Original Research Article

A Study on an Evaluation of Serum Total Sialic Acid in Oral Leukoplakia and Oral Squamous Cell Carcinoma

S. Jyotsna[1*], M. Supraja Chowdary[2]

[1]Associate Professor, Dental Depatment, Ayaan Institute of Medical Sciences, Moinabad, Hyderabad, Telangana, India.

[2]Senior Lecturer, Oral Medicine and Radiology, Narayana Dental College and Hospital, Nellore, Andhra Pradesh, India.

Received: 02-03-2021 / Revised: 10-04-2021 / Accepted: 25-05-2021

Abstract

Background and Objectives: Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer with a high morbidity rate and a 5-year mortality rate of about 50%.The incidence as well as mortality rate of cancer have shown a sharp acceleration since the last two decades Altered glycosylation of glycoconjugates is among the important molecular changes that accompany malignant transformation. Various studies in the past have shown the direct relation of Total Sialic acid in premalignant lesions and different malignancies. The purpose of this study is to investigate clinical usefulness of circulatory levels of Total Sialic acid in Leukoplakia, Squamous cell carcinoma and healthy controls of age and sex matched subjects.**Method:** Blood samples were collected from 20 untreated Leukoplakia, 20 untreated Squamous cell carcinoma and 20 healthy subjects. Total serum Sialic acid were evaluated by the simplified quick method by G Sydow and measured spectrophotometrically at 525nm.**Results:** Serum levels of Total Sialic acid were significantly elevated (P<0.001) in untreated Oral cancer patients as compared to healthy controls and Leukoplakia. Our data also inferred significant difference between 1)control group & Leukoplakia group, 2)control group & squamous group and 3)Leukoplakia group & squamous group with respect to the mean Sialic acid levels (P<0.001). The mean Sialic acid is found to be more in squamous group compared to Leukoplakia and control group and this difference is statistically significant. The mean Sialic acid in Leukoplakia group is higher than control group and this difference is also statistically significant.**Conclusion:** The data revealed direct relation between significant elevation of serum Total Sialic acid levels in Oral cancer patients and also an ascending order of increase of serum Total Sialic acid levels from healthy controls to Leukoplakia to Squamous cell carcinoma and suggested that potential utility of these parameters in initial diagnosis of Leukoplakia and Squamous cell carcinoma.

Keywords: Glycoprotein, Leukoplakia, Oral cancer, Sialic acid, Squamous cell carcinoma.

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer with a high morbidity rate and a 5-year mortality rate of about 50%.The incidence as well as mortality rate of cancer have shown a sharp acceleration since the last two decades . The cure rate is dismally low for such an accessible tumor, which makes it essentiality for further focus on them[1,2].Despite improvement in imaging and therapy, the survival rate for patients with these has not changed substantially for many years[3].

Oral cancer related morbidity and mortality is on a rise, globally with the incidence reaching high proportion in India, where there is 11.2% Prevalent in males and 11% in females with the site predilection of oral cavity as the second place in males and third in females . Clinical, epidemiology and laboratory studies suggest direct etiological relationship with prolonged tobacco use with Oral cancer[4].Despite the recent advances in tumour surgery and multimodal treatment regimes, the prognosis of head and neck squamous cell carcinoma (HNSCC) is still relatively poor. This may be because the symptoms that indicate the presence of the carcinoma often appear when the tumour is in an advance stage[5].Oral cancer is usually preceded in many cases by precancerous lesions or conditions like Leukoplakia or Sub mucous oral fibrosis, is attributed to different types of tobacco chewing[6].Early detection of oral cancer is of paramount importance to decrease the morbidity and mortality of the disease. This motivates the search of parameters which will help in the early diagnosis of oral cancer.Over the past several decades, with the studies on these tumours ,lead to the recognition of the significant role of glycoconjugates in malignant transformation. Terminal epitopes of carbohydrates play a significant role in cell – cell interactions in the development of cell adhesion and in malignant transformation. One of the most common changes in glycoconjugates during malignant transformation is the increase in size of oligosaccharides resulting in branching sites for incorporation of Sialic acid. The occupancy of Sialic acid at the terminal or near to terminal position underlies its vital role in determing surface characteristic of cells and secreted Glycoproteins. Being non-reducing terminal Sialic acid gained outstanding importance in cancer research[6].Sialic acid, a family of acylated derivatives of neuraminic acid, usually occurs as a terminal component at the nonreducing end of carbohydrate chains of glycoproteins and glycolipids are thought as important in determining the surface properties of cells and has been implicated incellular invasiveness, adhesiveness, and immunogenicity. Alterations in the sialic acid levels in cancer patients have stimulated interest in this sugar residue

