SALIVARY GLYCOPROTEINS AS INDICATORS OF ORAL DISEASES

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ABSTRACT

The present study deals with the estimation of total carbohydrate, protein bound carbohydrate, bound fucose and sialic acid along with total protein in disease conditions like gingivitis, periodontitis and their comparison with the normals.

The neutral hexose values in gingivitis (8.08 ± 2.20 mg/100mg protein) and periodontitis (12.5 ± 2.16 mg/100mg protein) decreased significantly when expressed per 100 mg protein compared to normals (19.8 ± 1.89 mg/100mg protein). This might be because of higher protein concentration in these two clinical conditions. The ethanol insoluble hexose values were significantly reduced in both these conditions compared to controls (3.71 ± 1.64 , 5.91 ± 1.63 , 7.65 ± 0.86 mg/100mg protein respectively). The ethanol soluble hexose values were also found to be drastically reduced. This decrease in saliva appears to be characteristic feature of oral diseases. In gingivitis and periodontitis fucose level was found to be increased compared to normals when expressed as a function of salivary volume. However in terms of protein concentration the values in gingivitis (2.95 ± 1.59), periodontitis (3.26 ± 0.98) and normals (3.20 ± 0.50 mg/100mg) were not different. Sialic acid in ethanol insoluble fraction of salivary samples mg/100mg protein was found to be significantly reduced in both gingivitis (0.78 ± 0.33) and periodontitis (0.95 ± 0.31) compared to controls (1.92 ± 0.33)

KEY WORDS

Salivary glycoproteins, fucose, sialic acid/analysis, periodontitis, gingivitis.

INTRODUCTION

Landquist showed that sugars in free form are present only in traces in fasting saliva(1). Most of the carbohydrate content of saliva is in bound form. The carbohydrate part of glycoproteins is readily split off from the proteins by ill defined bacterial enzymes (mucinases) in saliva, liberating reducing sugars, which cannot be readily distinguished from sugar being secreted by salivary gland(1).

The most prominent feature in periodontal diseases is connective tissue destruction. More specifically degradation of extracellular matrix (ECM)(2).

Enzymes capable of destroying all of the components of ECM are shown to be present within the periodontal environment. (3,4).

Arthur et al in their study, found a high gingival crevicular fluid fucose to protein ratio in periodontitis patients, compared to controls. This probably represents the

partial breakdown of glycoproteins, which may occur as a result of inflammation(5).

The aim of the present investigation was to compare the levels of total carbohydrate, bound fucose and sialic acid in saliva along with total protein in gingivitis and periodontitis

MATERIALS AND METHODS

O-cresol, mannose were the products of Aldrich chemical company, Milwaukee, U.S.A. Sulphuric acid used was analar grade (sp.gravity 1.84) from Ranbaxy laboratories, Punjab. N-acetyl neuraminic acid from Sigma Chemicals Co., U.S.A. Other reagents were analytical grade chemicals. Absorbance measurements were made in an Elico spectrophotometer (model SL-159) using cuvettes of one cm path length.

Patient selection

Saliva samples used in this study were collected from persons attending the out patient clinics of the department of Oral Diagnosis and Periodontology, S.D.M.College of Dental Sciences, Sattur, Dharwad. The control group consisted of student and staff volunteers who had normal gingiva and absence of loss of attachment as determined by probing. They were healthy with no systemic diseases. The second

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group consisted of patients, who presented symptoms of gingivitis (severe inflammation, marked redness, edema, ulceration, tendency to spontaneous bleeding and loss of attachment) as determined by the clinicians. The third group consisted of periodontitis patients who were selected based on radiographic evidence of alveolar bone loss and having at least 6 to 8 sites with a probing pocket depth of ≥ 5 mm.

Sampling technique

Unstimulated whole saliva samples were collected around 9-10AM, 2 hours after the subjects had breakfast. This was to ensure that the variability in salivary flow and composition be minimised due to diurnal variation. The subject was asked to rinse the mouth with distilled water thoroughly to remove any food debris, and then after 10 minutes directed to spit into a sterile glass beaker. Three ml of saliva was centrifuged for 10 minutes at 800 g to obtain a clear supernatant fluid, which was assayed immediately.

Salivary total protein level was estimated according to the method of Smith *et al* (6) using, bicinchoninic acid reagent using serum albumin as standard.

