studied extensively to aid in diagnosis, screening, prognosis, and treatment response monitoring. This study focuses on the role of beta 2 microglobulin (β2 M), a tumour biomarker found in OSCC and premalignant lesions such as leukoplakia and oral submucous fibrosis (OSMF). Serum was collected from 20 patients with OSCC (Group I), 10

patients with leukoplakia and 10 patients with OSMF leukoplakia (Group II), and 20 healthy controls (Group III). The IMMULITE 1000 kit was used to determine the β2 M levels. The β 2 M levels increased with the degree of differentiation of OSCC (p<0.001) and with the stages (<p0.05) and grades of dysplasia (p<0.001) in premalignant lesions. The average serum β2 M levels, however, did not differ significantly between the three study groups (p >0.05). Serum β2 M levels were higher in advanced OSCC than in premalignant lesions and controls. A comparison of the distribution of age, gender, and habits, as well as the sites involved, was also made. β2M can be used as a prognostic marker to track the treatment of patients who progress to advanced stages of OSCC.

Keywords: Beta 2 microglobulin, tumour biomarker, oral carcinoma, premalignant, serum
Tob Regul Sci.™ 2021;7(6-1): 7436-7448
DOI: doi.org/10.18001/TRS.7.6.1.65

1.Introduction

Oral cancer accounts for 2% of the cancer burden worldwide [1]. For both men and women, the highest percentage of oral cancer are reported in Melanesia, Central and Eastern Europe and South-Central Asia, while the lowest percentage are noticed in Eastern Asia, Africa, and Central America [2]. In developed nations, cancer is a leading cause of death, while in underdeveloped nations, it is the second major cause of death. In India, oral cancer is responsible for roughly 30-40% of all cancers. This is attributed to prevalent addictive oral habits such as tobacco chewing habits and poor nutritional status responsible for the high incidence of oral precancer and cancer [1].

Oral squamous cell carcinoma (OSCC) can develop as a result of pre-existing precancerous conditions such as oral erythroplakia, leukoplakia, lichenoid dysplastic lesions and submucous fibrosis or it can develop spontaneously [3]. Leukoplakia is the most common potentially malignant oral lesion, with a prevalence of 0.6%to 4.6% according to the epidemiological data and it is also possible that it may turn malignant, with transformation rates ranging from 3-33% over ten years. [4]. With most of the case finding studies in South and South East Asia, OSMF has a prevalence of 0.1 to 30% in Indian population [5].

The term "epithelial precursor lesions" is used in the most recent WHO monograph on Head and Neck Tumours (2005) [6]. The current study's authors were not in favour of subdividing precancer into conditions and lesions, and the general consensus was to refer to all clinical manifestations that convey a risk of cancer as "potentially malignant disorders" to represent their wide - spread anatomical allocation. In this study, leukoplakia and Oral Submucous Fibrosis (OSMF) were included in this category [7].

The ability of oral malignant lesions to progress to a malignant state is largely determined by histopathologic examination. However, this is subjective, and difficult for detecting predictors for carcinogenic processes, such as well-defined stages of onset, advancement, transformation, and progression. The biological markers related with each stage in the oncogenic process can be used to investigate the specific events that happen at each step [7]. Presently available and establishing tools for the same apart from biopsy, vital staining and histopathological examination are biomarkers, DNA ploidy (chromosomal polysomy), Brush biopsy and optical techniques [8]. Over the last ten years, scientists have carried out extensive research on these occurrences to explore diagnostic, biological and prognostic characteristics [9].

Tumor markers have a wide range of applications, including screening, diagnosis, prognosis, and monitoring therapeutic efficacy. As a result, the study of tumour markers has become critical in oncology research. Human 2 M is a protein with a low molecular weight (MW 11600) that consists of a single polypeptide chain of 99 amino acids. It is the small chain of the HLA-A, -B, and -C major histocompatibility complex antigens. 2 M is found on the surface of human lymphocytes in various biological fluids such as serum, urine, cerebrospinal fluid, saliva, and so on [10]. Serum 2 M levels have been found to be elevated in a significant proportion of patients suffering from malignant

conditions and diseases such as acute and chronic leukemias, myeloma, Non-Hodgkin's lymphoma and tumours of the colon, breast, lung, cervix, stomach, and uterus [11,12].
Plasma biomarkers have been shown to have a significant potential for helping in the early diagnosis and prevention of oral cancer growth or recurrence [13]. β2 M has gained prominence in recent years, owing primarily to the discovery of its close structural similarity toward certain domains of immunoglobulin G (IgG) molecules and its relation on cellular membrane with HLA immunologically specified antigens [1]. There is a paucity of literature on β2 M as a potential biomarker in human OSCC. So, this research was carried out to evaluate the role of biochemical parameter such as β2 M in potentially malignant oral lesions including leukoplakia and OSMF and in OSCC.