**Correspondence*
Dr. S. Jyotsna
Associate Professor, Dental Depatment, Ayaan Institute of Medical Sciences, Moinabad, Hyderabad, Telangana, India.
E-mail: jyots2k@yahoo.com

as a possible tumor marker[7].Numerous investigators have reported possible relation of increased Sialic acid levels with various malignancies. Total Sialic acid (TSA) consists of Glycoproteins and Gylcolipids bound Sialic acids. These glycoconjugates are released in to circulation through increase turnover, secretions and / or shedding from malignant cells[7].The idea of screening and following patients with malignancy by blood – based test is appealing from several point of view including its ease, economic advantage, non-invasiveness and possibility of repeated sampling.

Therefore, the present study is an attempt to investigate the serum levels of Glycoconjugate-the Sialic acid in patients with Leukoplakia and Squamous cell carcinoma for its early diagnosis.

Aims & Objectives

- To Asses serum Total Sialic acid levels in Leukoplakia.
- To Asses serum Total Sialic acid levels in Squamous cell carcinoma.
- To Asses serum total Sialic acid levels in controls.
- To correlate the Total Sialic acid levels in Leukoplakia and Squamous cell carcinoma with that of controls.

Source of the data: This study was carried out in Department of Dental Sciences, Ayaan Institute of Medical Sciences, Hyderabad, Telangana, India.

Methods of Selection of Data:

Sample Size:

1. Total number of subjects: 60
2. Patients with Leukoplakia: 20
3. Patients with Squamous cell carcinoma: 20
4. Age and sex matched controls for comparison of results: 20

Selection of Cases:

Inclusion Criteria:

1. 20 patients of Oral Leukoplakia (Histopathologically proven)
2. 20 patients of Squamous cell carcinoma (Histopathologically proven)

Exclusion Criteria: Patients were referred to General physician for opinion to evaluate for any systemic disease status like diabetes mellitus, Ischemic heart disease, different bone disorders like pyogenic arthritis, Rheumatoid arthritis, malignant bone tumors. Subjects with any of the above mentioned diseases were excluded from the study.

Selection of Controls: Included age and sex matched 20 healthy individuals with the same exclusion criteria as that of selection of cases.

Sample Collection, Storage and Handling: The subject is seated comfortably with the arm supported. Aseptic measures are used and tourniquet is applied 2 inches above the elbow of the upper arm. The site of the puncture is cleaned using sterile gauze dipped in 100% alcohol. Using a 5ml syringe with the needle size of 0.55 x 25mm. 5ml of blood is drawn from the anticubital vein. The blood is allowed to clot and the serum separated by centrifugation. Serum Sialic acid is estimated through a simplied quick method from G.Sydow.

Materials Required

1. Sialic acid Powder (Only for working Standard solution)
2. Ehrlich's Reagent (p-dimethylaminobenazaldehyde solution)
3. 5% perchloric acid ($HClo_4$ solution)
4. Adjustable Micropipette of 1000µl.
5. Centrifuge
6. Incubator
7. Spectrophotometer

Estimation of Serum Sialic Acid:

Principle:

Free Sialic acid in serum reacts with Paradimethylamino-benzaldehyde (Ehrlich's Reagent) to form a pink colored solution. The absorbance of the color developed in the sample at 525nm is proportional to the total Sialic acid concentration in the serum.

