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Original Research Article

A cross sectional study to compare serum beta 2 microglobulin levels in oral leukoplakia and oral squamous cell carcinoma patients

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ABSTRACT

Background: One of the major health problems in the world is cancer. More than 11 million people are diagnosed yearly with cancer. Oral cancer is one of the deadliest cancers. Oral cancer is generally preceded by precancerous lesions. India has highest number of oral cancer patients. β2 microglobulin (β2 m) is one of the tumor markers.
Methods: We are correlating serum levels of beta 2 microglobulin in leukoplakia and oral squamous cell carcinoma. In our study, the serum β2 m levels were estimated in 2 groups of 30 subjects each with oral squamous cell carcinoma (OSCC) designated as Group A, 30 subjects with oral leukoplakia designated as Group B.
Results: There were majority of participants from age group of 51 to 70 years (Group A-60%, Group B-53.33%). Group A had majority of females 20 (66.67%) while males were 10 (33.33%). Group B had majority of males 18 (60.00%) while females were 12 (40.00%). Major adverse habit was tobacco chewing (83.33% Group A and 73.33% in group B). Most common site was Left BM. Right BM was second. There was significant association between the groups and serum β2 m levels. Mean levels of Group A patients were 3.13±0.47 mg/L and Group B 1.43±0.29 mg/L.
Conclusions: Adverse habits like tobacco chewing, smoking and alcohol increase chances of oral cancers and precancerous lesions. Significant correlation between oral cell carcinoma cases and serum β2 m levels is seen.

Keywords: β2 microglobulin, Oral cancer, Tumor markers

INTRODUCTION

Cancer is a health problem worldwide. It affects all people: the young and the old, the rich and the poor, men and women. More than 11 million people are diagnosed with cancer every year. Oral cancer is one of the most life-threatening disease of oral tissues and it is currently frequent cause of cancer-related deaths, it is usually preceded by oral precancerous lesions like the leukoplakia.[1,2]

Over 95% of the oral cancer lesions are seen to be squamous cell carcinomas. Oral cancer development is the multistep and multifocal process involved of field carcinogenesis and intraepithelial clonal spread. The frequency of the oral cancer in India remains highest in the world (30-50% of global total cancer incidence), due to exposure to carcinogens, such as the tobacco and it is important that an aggressive and all-out fight be launched to overcome this dreaded disease.[3]

Tumor markers are substances, which change quantitatively in serum during tumor development. One such tumor marker is β2 microglobulin. β2 microglobulin (β2 m) is an 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. β2 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.[4]

The term 'oral cancer' encompasses all the malignancies that originate from the oral tissues.[5] while its incidence is relatively low in western countries there are some basic exceptions to this trend: in our Indian subcontinent and in the other parts of Asia, it remains one of the most common forms of cancer.[6] over 95% of oral cancer lesions are squamous cell carcinomas. It represents almost third most common form of malignancy in the developing countries while in developed countries it is eighth most common.[7]

In the carcinomas of oral cavity, various other serum markers have been studied: these include oncofetal proteins (alpha-fetoprotein, CEA), and other proteins like β-protein and enzymes (LDH) etc. One of such important markers is β2 m. β2 m is a low molecular weight, 11800 kD protein found on the surface of all cells except erythrocytes as the invariable light chain of the histocompatibility antigen. Increased concentrations of cell surface β2 m in potentially malignant and malignant epithelial tissues with normal concentration of MHC class i heavy chains has been found.[8]

METHODS

Study design: Cross sectional comparative study.

Study site: Tertiary care centre and Medical College in Maharashtra.

Study period: 6 months July 2018 to January 2019.

Sample size

60, two groups A and B. In our present comparative study, the serum β2 m levels were estimated in 2 groups consisting of 30 subjects each with oral squamous cell carcinoma (OSCC) designated as Group A, 30 subjects with oral leukoplakia designated as Group B.

Sampling technique: Convenience sampling.

Inclusion criteria

Inclusion criteria were patients with oral leukoplakia (histopathologically proven); patients with primary oral squamous cell carcinoma (histopathologically proven).

