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ESTIMATION OF SERUM TOTAL SIALIC ACID IN ORAL LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Objectives: 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 30 untreated Leukoplakia, 30 untreated Squamous cell carcinoma and 30 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 ($P<0.001$). **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: Leukoplakia, Oral cancer, Sialic acid, Squamous cell carcinoma.

INTRODUCTION

Cancer meaning Crab in Greek as these adheres to any part, which it seizes upon it as an obstacle manner. It was Hippocrates the father of medicine coined the term "Karkinos" (for non-healing neoplastic Ulcers) and "Karkinoma" (for solid malignant ulcers).[1] Oral cancer is a serious health problem world wide accounting not only for mortality but also responsible for extensive disfigurement, loss of function, behavioural changes, financial and sociologic hardship.[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 is the Sixth cause of Cancer related morbidity and mortality globally4 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]

Therefore the early diagnosis of Squamous Cell Carcinomas (SCC) would improve survival and quality of life, avoiding the mutilation that physician often have to make to save patients lives. The search for biological marker that could predict the changes in the pre-malignant Lesion would be useful in detecting patients with high risk for malignancy. Glycoproteins and gylcolipids, which form the major constituents of cells, have been implicated in cellular invasiveness, adhesiveness and immunogenesity. They are released in to circulation through increased turnover, secretion, and / or shedding from malignant cell. Changes in serum glycoprotein levels are characteristic of many pathological conditions including malignancy.[6] 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.[7]

The presence of Sialic acid at the terminal or near the terminal position underlies its importance in determining chemical and biological diversity, and characteristic of cell surface and secreted glycoprotein. Numerous investigators have reported possible relation of increased Sialic acid levels with various malignancies.[8]

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.

MATERIAL AND METHODS

This study was carried out in Department of Oral Medicine and Radiology tertiary care institute.

Methods of Selection Of Data

I. Sample Size.
1. Total number of subjects: 90.
2. Patients with Leukoplakia: 30.
3. Patients with Squamous cell carcinoma: 30.
4. Age and sex matched controls for comparison of results: 30.

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.

III. Selection of Controls

Included age and sex matched 30 healthy individuals with the same exclusion criteria as that of selection of cases**.**

Sample Collection, Storage and Handling

1. 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.
2. The blood is allowed to clot and the serum separated by centrifugation.
3. Serum Sialic acid is estimated through a simplied quick method from G. Sydow.

Estimation of Serum Sialic Acid

Principle

Free Sialic acid in serum reacts with Paradimethyl aminobenzaldehyde 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.

Stock Standard Solution

25 mg of SA powder was measured by physical balance and was dissolved in 10 ml of deionized water. The dissolved solution was transferred to a 25 ml volumetric flask. Water was added to make up the volume up to 25ml. (conc:1mg/ml).

Procedure

Simplified Quick method by G sydow

Preparation of Standard curve

In another set of 6 test tubes, 0.5 ml of standard with 2.0 ml of 5% Perchloric Acid and incubation for 5 min at 100oC. Cool down; centrifugation at 2500 x g for 4 mins. 1.0 ml of clear supernatant added to 0.2 ml of Ehrlich's reagents; Heated for 15 min at 100oC. Cooling the mixture and addition of 1.0 ml of water. Optical density measurement at 525 nm against reagent blanks in spectrophotometer. The OD values were plotted in a graph paper against Sialic acid concentrations of the standard solution to obtain a linear curve.

Calculation

The serum (0.5 ml) collected from all 60 cases and 30 controls were subjected to similar treatment. The OD values were plotted in the standard graph (curve) to obtain the serum levels of Sialic acid in controls and cases.[9]

Statistical analysis

Analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. Descriptive statistics were calculated. Level of significance was set at p= 0.05.

RESULTS

In our comparative study, Serum Sialic acid were estimated in three groups consisting of 30 subjects in Leukoplakia designated as Group-I, 30 subjects in Squamous cell carcinoma as Group-II, and age and sex matched 30 subjects as controls in Group III.

Age distribution

The age distribution in three different groups are; In Group I with Leukoplakia, the age distribution was between 20-70 years, It was inferred from our study that peak occurrence of Leukoplakia was between the age group of 20-30 years. In Group II with 30 cases of Oral Cancer (Squamous Cell Carcinoma), the distribution of age was between 30-75 years. 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 6 cases between the age of

20-30 years, 7 cases were within the age of 31-40 years, 41- 50 years, 51-60 years respectively.

Table 1: Age distribution in three different groups.

Age	Lp-Group-I		SCC Group-II		Controls Group-III	
	No	%	No	%	No	%
20-30	8	26.6	0	0	6	20
31-40	7	23.3	5	16.6	7	23.3
41-50	4	13.3	11	36.6	7	23.3
51-60	4	13.3	9	30	7	23.3
61-70	7	23.3	5	16.6	2	6.6
>71	0	0	0	0	1	3.3
Total	30	100	30	100	30	100

Gender distribution

In Group I, Gender predilection was noticed. Of the 30 patients, 24 cases were males and 6 cases were females. Of the 30 cases in Group II, 14 cases were males and 16 cases were females. In Group III of 30 subjects, 18 cases were males and 12 cases were females.

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. Our study showed the out numbering of LP in clinical Staging I. Clinical Staging of Oral cancer in 30 cases of Group II showed, Stage III of 20 cases and 10 cases of Stage IV.

TNM staging in 30 cases of Group II revealed 7 cases reported with Tumor size of T1, 15 cases with Tumor size of T2, 4 cases with Tumor size of T3, 4 cases with Tumor size of T4.

Serum Total Sialic acid

Group I showed the mean of 77.02 mg/dl with the standard deviation of 16.08 mg/dl; the interval of mean between 68.20 to 84.12 mg/dl. Group II showed the mean of 94.68 mg/dl. Group III showed the mean of 61.40 mg/dl.

There is a significant difference between control group & Leukoplakia group, control group & squamous group and Leukoplakia group & squamous group with respect to the mean Sialic acid levels The mean Sialic acid is found to be more in squamous group compared to Leukoplakia and control group and this difference is statistically significant.

DISCUSSION

Carcinoma of the oral cavity is one of the most frequent malignant tumors worldwide, with major predominance in South-East Asia and India. in our present study included 30 Leukoplakia cases, 30 Squamous cell carcinoma cases and 30 age and sex matched controls. Our study showed gender predilection for men in Leukoplakia up to 84% which was almost similar to other study.[10]

The site of predilection for Leukoplakia being buccal mucosa and then commissure of lip in different studies correlates with our study. In Oral Cancer of Group II including 30 patients were between the ages distribution of 31-40 years of age group. 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]

Substances like Glycoproteins and Gylcolipids are major constituents of cell membrane, and hence, cell-surface Glycoconjugates are important in malignancy.[8] Sialic acid is thought to be important in determining the surface properties of cells and has been implicated in cellular invasiveness, adhesiveness and immunogenesity[8]. 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.[9] 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 ($P<0.001$).

In order to find out among which pair of groups there exist a significant difference, Bonferroni's test was carry out between three Groups with multiple comparison and inferred that; there is a significant difference between control group & Leukoplakia group, control group & squamous group and 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 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. Our results are correlating with the different studies done by different people corresponding to the level of Total serum Sialic acid in different untreated malignancies and oral precancerous lesion of the oral cavity6.[7,8]

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.[11,12] The patients in our study did not have any systemic disease. Serum TSA can be used for initial diagnosis, monitoring therapy of premalignant and malignant lesions and even recurrence of malignancy.[13]

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 cannot 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

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.

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