Results

In our comparative study, Serum Sialic acid were estimated in three groups consisting of 20 subjects in Leukoplakia designated as Group-I, 20 subjects in Squamous cell carcinoma as Group-II, and age and sex matched 20 subjects as controls in Group III.

Age distribution-Result: The age distribution in three different groups are; In Group I with Leukoplakia, the age distribution was between 20-70 years, 6 cases (30%) were in the age group of 20-30 years, 5 cases (25%) were in the age group of 31-40 years, 3cases (15%) in the age group of 41-50 years ,2 cases (10%) in 51-60 years, 3 cases (15%) were in the age group of 61-70 years and 1 case (5%) were in the age group of >71 years (Table 1, Graph 1).It was inferred from our study that peak occurrence of Leukoplakia was between the age group of 20-30 years.In Group II with 20 cases of Oral Cancer (Squamous Cell Carcinoma), the distribution of age was between 30-75 years. 6 cases(30%) were between the age of 31-40 years, 7 cases (35%) were in the age group of 41-50 years, 4 cases(20%) were in the age group of 51-60 years, 3 cases(15%) were in the age group of 61-70 years (Table I, Graph I). It was noticed from our study that peak occurrence of SCC was between the age group of 41-50 years.The age distribution among Group III was between 20-75 with 4 cases (20%) between the age of 20-30 years, 3 cases (24%) were within the age of 31-40 years, 6 cases(30%) were in the age group of 41- 50 years, 5 cases(25%) were in the age group of 51-60 years , 1 case(5%) was in the group of 61-70 years and above 71 years respectively(Table 1).

Table 1: Age distribution in three different groups

Age	Leukoplakia Group-I		Squamous Cell Carcinoma Group-II		Controls Group-III	
	No.	%	No.	%	No.	%
20-30	6	30.0%	0	0	4	0.2
31-40	5	25.0%	6	30.0%	3	15.0%
41-50	3	15.0%	7	35.0%	6	30.0%
51-60	2	10.0%	4	20.0%	5	25.0%
61-70	3	15.0%	3	15.0%	1	5.0%
>71	1	5.0%	0	0.0%	1	5.0%
Total	20	100.0%	20	100.0%	20	100.0%

Gender distribution-Result: The Gender distribution observed among the three Groups are;In Group I, Gender predilection was noticed. Of the 20 patients, 17 cases (85%) were males and 3 cases (15%) were females (Table 2).

It was inferred from our study that Leukoplakia occurrence was predominant in males. Of the 20 cases in Group II, 9 cases (45%) were males and 11 cases (55%) were females (Table 2). In GroupIIIof 25 subjects, 12 cases (60%) were males and 8 cases (40%) were females (Table 2).

Table 2: Gender distribution between Three Groups

Gender	LP-Group-I	SCC-Group-II	Controls

	No	%	No	%	No	%
Male	17	85.0%	9	45.0%	12	60.0%
Female	3	15.0%	11	55.0%	8	40.0%
Total	20	100.0%	20	100.0%	20	100.0%

Habits distribution-Results: Habits distributions between three Groups are;The entire Group I subjects had deleterious oral habits. Of which 8 cases (40%) had smoking habits, 10 cases (50%) had smokeless tobacco(chewing) habit, 2 cases (10%) were alcoholic, 4 cases (20%) had the habit of smoking and smokeless tobacco habit, 6 cases(30%) had ST and alcohol habits, 3 cases(15%) had all the three habits i.e, Smoking, ST and alcohol habits(Table 4).

In Group II , only 1case (5%) did not have any oral deleterious habits. 7 cases (35%) had smoking habit, 12 cases(60%) had smokeless tobacco habit. 8 cases (40%) had both smoking and smokeless tobacco habit.(Table 3,graph 3).In both Group I and Group II subjects smokeless tobacco habit (chewing) was the predominant deleterious habit.The subjects in Group III i.e Control group did not have any deleterious oral habits.

