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Evaluation of salivary contents in oral cancer: A preliminary study

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Abstract

Introduction: Salivary levels have been measured in many infections, autoimmune or psychiatric diseases and in various cancers. Salivary components such as kallikrein, epidermal growth factor, CA-125and p53 are used as tumor markers in breast, ovary or lung malignancies. There is short of studies using saliva as a diagnostic fluid for oral cancer. **Aim and objective:** Estimation of salivary total protein, sialic acid and total sugar in the oral cancer patients. **Material and method:** Unstimulated saliva was collected from 50 controls (Group I) and 50 oral squamous cell carcinoma patients (group II) and sialic acid, total protein, and total sugar were estimated and compared with the different histological grades. **Statistical analysis and Results:** Student's t test and ANOVA was performed for the statistical analysis. The level of salivary protein was lower in Group II compared to Group I. The level of total sugar, free sialic acid and protein bound sialic acid was elevated in Group II than Group I. The total protein and sugar and sialic acid were significantly high in well differentiated squamous cell carcinoma. **Conclusion:** We conclude that analysis of saliva can help in early diagnosis of oral cancer.

Keywords: Tumor marker, non-invasive, serum, histopathology.

INTRODUCTION

Cancer of head and neck region accounts for about 30-40% of all malignancies in India and is standing 6th world-wide for cancer-related mortality, with an expected 500,000 new cases diagnosed yearly [1]. Tansformed cell surface morphology is the seal of malignant cell. Early detection is the key to treat oral cancer [2]. Attempts are on to develop sensitive, specific and reliable biochemical tests for the early detection and diagnosis of the disease.

Saliva, a normal constituent of oral cavity, is a complex fluid comprising of different inorganic and organic elements which together alter the oral environment. It has a promising role in diagnosis of certain diseases, due to its ease of access, non-invasiveness and without use of any special instrumentation [3].

Certain glycoprotein tumor markers have been reported in saliva. Deviant glycosylations are the common feature of cancer and glycoconjugate levels increase as the cancer advances [4].

Patients suspected with any pathology show a change in total sugar (TS) and total protein level, hence it can abet in early diagnosis. So the present study is conducted to evaluate role of these constituents in oral cancer patients.

AIM AND OBJECTIVE

To estimate sialic acid, total protein, and total sugar in saliva of oral cancer patients and evaluate the role in diagnosis and prognosis of oral cancer.

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MATERIAL AND METHOD

Histopathologically confirmed oral squamous cell carcinoma (OSCC) cases with individuals in age group of 26- 73 years were selected. Cases with xerostomia and any other chronic systemic illness were excluded. The study group comprised of 50 healthy individuals (Group I) and 50 Oral squamous cell carcinoma patients (Group II).

Unstimulated saliva samples were collected from the histopathologically confirmed cases of OSCC. The subjects were asked to rinse with water to remove any food debris from oral cavity. Spitting forcibly was avoided to prevent blood contamination from the ulcerated lesion. 2ml of saliva was taken in plastic container and was sent for biochemical examination.

Estimation of biochemical factors in saliva

Sample was centrifuged for 15 min at 3000 rpm. Sialic acid with total protein and total sugar were analysed from the supernatant. Proteins were precipitated with ethanol. On the basis of reaction with ninhydrin reagent sialic acid level of precipitate and supernatant were calculated and matched to N-acetyl neuraminic acid values in range of 20.100 mg/ml. Total sugar content was evaluated by sulphuric acid and phenol reaction of sugar.

Statistical analysis

Analysis was done by Student's t test and one way analysis of variance (ANOVA). Mean difference between the groups was done by Tukey's post hoc test. Categorical groups were compared by chi-square test. p value less than 0.05 ($p<0.05$) was considered significant. Analysis was performed on SPSS software (windows version 17.0).

RESULT

Comparing the mean age of two groups, Student's t test showed significantly different and higher age of Group II as compared to Group I (35.60 ± 1.12 vs. 48.50 ± 1.13, t=6.25, $p<0.001$). In both groups, the incidence of males was higher than females and was higher in Group I than Group II. [Fig1a, 1b]

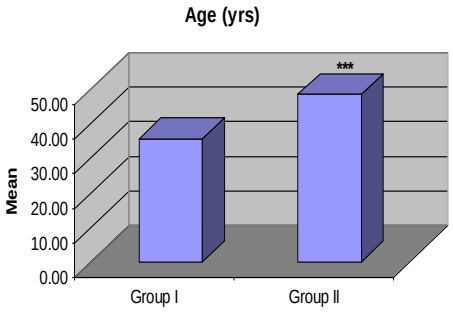

Figure 1a: Mean age of the patients.