2. Materials and methods

2.1 Screening and Patient selection

Of the 70 patients screened for the study, the samples were collected consecutively as per the number and age matched and diseased patients that is patients with Potentially Malignant Oral Lesions and Oral Cancer who came to the department of Oral Medicine and Radiology , MA. Rangoon Wala College of Dental Sciences and Research Institute. The present study included 60 patients, of which 6 were excluded owing to ongoing treatment to potentially malignant lesions and 4 dues to history of cardiovascular disease. The study protocol was clearly explained to all patients. The research was carried out in strict compliance with the ethical standards outlined in the World Medical Association Declaration of Helsinki, Version VII, 2013. MA Rangoon Wala Dental College and Research Pune Centre's self-governing ethics committee provided approval for the study; the college is registered under the Maharashtra University of Health Sciences before commencement. Following verbal consent, each patient was asked to sign a written consent form.

2.2 Study protocol and criteria for patient selection

The patients were grouped into 3 categories as illustrated in the study protocol in Figure 1.

Inclusion criteria for the study were: 1) Patients who were clinically and histopathologically diagnosed as potentially malignant oral lesions and Oral Squamous Cell Carcinoma (OSCC) including leukoplakia and oral submucous fibrosis(OSMF). The healthy subjects included did not have any habits such as Tobacco(chewing/smoking) and alcohol or any lesions that could be responsible for the etiology of OSMF or leukoplakia.

Exclusion criteria were: 1) patients who have undergone any treatment for OSCC or the potentially malignant lesions; 2) patients with i) lymphatic malignancy(like lymphoma, multiple myeloma, leukemia), ii) lymphoproliferative disease (Sjogren's syndrome) and other autoimmune disorder such as Systemic Lupus Erythomatosus, iii) any renal diseases and iv) Cardiovascular diseases or hepatobiliary disease.

2.3 Data collection

The initial appointment consisted of collecting the demographic data as per the case history proforma. A biopsy was performed and was sent for histopathologic examination to the Department of Oral Pathology. Clinical staging of OSCC patients was done using the TNM classification provided by the American Joint Committee on Cancer (AJCC) for Organising Atlas (2006) [14]. Staging of leukoplakia was undertaken as described by Van der Waal and Schepman KP et al(2000) for the definitive histopathological diagnosis [15]. Staging of OSMF was done based on the clinical features as suggested by Nagesh and Bailoor (2005) [16].
After confirmation of diagnosis by histopathological report, venous blood was taken and then this sample was used to measure the serum β2 M.

2.4 Biopsy procedure

Patients in Group II and Group III were subjected for biopsy procedure. The patients were explained about the surgical procedure and they were asked to sign the informed consent form prior to the procedure. Toluidine blue test was done to select the biopsy site. It is commonly available for use as a three-component system, along with a kit.

The patient was instructed to wash his or her mouth with water two times (20 seconds each). After washing with water, the patient was instructed to wash with 1% acetic acid (20 seconds). Gauze was used to gently dry the suspected mucosal regions. While drying, care has been taken not to damage the tissue. A cotton swab was used to apply a 1 percent toluidine blue solution to the lesion. The patient was instructed to wash with acetic acid once more (approximately 150 ml for 1 minute). The patient was instructed to wash with water after washing with acetic acid. If the mucosa was stained positively, the procedure was repeated after 1-2 weeks. Under local anaesthesia, a biopsy of the sites that stained positive on two consecutive visits was performed and later the specimen was subjected for histopathological examination. Excised site was later sutured with 3-0 BBS suture material. Post-surgical instructions were given. Patients were later recalled after 8 days for suture removal.

2.5 Collection of serum samples

After taking thorough clinical history and confirmation of diagnosis by histopathological report, blood was collected from both OSCC and PML group of patients. A tourniquet was applied midway between the elbow and shoulder. About 4 ml venous blood was drawn from midcubitial / antecubital vein, with the help of a 5 ml sterile disposable syringe and a 22-gauge needle 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 specimen as presence of fibrin may erroneous result and then centrifuged for 15 minutes at 1000 rpm at 370C to get the serum separated. The sample were collected with the similar method as that of diseased Patients from age and sex matched healthy individuals.