Table 3: Habits distribution between Two Groups

Habit	LP Group - I		SCC Group - II	
	No	%	No	%
None	0	0.0%	1	5.0%
Smoking-A	8	40.0%	7	35.0%
ST-B	10	50.0%	12	60.0%
Alcohol-C	2	10.0%	0	0.0%
TOTAL	20		20	
A+B	4	20.0%	8	40.0%
B+C	6	30.0%	0	0.0%
C+A	3	15.0%	3	15.0%
A+B+C	3	15.0%	6	30.0%

Site distribution - Result

The Site distribution between Group I and Group II are;

In Group I, the lesion showed different site of involvement in the oral cavity. 17 cases (41%) involved buccal mucosa, 8 (20%) cases involved commissure of lip, 4 (10%) cases in retromolar area, 3 cases (7%) each in Gingivobuccal sulcus, Buccal mucosa and gingivobuccal sulcus, Lip and Gingiva respectively (Table4).

The lesions in Group II were distributed at different sites in the Oral cavity. 12 cases (26%) involved buccal mucosa. 2 cases (4%) in alveolus, 12 cases (26%) in retromolar area, 1 case (2%) in floor of the mouth and Tongue, 3 cases(7%) in Gingivobuccal sulcus, 6 cases(13%) in Gingivobuccal sulcus and Buccal mucosa, 2 cases (4%) in commissure of lip, 3 cases(7%) in gingiva, 2 cases (4%) in Tongue and floor of the mouth. 2 cases (4%) in palate (Table 4).

It was noticed from our study that both in Group I and Group II, Buccal mucosa is the site of predilection for LP and SCC respectively.

Table 4: Site distribution between Two Groups

Sites	LP Group - I		SCC Group - II	
	No	%	No	%
BM	17	41%	12	26%
ALVEOLUS	0	0%	2	4%
RMA	4	10%	12	26%
RMA+SP	0	0%	0	0%
FOM	0	0%	1	2%
TONGUE	0	0%	1	2%
GBS	3	7%	3	7%
BM+GBS	3	7%	6	13%
ALV+FOM	0	0%	0	0%
COL	8	20%	2	4%
LIP	3	7%	0	0%
GINGIVA	3	7%	3	7%
TONGUE+FOM	0	0%	2	4%
PALATE	0	0%	2	4%
TOTAL CASES	41	100%	46	100%

Staging in Leukoplakia - Result

Of the 20 Subjects in Group-I, 19 cases (98%) were in stage I and 1 case (2%) was in Stage II of clinical staging (Table 5). Our study showed the out numbering of LP in clinical Staging I.

Table 5: Staging in Leukoplakia – Group-I

LP	No	%
Stage 1	19	98.00%
Stage 2	1	2.00%

Total	20	100%

Staging in SCC-Result

Clinical Staging of Oral cancer in 20 cases of Group II showed, Stage III of 14 cases (70%) and 6 cases(30%) of Stage IV(Table 6, Graph 6). Our study showed the out numbering of SCC in Clinical Staging III.

Table 6: Staging in Oral Cancer- Group-II

SCC	No	%
Stage I	0	0.00
Stage II	0	0.00
Stage III	14	70.00
Stage IV	6	30.00
Total	20	100

Tumor size in SCC-Result:

TNM staging in 20 cases of Group II revealed 4 cases (20%) reported withTumor size of T1, 10 cases(50.00%) with Tumor size of T2, 3 cases(15%) with Tumor size of T3, 3 cases(15%) with Tumor size of T4 (Table 7,Graph 7).The study showed the Outnumbering of SCC subjects in T2 Tumor size.