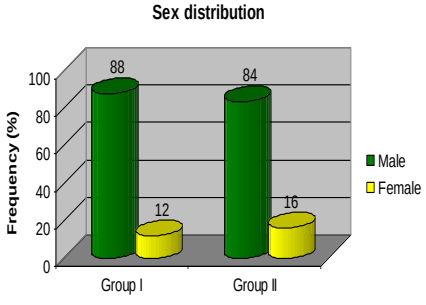

Figure 1b: Showing the males most commonly affected compared to females

Comparing the mean total protein of two groups, Student's t test showed significantly different and lower (50.8%) total protein of Group II as compared to Group I (99.80 ± 2.45 vs. 49.11 ± 2.19, t=12.51, $p<0.001$). [Fig 2]

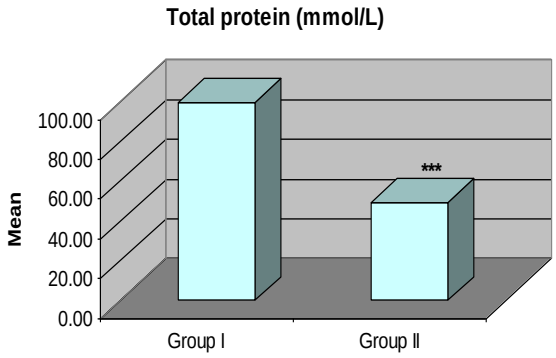

Figure 2: Mean protein of 2 groups

Comparing the mean total sugar of two groups, Student's t test showed significantly different and higher (87.2%) total sugar of Group II as compared to Group I (25.58 ± 0.91 vs. 200.07 ± 3.55, t=28.22, $p<0.001$).[Fig 3]

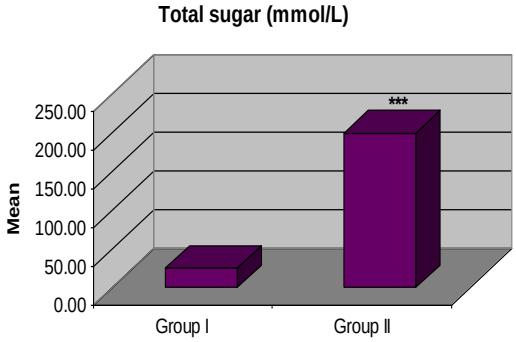

Figure 3: Mean total sugar of 2 groups

Comparing the mean free sialic acid of two groups, Student's t test showed significantly different and higher (20.3%) free sialic acid of Group II as compared to Group I (3.26 ± 0.09 vs. 4.10 ± 0.06, t=7.30, $p<0.001$). [Fig 4]

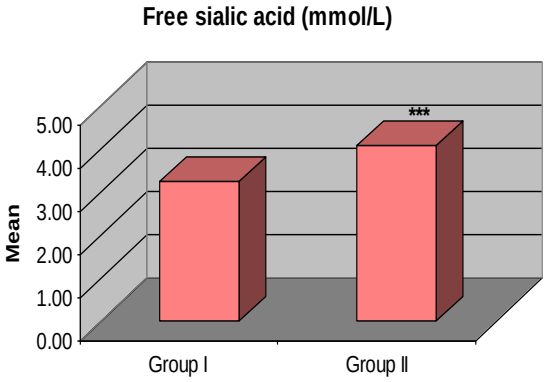

Figure 4: Mean free sialic acid of 2 groups

Comparing the mean protein bounded sialic acid of two groups, Student's t test showed significantly different and higher (27.7%) protein bounded sialic acid of Group II as compared to Group I (2.11 ± 0.09 vs. 2.92 ± 0.03, t=10.46, $p<0.001$). [Fig 5]

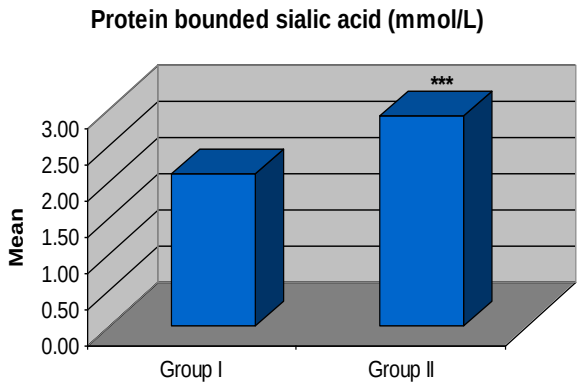

Figure 5: Mean protein bound sialic acid of 2 groups

Association between outcome measures and histopathological findings

The mean total protein in WD patients was the highest followed by MD and PD the least but was non significant. [Fig 6]

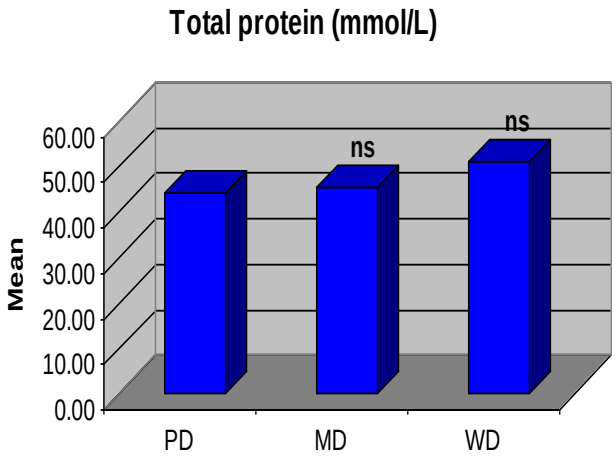

Figure 6: Mean total protein of 3 groups

The mean total sugar in WD patients was the highest followed by PD and MD the least, but was insignificant. [Fig 7]