Salivary total carbohydrates was determined by o-cresol: H_2SO_4 method(7). Clear saliva (0.1ml) was made up to one ml with distilled water, followed by the addition of 3ml concentrated sulphuric acid. The solution was mixed thoroughly, vortexed and kept at room-temperature for 30 minutes. 0.1ml of ethanolic o-cresol (10mg) was added and mixed. The colour formed was measured after 30 minutes at 500 nm. Total carbohydrate values are expressed in terms of mannose units, for which D-mannose was used as standard.

Salivary bound carbohydrates were estimated by precipitating salivary proteins with ethanol and the precipitate was taken for the assay.

Salivary bound fucose was quantitated in saliva according to the method developed by Pattabiraman(8).

The precipitate obtained after ethanol treatment was suspended in 1.6ml distilled water. To this, 2.4 ml concentrated sulphuric acid was added and mixed. The solution was kept at room temperature for 30 minutes. Freshly prepared L-cysteine hydrochloride (50 μ l, 5mg) was added followed by 0.1 ml of ethanolic o-cresol (20 mg). After 30 minutes at room temperature, the chromogen formed was analysed at 490nm. The salivary protein bound fucose was calculated by running standard L - fucose simultaneously with the test.

The method developed by Yao and associates(9) was adapted for the estimation of protein bound sialic acid. To the precipitate obtained after ethanol treatment,

1.0ml of distilled water was added and mixed thoroughly. To this, 1.0ml glacial acetic acid was added followed by 1.0ml of acid ninhydrin reagent. The reaction mixture was heated in a boiling water bath for 10 minutes, cooled under tap water and absorbance was measured at 470nm. The salivary sialic acid was calculated by running standards containing N-acetyl neuraminic acid simultaneously with test.

RESULTS

Salivary neutral hexose values in healthy adults (n=20), varied from 16.5 to 26.2 mg /dl. There was no significant difference between males (n=10) and females (n=10) (data not shown). In periodontitis (n=20) total neutral hexose values varied from 12.8 to 25.4 mg / dl. Statistically there was no difference between normal healthy adults and periodontitis patients. In gingivitis (n=20) the values were in the range 12.6 to 24.0 mg/dl. The mean $\pm$ S. D. values were slightly lower in gingivitis than in normal adults and the difference was found to be statistically significant ($p < 0.01$).

In Table 1, neutral hexose values as functions of protein concentration are depicted. Compared to healthy adults, in periodontitis and gingivitis there was a significant decrease in values per 100 mg protein. This is because of increased protein levels in these two disease conditions as reported in our earlier publication(10)

In Table 2, neutral hexose values in ethanol precipitable fraction in different groups are given. There was no difference between the values in different groups. The values given for ethanol soluble neutral hexose are the calculated values based on the differences between total neutral hexose and ethanol precipitable neutral hexose. In gingivitis and periodontitis the decrease is statistically significant. The ethanol insoluble hexose values and also soluble hexose values when expressed per 100mg protein, drastic reduction was observed.

Fucose was determined in protein bound fraction (ethanol insoluble) in salivary samples. The data are shown in Table 2. In gingivitis and periodontitis fucose level found to be increased compared to normals, when expressed as function of salivary volume. However, in terms of protein concentration, there was no difference among the three groups.

In Table 2, levels of sialic acid in the ethanol insoluble fraction of salivary samples are indicated. Sialic acid per 100mg protein was found to be significantly reduced in both gingivitis and periodontitis.

DISCUSSION

Salivary total neutral hexose value (per unit volume) was found to be marginally decreased in gingivitis but

Table 1: Salivary total protein and neutral hexose in normals, gingivitis and periodontitis (as mannose units)

Group	Total protein level	Total hexose level	Total hexose level mg/100 mg
	Mg/100 ml, Mean $\pm$ S.D.	Mg/100 ml, Mean $\pm$ S.D.	protein, Mean $\pm$ S.D.
Normals (n=20)	92.59 $\pm$ 14.11	21.9 $\pm$ 2.71	19.8 $\pm$ 1.89
Periodontitis (n=20)	168.69 $\pm$ 32.67	20.4 $\pm$ 4.01	12.5 $\pm$ 2.16
Gingivitis (n=20)	217.88 $\pm$ 39.89	18.0 $\pm$ 2.97	8.08 $\pm$ 2.20

Table 2: Salivary ethanol precipitable, ethanol soluble neutral hexose, protein bound fucose and protein bound sialic acid level in normal gingivitis and periodontitis