Table 7: Tumor size in Oral Cancer

SCC-Group-II Tumor size-T	No	%
T1	4	20.00
T2	10	50.00
T3	3	15.00
T4	3	15.00

Nodal status in SCC – Result:

The Nodal status of group II showed a significant number of 15 cases (75%) in N1 stage, 1 case(5%) in N2a stage, 2 cases (10%) in N2b stage , 2 cases(10%) in N2c stage (Table 8 , Graph 8). The study showed the Outnumbering of SCC subjects in N1 Nodal state.

Table 8: Nodal involvement in Oral cancer

SCC-Group-II Nodal involvement-N	No	%
N1	15	75.00
N2a	1	5.00
N2b	2	10.00
N2c	2	10.00

Metastasis was not noticed in any of the cases.

Serum Total Sialic acid –Result

The Serum Total Sialic acid levels were estimated in all the three Groups and the arithmetic mean along with Standard deviation was calculated. (Table 9).Group I showed the mean of 78.36 mg/dl with the standard deviation of 16.81 mg/dl; the interval of mean between 72.13 to 84.25 mg/dl, with the minimum of 48.00 mg/dl to a maximum of 109.00 mg/dl. Group II showed the mean of 96.78 mg/dl with the standard deviation of 21.45 mg/dl; the interval of mean between 91.11 to 111.30 mg/dl , with the minimum of 53.00 mg/dl to a maximum of 128.00 mg/dl.Group III showed the mean of 61.12 mg/dl with the standard deviation of 4.88 mg/dl; the interval of mean between 58.19 to 62.25 mg/dl, with the minimum of 51.50mg/dl to a maximum of 69 mg/dl.Our study showed the ascending order of Serum Total Sialic acid levels in Controls, LP and SCC.

Table 9: Descriptive Statistics

Group	N	Mean	Std. Deviation	95% Conf Interval for Mean		Min	Max
				Lower Bound	Upper Bound		
Control	20	61.12	4.88	56.34	64.35	52.40	71.00
Leukoplakia	20	78.36	16.81	72.13	84.25	48.00	109.00
Squamous Cell Carcinoma	20	96.78	21.45	91.11	111.30	53.00	128.00

Discussion

Carcinoma of the oral cavity is one of the most frequent malignant tumors worldwide, with major predominance in South-East Asia and India[8].Among the oral tumors, 90% are squamous cell carcinoma (SCC), which arises from the mucosal lining. This high incidence of oral cancer in India is due to the widespread habits of tobacco chewing and smoking and alcohol[9].The transformation rate from premalignant to malignant varies from 0.6 to 36 %.Leukoplakia is the most common premalignant, potentially malignant or precancerous lesion of the oral mucosa[10].In our present study conducted in Ayaan Institute of Medical Sciences, Dental Department included 20 Leukoplakia cases, 20 Squamous cell carcinoma cases and 20 age and sex matched controls.Among 20 Leukoplakia cases the age distribution was between 20-70 years. The existence of Leukoplakia even in earlier ages up to 15 years was noticed which was related with the usage of tobacco[11].Our study showed gender predilection for men in Leukoplakia up to 85% which was almost similar to other study. This can also vary depending on the geographical variation and usage of tobacco[12].Every case of Leukoplakia had the habit of usage of tobacco either as smoking or smokeless (chewing) form or in some with addition usage of alcohol. Smokeless tobacco usage was most common.Oral Leukoplakia frequently precedes oral cancer.