2.6 Method of measuring serum Beta 2- Microglobulin (β 2 M)

Measurement of serum β2 M was done using chemiluminescence immunometric assay (CLIA) (IMMUlLITE 1000 β 2 microglobulin kit). CLIA is a method in which, the concentration of samples is determined according to the intensity of the luminescence that the chemical reaction emits. The 2 M CLIA analysis is a two-site solid step immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells, and the detector is used on another monoclonal antibody labelled with horseradish peroxidase. The two antibodies "sandwich" the 2 M molecules present in the standard solution or serum. Washing removes the unbound antibody-enzyme labels after the coated antibody-antigen-antibody-enzyme complex is formed.

The activity of horseradish peroxidase obligated in the wells is then measured by adding substrate reactants and performing chemiluminescent reactions. The intensity of the light emitted by the associated well is relative to the quantity of enzyme present and is directly proportional to the amount of β2 M antigen in the sample. The concentration of β2 M in the unknown sample is quantified by referencing a series of β2 M standards assayed in the same manner.

At first, test samples (Serum: quantity required 5 L) (dilution of serum) were incorporated to black opaque microplates covered with antibodies. The serum was pre-diluted using the kit's diluents. The unreacted ingredients are then washed away, followed by the addition of a specific antibody conjugated with horseradish peroxidase. Finally, chemiluminescent substrate was decided to add to the microwells, and the photon counter reader was used to scan the relative luminosity values (RLU).

2.7 Statistical analysis

The age distribution of cases studied across three study groups was analysed by one-way analysis of variance (ANOVA) with Post-hoc Bonferroni's correction. The comparison of gender distribution, the intergroup comparison for

distribution of sites involved, the habits studied and the intergroup comparison for distribution according to histopathological diagnosis between the three study groups was analysed by using Chi-square test. The intergroup comparing of serum β2 M across three study groups and the comparison of serum β2M across various stages of oral cancer was done by using one-way ANOVA with Post-hoc Bonferroni's correction for comparison of multiple group. The independent sample 't' test was used to compare serum 2M in histopathological variants of the OSCC group. For statistical significance, P<0.05 was used. The entire set of data was statistically analysed with the statistical package for social sciences (SPSS, version 11.5) for Microsoft Windows.

3. Results

3.1 Distribution of age, gender, sites involved, and habits studied across the study groups

(Table 1)

The average age of studied cases differed between OSCC and PML groups (p<0.05) which was significant statistically. The average age of cases studied differed between OSCC and control group (p <0.05) and it was statistically significant. The average age of the studied cases did not significantly differ (p >0.05) between the PML and Control groups.

The gender distribution did not significantly differ across three study groups (p>0.05)

The distribution of site involved differed across OSCC group and PML groups (p<0.01) and it was statistically significant. Significantly higher proportion of PML cases had buccal mucosa site involvement.

Tobacco habit differed across PML and control group (p<0.01); it also differed between oral cancer and control group (p<0.01) and oral cancer and PML groups (p <0.01) and it was found to be statistically significant across all three groups.

Gutka habit differed between PML and Control group(p<0.01), it also differed between OSCC group and PML group (p< 0.01) and it was statistically significant. The gutka habit did not significantly differ between OSCC group and control group (p>0.05).

Alcohol habit differed between PML and control group (p<0.01); it also differed between control group and OSCC group(p<0.01) and it was found to be statistically significant. Alcohol habit did not differ significantly between PML and OSCC group (p>0.05) (Table 4)

3.2 The distribution of studied cases according to clinical staging between three study groups

In the OSCC group, 2 patients were in stage IV(10%) ,8 patients were in stage III (40 %), in stage II, 4 patients (20%) and in stage I, 6 patients(30%). In the PML group, of 10 patients of OSMF, Grade I have 4 patients (20%), Grade II have 4 patients (20%) and grade III OSMF have 2 patients (10%). Out of 10 patients of leukoplakia, 8 patients (40%) were in Stage 3 and 2 patients (10%) were in stage 4 (Table 2).