Group	Salivary ethanol precipitable neutral hexose		Salivary ethanol soluble neutral hexose		Protein bound fucose		Protein bound sialic acid	
	Mg/100ml	Mg/100ml protein	Mg/100ml	Mg/100ml protein	Mg/100ml	Mg/100ml protein	Mg/100ml	Mg/100ml protein
Normal (n=20)	8.44 $\pm$ 1.22	7.65 $\pm$ 0.86	13.50 $\pm$ 1.71	12.2 $\pm$ 1.28	3.51 $\pm$ 0.55	3.20 $\pm$ 0.50	2.10 $\pm$ 0.34	1.92 $\pm$ 0.33
Periodontitis (n=20)	9.47 $\pm$ 2.08	5.91 $\pm$ 1.63	10.80 $\pm$ 3.27	6.35 $\pm$ 1.33	5.40 $\pm$ 1.65	3.26 $\pm$ 0.98	1.57 $\pm$ 0.47	0.95 $\pm$ 0.31
Gingivitis (n=20)	8.16 $\pm$ 3.02	3.71 $\pm$ 1.64	9.46 $\pm$ 3.33	4.20 $\pm$ 1.55	6.48 $\pm$ 2.69	2.95 $\pm$ 1.59	1.76 $\pm$ 0.71	0.78 $\pm$ 0.33

Values are expressed as mean $\pm$ S.D.

not in periodontitis. In both these conditions salivary protein level was found to be elevated. This non-specific increase is not of much diagnostic significance since it has been shown that in diabetes also salivary protein level is increased(11).

However when corrected to protein level, neutral hexose value was found to be significantly reduced in gingivitis and periodontitis. This is in sharp contrast to the observation in diabetes, where total neutral hexose level (units / mg protein) was not altered(12).

The neutral hexose in ethanol soluble fraction represents free salivary hexose (glucose) as well as glycopeptides and oligosaccharides not precipitated by ethanol. The reported salivary glucose value is around one mg/dl (12). In terms of mannose units, this will be close to 0.5mg/dl by the method used. Thus, the bulk of ethanol soluble neutral hexoses represent oligosaccharides and glycopeptides.

Ethanol insoluble (protein bound) neutral hexose levels

were found to be significantly decreased in gingivitis and periodontitis. Similarly, ethanol soluble neutral hexose levels were also found to be drastically reduced in these conditions when correlated to protein levels. In contrast protein bound hexose in saliva was reported to be elevated in diabetes(11). Thus decrease in ethanol soluble and ethanol insoluble hexose values in saliva appears to be a characteristic feature of oral diseases. It is probable, the decrease is due to increased level of glycosidases and increased utilization of released oligosaccharide units by microorganisms.

The level of glycosidases in saliva is reported to be increased in periodontitis and it returns to normal level on treatment(13). Exoglycosidases for the preferred anomeric links of the principal monosaccharide units of salivary glycoproteins are produced by the oral bacteria *in vitro* and in dental plaque *in vivo*. They may be responsible for the decreased bound carbohydrate level in oral diseases. There is indirect evidence to indicate that oral bacteria utilise the released

carbohydrates as metabolic fuel. Beighton and others(14) and Smith and Beighton(15) showed that withdrawal of dietary carbohydrate did not greatly influence the number of bacteria in dental grooves in periodontitis, 18 hours after they have been cleaned. According to Arthur(5) and others the increased fucose: protein ratio can be used as an indicator for periodontal disease progression. Dharapur(11) reported an increase in fucose and protein levels in diabetes in saliva. The alteration in basement membrane permeability as proposed by Mandel (16) is one of the causes for increased protein and fucose values in saliva in diabetes. The present study show that fucose level in saliva is increased both in periodontitis and gingivitis. However when normalised to protein concentration, fucose values did not differ in gingivitis and periodontitis compared to normal subjects unlike in diabetes. It is probable the increased glycoprotein level is offset by increased fucosidase activity leading to comparable level of fucose per unit of protein in gingivitis and periodontitis. The glycosidases could be of bacterial origin since adhesion of materials containing microorganisms at the tooth surfaces is considered to be a primary factor in the development of periodontal diseases (17-22).

The microorganisms either can release hydrolytic enzymes or agents that may cause direct damage to periodontal tissue by stimulating host mediated responses.

Shinohara and co-workers(23) studied the relationship between the sialic acid concentration in serum and whole saliva in rats with naturally occurring gingivitis. They suggested that the amount of sialic acid in saliva can be a useful index of the severity of periodontal disease. The present studies indicate that protein bound sialic acid level per se was found to be decreased in gingivitis and periodontitis. When correlated to protein level the decrease was found to be highly significant. The data suggest that increased sialidase activity could be the cause for the observation.

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