They both has similar etiologic factors and can be induced and promoted by tobacco; There are about 300 carcinogenic compounds present in tobacco out of which tobacco-specific nitrosamines have been identified as the most important carcinogens in tobacco. The other carcinogenic compounds in tobacco metabolites are polycyclic aromatic hydrocarbons, a-particle-emit-ting[11]Po (polonium), trace metals, carbon monoxide, hydrogen cyanide, and phenols[9].The site of predilection for Leukoplakia being buccal mucosa and then commissure of lip in different studies[13] correlates with our study. Leukoplakia in stage I clinical staging has out numbered.In Oral Cancer of Group II including 30% patients were between the age distribution of 31-40 years with 35% of the patients were in the age group of 41-50 years , 20% were in the age group of 51-60 years and 15% were in the age group of 61-70 years. The similar study was showing the wider range of age distribution between 14-80 years of age with the median age of 43 years[7].Almost equal predilection for males and females in our study which is contradictory to the sex predilection in India which in turn depends on The usage of tobacco.[8,9]. In India, the buccal mucosa (cheek) is the primary site for cancer development as against the tongue and the floor of the mouth in Western countries, which may be due to the habit of keeping the betel-quid and tobacco in contact with the cheek for a long time9 which is similar to our study. Only one patient did not have any oral deleterious habit.In our study, majority of the patients were in Stage III clinical staging of SCC with T2 tumor size, including Lymph node involvement in all the cases. This may be due to lack of awareness as majority of the cases are poorly educated and are from rural background.Tumor markers are substances specific for certain tumor or cancer cells and thus could be of appreciable diagnostic and prognostic value in cancer patients[14].

In our present study, Total serum Sialic acid was estimated using a simplified quick method by G Sydow. The OD values were plotted in the standard graph (curve) to obtain the serum levels of Sialic acid in controls and cases. The values obtained are within the range which was noticed in different studies[15].The interval of mean was between 72.13 to 84.25 mg/dl in Group I, between 91.11 to 111.30 mg/dl in Group II and between 58.19 to 62.25 mg/dl in Group III.

Analysis of variants was carried out between three groups and the results was conclude that there is a significant difference in the mean Sialic acid levels in the 3 groups ($P<0.001$) (Table 10).The mean Sialic acid is found to be more in squamous cell carcinoma group compared to Leukoplakia and control group and this difference is statistically significant. The mean Sialic acid in Leukoplakia group is higher than control group and this difference is also statistically significant (Table 11).Our results are correlating with the different studies done by different authors corresponding to the level of Total serum Sialic acid in different untreated malignancies and oral precancerous lesion of the oral cavity[6,7].SCC of different sites other than oral cavity also showed significant increase in the level of Total Sialic acid due to its increased turn over, secretion and shedding from malignant cells[16,17].In our study, Group III did not have any deleterious habits compared to Group I and II except only one patient in Oral cancer, who had either smoking or smokeless tobacco oral habit- which is one of the risk for developing Leukoplakia and Oral cancer in India. The rise in the level of Sialic acid was also noticed in both smoking and smokeless tobacco habits indicating its harmful effects[18].The changes in serum TSA was noticed in different systemic diseases like diabetes mellitus, Ischemic heart disease, and different bone disorders like pyogenic arthritis, Rheumatoid arthritis, malignant bone tumors[19].The patients in our study did not have any systemic disease. So, the increase in the TSA in both LP and SCC is due to increased turnover, secretion, loss of adhesiveness and / or shedding from Premalignant and malignant cells. Serum TSA can be used for initial diagnosis, monitoring therapy of premalignant and malignant lesions and even recurrence of malignancy[20].Several studies by Baxi *et al*[21] , Rao *et al*[22] Raval*et al*.[23]and Joshi and Patil et al[24] demonstrated elevated levels of serum sialic acid in oral cancer group as compared to premalignant and control group. Xing *et al*.[25] and Bathi *et al* also showed elevated levels of serum sialic acid in oral cancer group as compared to the control group but did not include premalignant group.According to the above-mentioned studies and the findings of our study, a definite relationship is established between the concentration of sialic acid in serum in cancer patients. Some of these studies also evaluated salivary Sialic acid levels and found a positive correlation with Oral cancers. This is due to the fact that saliva isultimately an ultrafiltrate of serum; thus, saliva can be used as a potentially diagnostic tool as compared to serum. Increased levels of sialic acid in cancer patients can be explained by spontaneous release or shedding of aberrant sialic acid-rich glycoproteins.The idea of screening and following patients with malignancy by blood test using simplified quick methodology by G Sydow is appealing from several point of including its ease, economic advantage, non-invasiveness, and possibility of repeated sampling.But the disadvantage is that since TSA is elevated in different disease entities, it can not be considered as specific Tumor marker. Further studies are required to include Total Sialic acid would be helpful as a specific Tumor marker in the initial diagnosis of Leukoplakia and Squamous cell carcinoma.