3.3 Distribution of studied cases according to histopathological diagnosis between three study groups

In the OSCC group, 65% of patients revealed squamous cell carcinoma which was moderately differentiated and 35% exhibited squamous cell carcinoma that was well differentiated . In the PML group, 35% of patients exhibited mild dysplasia, 15% with moderate dysplasia and 50% had OSMF. In the control group no lesion was seen in any of the volunteers. The distribution of histopathological diagnosis differed between OSCC and PML group (p = 0.001) and it was statistically significant (Table 3).

3.4 The distribution of studied biochemical parameters between the study groups

Despite the fact that the mean of serum β2 M values in the OSCC group were significantly high, the average serum β2 M levels did not significantly differ between the three study groups (p >0.05) (Figure 2).

3.5 The distribution of serum β2 M across various clinical staging and histopathological diagnosis in OSCC group (Table 4)

When stage I was evaluated to compare with stage II, there was no statistically significant difference ($p>0.05$). When stage I was evaluated to compare with stage III, no statistically significant different was found ($p>0.05$).When stage I was evaluated to compare with stage IV, there was statistically significant ($p<0.05$).When stage II was evaluated to compare with stage III no significant difference was seen ($p>0.05$). When stage II was evaluated to compare with stage IV, they were not statistically significant ($p>0.05$). When stage III was evaluated to compare with stage IV, they were not statistically significant ($p>0.05$). The average serum β2 M differed significantly between Stage I and Stage IV OSCC ($p<0.05$). The average serum β2 M levels did not differ significantly across four groups ($p>0.05$).

When moderately differentiated carcinoma group was compared with well differentiated carcinoma, a statistically significant difference was seen ($p <0.05$).

The average serum β2 M differed significantly across moderately differentiated and well differentiated groups ($p<0.05$).

4. Discussion

Oral cancer is one of the most prevalent disease, with approximately 95% of neoplasms happening after 45 years of age [6]. The patient's age in the present study ranged from 25- 76 years and the OSCC patients had a higher mean age (54.7±13.2 years), which is coherent with the previous report [6].

In the current study, number of males were more than females. Similarly, many authors have reported the male preponderance [17,18]. In the PML group , 80% were males and 20% were females' patients. This was in agreement with previous studies reported by Bancozy J et al [19]. The higher incidence among males compared to females associated with PML lesions could be co-related to the prevalent tobacco chewing habits and smoking among the males. However, the gender distribution did not differ significantly between three groups. In this study we found most common site to be alveolus (40%), this agree with the study by Khandekar SP et al [20]. In our study, the site distribution differed significantly across PML and OSCC and significantly high proportion of PML cases had buccal mucosal involvement. This study revealed site specific relationship in areas where tobacco quid is kept in lower gingivobuccal sulcus, causing cancer of buccal mucosa, and also involving alveolus of associated site.

Tobacco, smoking (both cigarettes and e-cigarettes) and smokeless, and alcohol are all known causes of oral cancer. The current study found that 95% of the participants were habitués, which is like Murthy NS et al's findings [21]. All the 20 patients in the PML group (100%) were habitués. Tobacco (either Smokeless form or smoking, Gutka) was strongly associated with PML, which was in accordance with studies of Napier SS et al [22]. All reported potentially malignant lesions were associated with habits. The tobacco habit differed significantly across the three study groups ($p<0.01$). It is evident that tobacco ,gutka and alcohol play a major role in causation of PML and OSCC.

In OSCC group, maximum patients were seen in stage III, which was in accordance with study by Sankarnarayan R [23] .This could be attributed to the lack of awareness and concern in the Indian population. Further, it was found that, most patients (65%) exhibited well differentiated carcinoma and 35% patients exhibited moderately differentiated carcinoma, consistent with studies of Khandekar SP et al [20].This could be due to lack of symptoms early in disease leading to a delayed diagnosis. In contrast to this Sherin N et al [24] reported most patients with moderately differentiated carcinoma in their study .

β2M protein is an invariant part of human leukocyte antigen (MHC I molecule) found on cell surface of all nucleated cells except erythrocyte [25]. As a shedding product of cellular turnover, it can be found in small amounts in regular human urine, serum, and cerebrospinal fluid [26]. 99% of β2M is reabsorbed and metabolized by the kidneys and excreted in urine in small amount under normal physiological conditions. It ranges from 670- 2143ng/ml in serum in adults and about 300 ng/ml in urine under normal physiological condition. In this study β2 M was

estimated by chemiluminescence immune assay method which is more sensitive than enzyme linked immune assay and radioimmunoassay(RIA) [27] methods and it was evaluated as in ng/ml units.