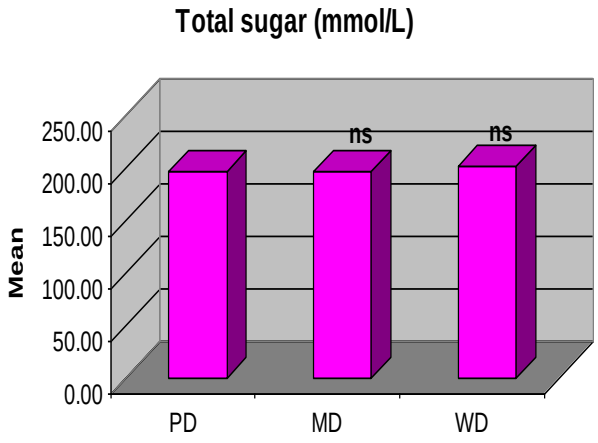

Figure 7: Mean total sugar of 3 groups

The mean free sialic acid in WD patients was the highest followed by MD and PD the least, but findings were non significant. [Fig 8]

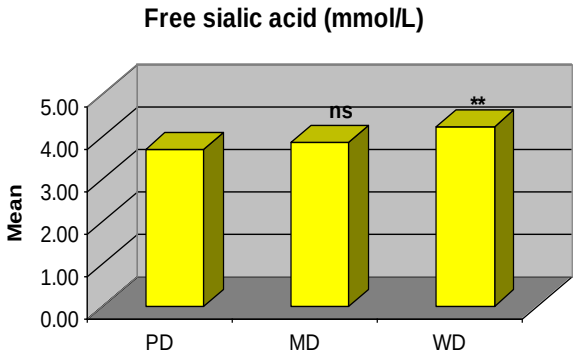

Figure 8: Mean free sialic acid of 3 groups

The mean protein bounded sialic acid in WD patients was the highest followed by MD and PD the least and was significant. [Fig 9]

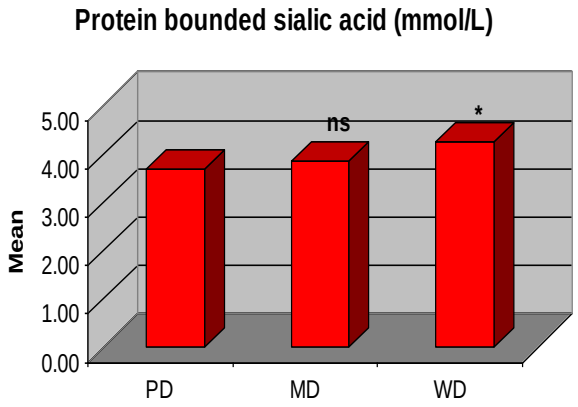

Figure 9: Mean protein bound sialic acid of 3 groups

DISCUSSION

In the present study, role of salivary parameters as bimomarker has been attempted. We found an increased level of total sugar, free and bound sialic acid and a decreased total protein. Stoyloff J and Ivanov SX (2005) in their study found a significant rise in serum sialic acid levels in head and neck tumors which is confluent with the present study [5, 6].

Rajpura K.B (2005) found significantly elevated serum levels of total and lipid bound sialic acid in oral cancer patients as well as in oral precancer similar to our study [7].

Koc (1996) in their study on salivary sialic acid between the cancer patients and controls found sialic acid levels were higher in the cancer group compared to controls [8].

Baxi in few of his studies has shown increased sugar and protein levels in OSCC which is similar to our study [1].

Dablesteen reported elevated protein and altered carbohydrate expression in OSCC contrast to present study in which protein level were significantly higher [9].

Sialic acid is a protein-bound monosaccharide which occurs in combination with other mono-saccharides like galactose, mannose, glucosamine and fucose. It is a constituent of many proteins in saliva and is mainly present in salivary mucin [3].

Aberrant glycosylations are the collective feature of cancer and level of glycoconjugates increases as the cancer advances [2]. Changes in level of serum sialic acid of cancer patient are associated with diminution in

tumor mass, recurrence and metastasis and are believed to be an important tumor marker in observing the clinical status of the cancer patient [4].

Glycoproteins and glycolipids are chief elements of cell membrane. Glycoconjugates can be released into the circulation by augmented turn over or shedding from malignant cells [5].

Aberrant glycosylation of tumor cells adds to the production of certain oligosaccharides.So, malignant cells contain a higher sialic acid residues on their surface [1].

CONCLUSION

The rise of serum sialic acid in oral cancer proposes the potential effectiveness of this factor in diagnosis as well as establishing clinical phase of malignant disease. Further studies need to be done in order to establish role of salivary glycoconjugates in oral cancer.

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