Conclusion

Our Present study has gathered following Conclusions from the Assessing of Serum Total Sialic acid in Oral Leukoplakia, Squamous cell carcinoma and age and sex matched controls:

- When the patients age and sex attributes were considered, the highest incidence was observed in the 3rd decade in Leukoplakia and 5th decade in OSCC. Males out numbered in Leukoplakia and almost equal sex predilection in OSCC.
- Definitive association was found between the harmful habits of tobaccochewing with smoking and alcohol consumption to the incidence of LP and SCC.
- The Smokeless tobacco usage was found to be related to the occurrence of LP and SCC with the site predilection of Buccal mucosa.
- Significant elevation of serum Total Sialic acid in Leukoplakia and Squamous cell carcinoma was assessed. So TSA in serum can be used as Tumor marker in LP and SCC.
- The level of Serum Sialic acid in LP with mild dysplasia (1 case) is not significant compared to LP without dysplasia but the level of serum TSA in poorly differentiated OSCC compared to well differentiated and moderately differentiated OSCC is significant. So more sample assessment of LP with dysplasia may be significant in initial diagnosis.
- Correlation of sialic acid levels in saliva with histopathologic grading of oral cancer is required in further studies.
- The levels of salivary sialic acid should also be correlated with clinical staging of leukoplakia lesions.

The Simplified quick methodology by G.Sydow in the assessment of serum TSA is less time consuming, less invasive, cost effective and values were within the levels found by different methodology. So, this method can be considered as a standardized method for the evaluation of TSA in serum for initial diagnosis of LP and SCC.

Acknowledgment

The author is thankful to Department of Dental Sciences for providing all the facilities to carry out this work.

References

1. Diamondopoulos, Meissner. Anderson's pathology, 8th edition, 1985; 1:15, Neoplasia.
2. Norman K Wood, Paul W Goaz. "Differential Diagnosis of Oral and maxillofacial lesions". Fifth edition, Elsevier publication, 35:587-594.
3. James D, Cox K, Kian Ang. "Radiation Oncology". Eighth edition, 10:219-254.