The present study reports a rise in serum β2 M in the OSCC group when compared to the control group, which is also reported by many previous studies [8,12]. The variation in levels between both the OSCC and the control group, however, was not statistically significant. In PML group, the mean serum β2 M was almost similar to the levels of control group and was not statistically significant, similar to the findings of Wilma C R et al [28]. It is important to note that, the average serum β2 M did not differ significantly across three study groups. The rise in levels found in oral cancer may be either due to increased cell turn over or escape from cell recognition phenomenon [29] or could be due to disturbance in the cell surface [30]. In contrast to this, significant results have been reported by various studies and predictive results in other type of malignancies [31].

From stage I to stage IV, there had been a steady rise in serum β2 M in the OSCC group, indicating a significant increase in levels as the disease progressed ($p<0.05$). Wilma CR et al [28] and Vaishali N et al [10] also found a positive correlation of increased level of β2 M with advancing stage of OSCC. Further, it was also found that, patients who were grouped under well differentiated carcinoma exhibited lower serum β2 M range in comparison to the moderately differentiated carcinoma. This further emphasizes that, as the disease progresses, due to accelerated cell membrane turn over or accelerated cell division and with increased tumor burden, there is a considerable rise in serum β2 M levels. These findings are consistent with those reported by Vaishali N et al [10]. This can be attributed to the fact that β2 M, like HLA-A, is a cell membrane subset, and thus accelerated membrane turnover or expedited cell division might rise serum β2 M shedding [28].

This study showed no significantly elevated serum β2M level in potentially malignant lesions. Therefore, the authors of the present study suggest that, contrary to the other studies reported in literature, β2M is not a specific biomarker for potentially malignant disorders. The significant increase in serum β2 M as reported in other studies could be attributed to the methods which was used for evaluation of serum β2M. CLIA method was used in this study and very few studies have been done with this method and CLIA is considered a sensitive method [31] compared to other methods. Since there are very few studies utilizing the CLIA method and the present study reports insignificant results pertaining to β2M levels, further studies need to be carried out with larger samples of population. However, we also found increase in serum β2 M elevated with increase in clinical staging and also found significant increase from well differentiated carcinoma to moderately differentiated carcinoma. Therefore, it can be used as a progression marker for the progression of OSCC and can thus be useful as a prognostic biomarker and for planning the management of OSCC.

However, because serum 2 M appears to lack precision for oral carcinoma as an independent marker and is raised in other inflammatory disorders as well, more research with larger samples is needed to determine whether serum 2M could be utilized as a biomarker in the early diagnosis and detection of oral cancer.

Future implications

Further studies needed to study translocation of β2 M from cell surface to cytoplasm in advanced tumor that may shed light on mechanism of β2 M mediated tumorigenesis, as β2 M is found on the plasma membrane of normal oral mucosa. Also, segregation needs to be done in various stages of oral cancer and needs to be studied thoroughly with larger samples for its possible role in the immunotherapy of oral cancer.

Funding- None

Declaration

The authors state that they have no conflicting interests in the publication of this paper.

Acknowledgements - None

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Table 1: Distribution of age, gender, sites involved and habits studied across the study groups

Age				
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)			
Gender				
Group	**Male**		**Female**	
Control (n=20)	30 (75.0)		10 (25.0)	
PML (n=20)	32 (80.0)		8 (20.0)	
Oral Cancer (n=20)	30 (75.0)		10 (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)			
Site involved				
Group	**Buccal Mucosa**	**Labial Mucosa**	**Tongue**	**Alvelous**
Control (n=20)	0	0	0	0
PML (n=20)	32 (80.0)	6 (15.0)	2 (5.0)	0
Oral Cancer (n=20)	14 (35.0)	4 (10.0)	4 (15.0)	16 (40.0)

Inter Group Comparisons			
PML v/s Cancer	0.005 (S)		
Habits			
Group	**Tobacco**	**Gutka**	**Alcohol**
Control (n=20)	0	0	0
PML (n=20)	22 (55.0)	22 (55.0)	14 (35.0)
Oral Cancer (n=20)	38 (95.0)	0	20 (50.0)
Inter Group Comparisons			
Control v/s PML	0.001 (S)	0.001 (S)	0.004 (S)
Control v/s Oral Cancer	0.001 (S)	0.999 (NS)	0.001 (S)
PML v/s oral Cancer	0.003 (S)	0.001 (S)	0.523 (NS)

P-value<0.05 is considered to be statistically significant. S: Statistically Significant, NS: Statistically Non-Significant.

