Original Research Article

An Assessment of Serum Total Sialic Acid in Oral Leukoplakia and Oral Squamous Cell Carcinoma at Nadiad, Gujarat

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Abstract

Background and Objectives: 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 25 untreated Leukoplakia, 25untreated Squamous cell carcinoma and 25 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.

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

Introduction

Oral cancer is a serious health problem worldwide accounting not only for mortality but also responsible for extensive disfigurement, loss of function, behavioral changes, financial and sociologic hardship. Frustration abounds because the cure rate is dismally low for such an accessible tumor.[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 globally[4] 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 tumor 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 tumor 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 glycolipids, which form the major constituents of cells, have been implicated in cellular invasiveness, adhesiveness and immunogenicity. 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]

Sialic acids frequently occupy the terminal, non-reducing position on membrane glycoproteins. 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 glycoproteins. 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, Faculty of Dental Science Nadiad, Gujarat, India. Age distributions of the patients were 20 to 70 years.

Sample Size was calculated as follows: Total number of subjects 75, Patients with Leukoplakia 25, Patients with Squamous cell carcinoma 25, Age and sex matched controls for comparison of results: 25.

Exclusion Criteria were 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 anyof the above mentioned diseases were excluded from the study. Controls were selected as included age and sex matched 25 healthy individuals with the same exclusion criteria as that of selection of cases**.**

Sample collection

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. SERUM SIALIC ACID was estimated as follows Free Sialic acid in serum reacts with Paradimethylaminobenzaldehydeto 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. 25 mg of SA powder was measured by physical balance and was dissolved in10 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).

Standard curve was prepared as follows. In another set of 6 test tubes, 0.5 ml of standard with 2.0 ml of 5% Per chloric 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. The serum (0.5 ml) collected from all 50 cases and 25 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]

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. The variables were assessed for normality using the Kolmogorov-Smirnov test. 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 25 subjects in Leukoplakia designated as Group-I, 25 subjects in Squamous cell carcinoma as Group-II, and age and sex matched 25 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, 7 cases (28%) were in the age group of 20-30 years, 6 cases (24%) were in the age group of 31-40 years. In Group II with 25 cases of Oral Cancer (Squamous Cell Carcinoma), the distribution of age was between 30-75 years. The age distribution among Group III was between 20-75 with 5 cases (20%) between the age of 20-30 years, 6 cases (24%).

Gender distribution: In Group I, Gender predilection was noticed. Of the 25 patients, 21 cases (84%) were males and 4 cases (16%) were females. Of the 25 cases in Group II, 12 cases (48%) were males and 13 cases (52%) were females. In Group III of 25 subjects, 15 cases (60%) were males and 10 cases (40%) 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.

TNM staging in 25 cases of Group II revealed 5 cases (20%) reported with Tumor size of T1, 13 cases(52.00%) with Tumor size of T2, 4 cases(16%) with Tumor size of T3, 3 cases(12%) with Tumor size of T4.

Serum Total Sialic acid: Group I showed the mean of 76.18 mg/dl with the standard deviation of 17.08 mg/dl; the interval of mean between 69.13 to 83.23 mg/dl, with the minimum of 45.00mg/dl to a maximum of 107.00 mg/dl. Group II showed the mean of 95.68 mg/dl with the standard deviation of 18.45mg/dl; the interval of mean between 88.06 to 103.30 mg/dl, with the minimum of 54.00 mg/dl to a maximum of 125.00 mg/dl. Group III showed the mean of 60.22 mg/dl with the standard deviation of 4.92mg/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.

In order to find out among which pair of groups there exist a significant difference, Annova and 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. 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.

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 conducted in faculty of dental science, Nadiad, Gujarat, included 25 Leukoplakia cases, 25 Squamous cell carcinoma cases and 25 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 25 patients were between the ages distribution of 31-40 years, similar study was showing the wider range of age distribution between 14-80 years of age with the median age of43 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].

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). 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 cavity[6,7,8].

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. 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[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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