4. Manisha H Shah, Shaila D Telang, Pankaj M Shah, Prabhudas S Patel. "Tissue and serum α 2-3 and α 2-6- linkage specific sialylation changes in oral carcinogenesis. Glycoconj J. 2008; 25:279-290.
5. Daniel Ayude, Gloria Gacico, Maria Paez De La Cadena, Esterella Pallas, Vicenta Soledad Martinez-Zorzano et al. "Combined use of established and novel tumor markers in the diagnosis of head and neck squamous cell carcinoma. Oncology Reports. 2003; 10:1345-1350.
6. Vasanti R Rao, Kumaraswamy SV,Girija Ramaswamy. "Cancer Detection and Prevention. 1998; 22(3):237-240.
7. KinnariB Rajpura, Prabhudas S Patel, Jyoti G Chawla, Raksha M Shah. "Clinical significance of total and lipid bound Sialic acid levels in oral precancerous conditions and oral cancer. J Oral Pathol Med. 2005; 34:263-
8. Anak Lamaroon, Surawut Pongsiriwet, sumana Jittidecharaks,. "Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma". J Oral Pathol Med. 2003; 32:195-9.
9. Takashi Hase, Shuichi Kawashiri, Akira Tanaka, Shinichi Nozaki. "Correlation of basic fibroblast growth factor expression with the invasion and the prognosis of oral squamous cell carcinoma". J Oral Pathol Med. 2006; 35:136–9.
10. Van Der Waal, Axell T."Review Oral Leukoplakia: a proposal for uniform reporting". Oral Oncology. 2002; 38:521–526.
11. Fali S Mehta, Pindborg JJ,Dr.Odont, Gupta PC,Daftary DK. "Epidemiologic and histologic study of oral cancer and Leukoplakia among 50915 villagers in India. Cancer. 1969; 24:832-849.
12. Eliza Carla Barroso Duarte, Marina Sena Lopes Da Silva, Marcus Vinicius Gomez, Ricardo Santiago Gomez. "GSTM1 polymorphism and oral Leukoplakia". J Oral Pathol Med. 2006; 35:202–5.
13. MousumiMajumder, NilabjaSikdar, RanjanRashmi Paul, Bidyut Roy. "Increased Risk of Oral Leukoplakia and Cancer Among Mixed Tobacco Users Carrying XRCC1 Variant Haplotypes and Cancer Among Smokers Carrying Two Risk Genotypes: One on Each of Two Loci, GSTM3 and XRCC1 (Codon 280). Cancer Epidemiol Biomarkers Prev, 2005, 14(9):1.
14. Tewarson SL, Mittal VP, Singh M, Gupta GP. "Serum Sialic acid-An important cancer marker". Indian Journal of Cancer. 1993; 30:125-131.
15. Singh R, Sur BK, Agarwal SN, RamrajuB. "Serum Sialic acid in malignancy". Ind. Jour. Med. Res. 1967; 55:3.
16. Mrochek JE, Dinsmore SR, Tormey DC, Waalkes TP. "Protein –bound Carbohydrates in Breast Cancer. Liquid – Chromatographic analysis for Mannose, Galactose, Fucose and Sialic Acid in Serum. Clinical Chemistry, 1976, 22(9):1
17. NaciyeKurtul M, YasarCil,SefaDogrulukPacac. "Serum total Sialic acid levels in smokers and users of smokeless tobacco in form of Oral powder (maras powder).Journal of Biomedical science. 2005; 12:559-563.
18. ShivanandNayak B, GeetaBakta. "Relation between Sialic acid and metabolic variables in India Type II Diabetic Patients". Lipids in health and diseases. 2005; 4:15.
19. Helbert KB, Silver R, Nelvin Murthy, Ann J Worth, Fernando A et al. "Prediction of malignant melanoma recurrence by serum N-Acetylneuraminic acid". Int.J. Cancer. 1982; 31:39-43.
20. Baxi BR, Patel PS, Adhvaryu SG. A report on clinical importance of serum glycoconjugates in oral cancer. Indian J Clin Biochem. 1990;5:139–44
21. Rao VR, Krishnamoorthy L, Kumaraswamy SV, Ramaswamy G. Circulating levels in serum of total sialic acid, lipid-associated sialic acid, and fucose in precancerous lesion and cancer of the oral cavity. Cancer Detect Prev. 1998; 22(3):237-40
22. Raval GN, Patel DD, Parekh LJ, Patel JB, Shah MH, Patel PS. Evaluation of serum sialic acid, sialyltransferase and sialoproteins in oral cavity cancer. Oral Dis. 2003; 9:119–28
23. Joshi M, Patil R. Estimation and comparative study of serum total sialic acid levels as tumor markers in oral cancer and precancer. J Cancer Res Ther. 2010;6:263–6.
24. Xing RD, Chen RM, Wang ZS, Zhang YZ. Serum sialic acid levels in patients with oral and maxillofacial malignancy. J Oral Maxillofac Surg. 1991;49:843–7.
25. Bathi RJ, Nandimath K, Kannan N, Shetty P. Evaluation of glycoproteins as prognosticators in head and neck malignancy. Indian J Dent Res. 2001;12:93–9.

Conflict of Interest: Nil
Source of support:Nil