Table 2 :The distribution of cases studied according to the clinical staging between three study groups

Staging	Group 1 Control Group (n=40)	Group 2 PML Group (n=40)	Group 3 Oral Cancer Group (n=40)
Grade I	0	8 (20.0)	0
Grade II	0	8 (20.0)	0
Grade III	0	4 (10.0)	0
Stage 1	0	0	12 (30.0)
Stage 2	0	0	8 (20.0)
Stage 3	0	16 (40.0)	16 (40.0)
Stage 4	0	4 (10.0)	4 (10.0)
NA	4 0 (100.0)	0	0

Values are n (% of cases). NA: Not applicable.

Table 3: The distribution of cases studied according to the Histopathological diagnosis between three study groups

Diagnosis	Group 1 Control Group (n=40)	Group 2 PML Group (n=40)	Group 3 Oral Cancer Group (n=40)	P-values (Inter-Group)		
				Group 1 v/s Group 2	Group 1 v/s Group 3	Group 2 v/s Group 3
OSMF	0	20 (50.0)	0	--	--	0.001 (S)
Mild Dysplasia	0	14 (35.0)	0			
Moderate Dysplasia	0	6 (15.0)	0			
Moderately differentiated	0	0	13 (65.0)			
Well differentiated	0	0	14 (35.0)			
NA	4 0 (100.0)	0	0			

Values are n (% of cases). P-values are obtained using Chi-Square test. P-value<0.05 is considered to be statistically significant. NA: Not applicable. S: Significant.

Table 4: The distribution of serum β2 microglobulin across various clinical staging and histopathological diagnosis in OSCC group

Stages	Serumβ2 Microglobulin (ng/mL)	
	Mean (ng/mL)	SD (ng/mL)
Stage I (n=6)	2072.5	316
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)	
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
Inter Group Comparisons		
Moderately v/s Well Differentiated	0.012 (S)	

P-value<0.05 is considered to be statistically significant. S: Statistically Significant, NS: Statistically Non-Significant.