Exclusion criteria

Exclusion criteria were patients with any other systemic diseases who were referred to the general physician to evaluate for such diseases; patients not willing to participate.

Principle of the test for β2

Highly purified anti-human-β2-m antibodies are bound to the microwells. β2 m, if present in diluted serum or urine, binds in the microwells. Washing of the microwells removes unreactive serum components. Anti-human-β2-m-horseradish peroxidase conjugate solution is pipetted into the wells to recognize β2-microglobulin bound to immobilized antibodies by forming sandwich complexes.

Horseradish peroxidase (HRP) conjugated anti-human β2-m immunologically binds to the bound patient β2 m forming a conjugate/ β2 m/antibody complex. It is used to calculate the concentration of β2 m present in the original sample.

Equipment

- Microplate reader capable of endpoint measurements at 450 nm.
- Multi-channel dispenser or repeatable pipette for 100 µl.
- Vortex mixer.
- Pipettes for 10 µl, 100 µl and 1000 µl.
- Laboratory timing device.
- Data reduction software.

Procedure

The standard procedure for performing the test includes following steps–preparation of reagents, preparation of sample buffer, wash solution, sample preparation, and finally conduction of test.

Ethical approval

Ethical approval was taken from the institutional ethics committee.

Statistical analysis

A predesigned pretested questionnaire was used to collect the data. Data Collected was entered in Microsoft Excel. Data is represented in frequencies and percentages, charts and graphs. Mean and standard deviation of quantitative variables is shown. Appropriate statistical tests are applied using EpiInfo version 7.2 and SPSS software version 20 for analysis. Students t test was used for comparison of means.

RESULTS

In our present comparative study, the serum β2 m levels were estimated in 2 groups consisting of 30 subjects each with oral squamous cell carcinoma (OSCC) designated as Group A, 30 subjects with oral leukoplakia designated as Group B.

There were majority of the participants from the age group of 51 to 70 years in both the groups (Group A- 60%, Group B– 53.33%). Next common age group was 31 to 50 years (Group A- 33.33%, Group B– 26.67%). Group A had majority of females 20 (66.67%) while males were 10 (33.33%). Group B had majority of males 18 (60.00%) while females were 12 (40.00%) (Figure 1).

Table 1: Age distribution.

Age (in years)	Group A	Group B
	N (%)	N (%)
≤30	1 (3.33)	4 (13.33)
31-50	10 (33.33)	8 (26.67)
51-70	18 (60.00)	16 (53.33)
>70	1 (3.33)	2 (6.67)
Total	30 (100)	30 (100)

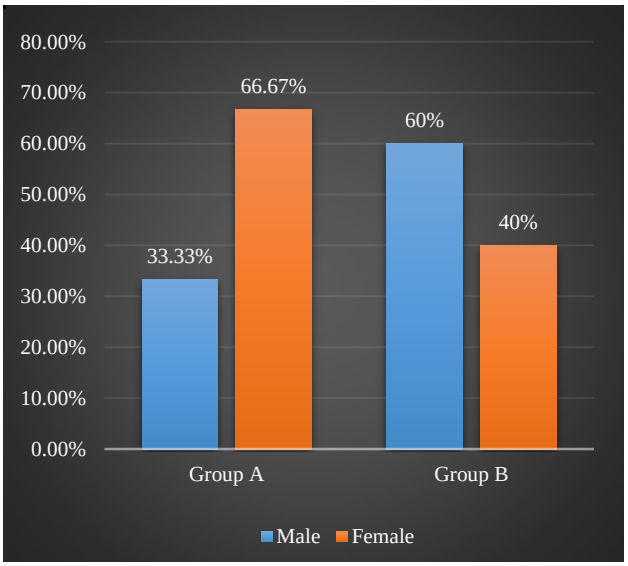

Figure 1: Sex distribution of the participants.

Table 2: Adverse habits.

Habits	Group A (n=30)	Group B (n=30)
	N (%)	N (%)
None	1 (3.33)	-
Alcohol	5 (16.67)	7 (23.33)
Smoking	13 (43.33)	12 (36.67)
Tobacco chewing	25 (83.33)	22 (73.33)

Major adverse habit found in both the group was tobacco chewing (83.33% in Group A and 73.33% in Group B) while others were Smoking (43.33% in Group A and 36.67% in Group B) and alcohol drinking (16.67% in Group A and 23.33% in Group B). Group A had one patient (3.33%) with no any adverse habits.