Figure 1: Flow chart for recruitment of patients

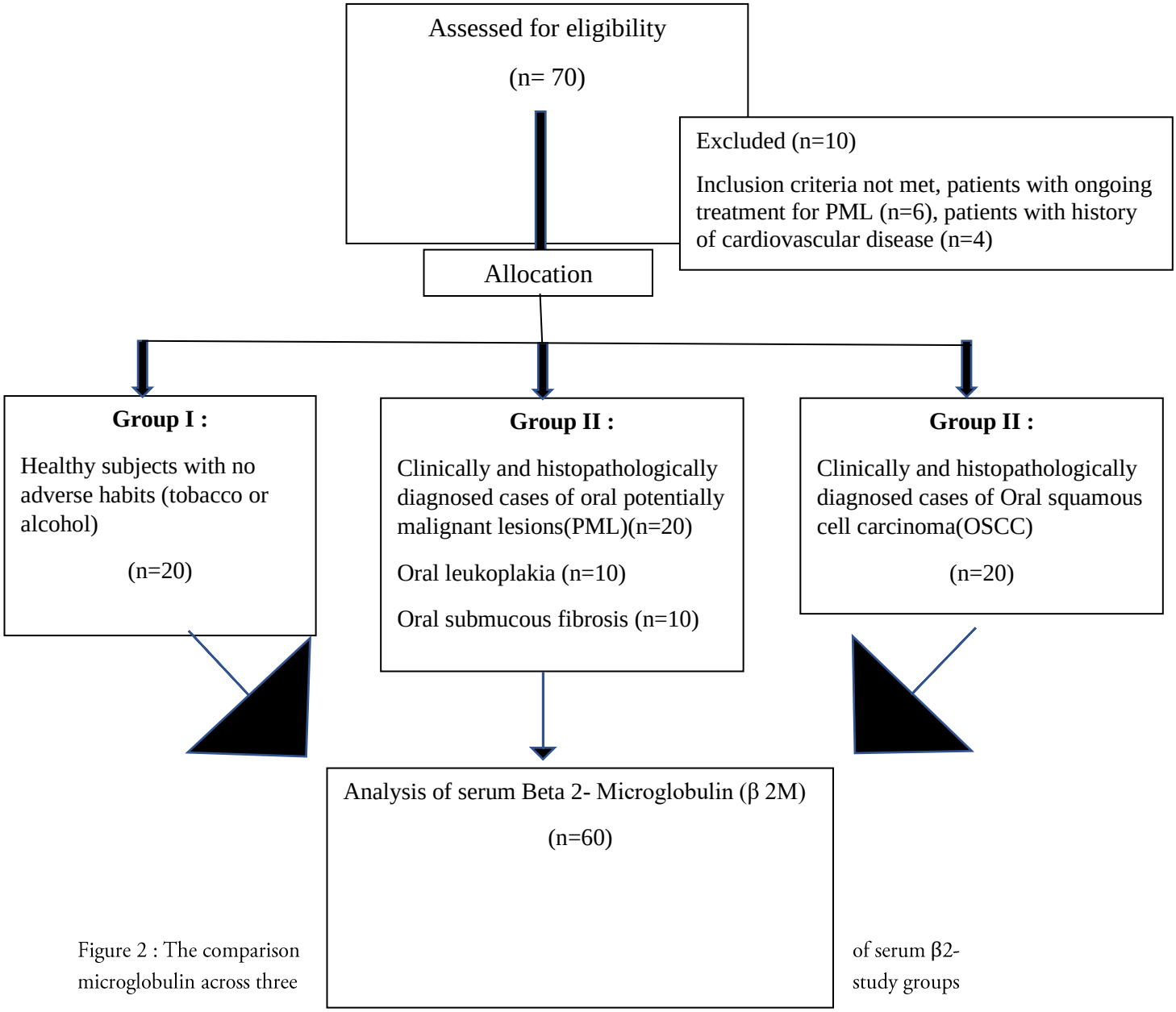
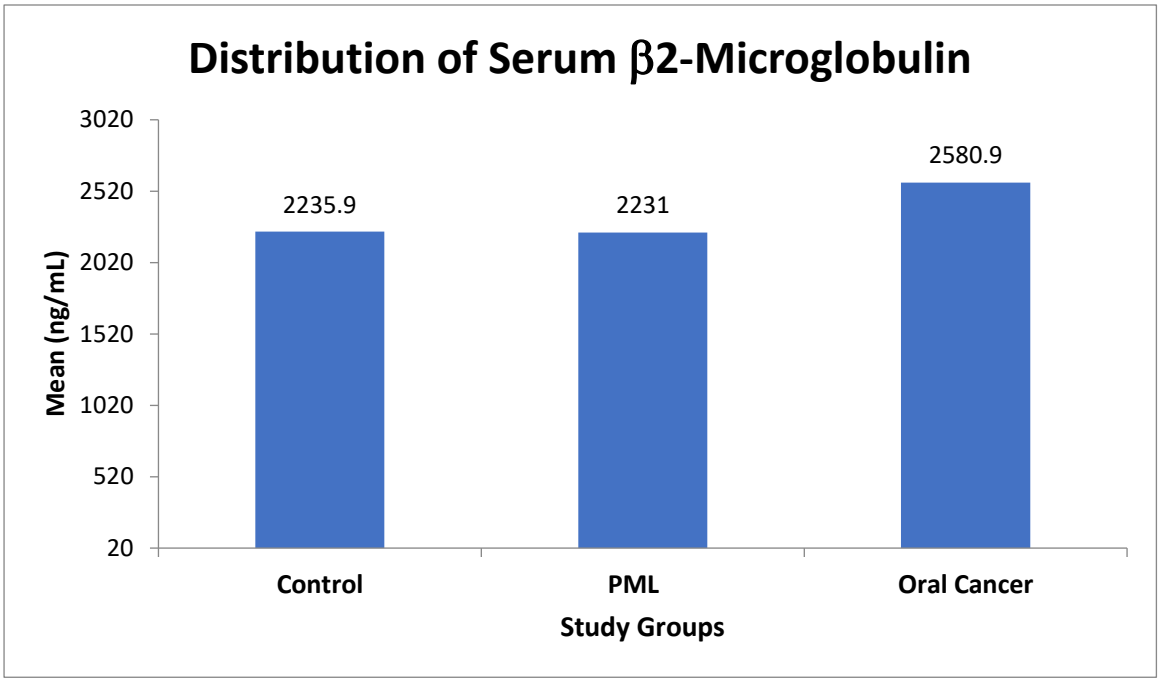


Figure 2 : The comparison of serum β2-microglobulin across three study groups



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.