Most common site was left BM, with 5 cases from Group A and 13 cases from Group B. Right BM was second most common with 5 cases from Group A and 7 cases from Group B. Distribution of different lesions are depending on the site was shown in Table 3.

There was significant association between the groups and serum β2 m levels. Mean levels of Group A patients were 3.13±0.47 mg/L and Group B were 1.43±0.29 mg/L (Table 4).

Table 3: Distribution of site of lesions of OSCC and oral leukoplakia.

Lesions	Group A (n=30)	Group B (n=30)
BM and gingiva	-	1
Commisures	-	1
FOM	2	-
Left alveolus	2	-
Left GBS	3	1
Left BM	5	13
Left BM and GBS	3	1
LIP	2	-
Lips and commissures	-	1
Left and right BM	1	2
Right alveolus	2	-
Right BM	5	7
Right BM and GBS	-	1
Right GBS	1	2
Right Commisure	1	-
Tongue	3	-

Table 4: Comparison of serum β2 m levels in mg/l between the groups.

Groups	β2 m levels in mg/L	
	Range	Mean±SD
Group A	2.06 -3.68	3.13±0.47
Group B	1.08-1.94	1.43±0.29
Comparison	Group A–Group B= p<0.001	

DISCUSSION

Oral cancer constitutes a major part of cancer deaths in India. Still, the great morbidity and the mortality rates of this dangerous disease have not improved in last decades.[9] Hence, early recognition is important. Which can improve the oral cancer survival rates, preservation of function and enhance the aesthetic and psychological outcomes in patients.[10]

It is also clear from our study that female patients are almost equal to the males which is similar to sex wise incidence rates observed in Indian studies.[11,12]

In present study patients had habits of using some or the other form of tobacco products most commonly chewing the betel-liquid with tobacco and this has been demonstrated as the major risk factor for cancers of oral cavity. In the male patients usage of pan masala, chronic smoking and abnormal amount of alcohol consumption were observed in all cases except in 1 patient who had no deleterious habit at all.

In addition, there is also a site-specific relationship as example, in areas where tobacco quid is kept in the lower gingivobuccal sulcus; cancer at that site is very common. Similarly, we have also found in our study group that

incidence of cancer from the buccal mucosa have been the highest. With these facts the relevance of our study cannot be over emphasized considering the highest rates of oral cancers especially the cancers of buccal mucosa and alveolus along with the gingiva which have been termed as the 'Indian oral cancers'.[12]

In our present study, the range of serum β2 m in OSCC patients was estimated by the enzyme linked immuno-sorbent assay employing sandwich technique and range assayed in the serum samples of Group A patients was 2.06-3.68 mg/L. Mean was 3.13 mg/l with a standard deviation of 0.47 mg/l. The range assayed in the serum samples of Group B patients was 1.08-1.94 mg/l. Mean was 1.43 mg/l with a standard deviation of 0.29 mg/l.

Comparison of serum β2 m in oral squamous cell carcinoma that is Group A with Group B patients ($p<0.001$) demonstrated that the elevation of serum β2 m in OSCC was highly significant compared to leukoplakia patients. This noteworthy finding was reported previously by Manzar et al, 1992 who studied serum β2 m in 50 cases of OSCC and 20 normal subjects. Similar results have also been obtained by Anil et al, 1995 who studied serum β2 m in oral squamous cell carcinoma, oral submucus fibrosis and oral leukoplakia. Other studies too have reported similar findings.[13-20]

CONCLUSION

From the observations we gathered in our present study on evaluation of serum β2 m levels in oral leukoplakia, oral squamous cell carcinoma we conclude that the Oral cancers are more common in increasing ages and are seen both in males and females equally. Adverse habits like tobacco chewing, smoking and alcohol drinking increases the chances of oral cancers and precancerous lesions. There is a significant correlation between the oral cell carcinoma cases and serum β2 m levels as compared to the precancerous lesions like leukoplakia.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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