Evaluation of salivary α-L-Fucose and its related parameters in periodontitis

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Abstract

Background and objective: Periodontitis is one of the most widespread oral diseases in Kurdistan. Saliva can be used as a noninvasive diagnostic fluid to measure chemical biomarkers released during oral disease, so this work is directed to focus on the study of salivary fucose (a glycoprotein component) and its related parameters in periodontitis, to assess the possibility of using them as indicators for the disease and its progress.

Methods: The present work included 79 individuals. They were grouped into healthy (32), advanced periodontitis (20) and moderate periodontitis(27) subjects. Their age was ranged between (20-60) years. Unstimulated whole saliva samples were collected from all the groups. The samples were used for the estimation of salivary; total fucose (TF), protein bound fucose (PBF), protein bound hexose (PBH), lipid associated fucose (LAF), total protein (TP), total calcium (TCa), zinc (Zn), and salivary magnesium (Mg).

Results: Salivary TF, LAF, FF, PBF, TP and TCa were significantly increased in both advanced and moderate periodontic groups comparing to healthy individuals, while there were no significant differences in salivary PBH, Mg and Mg/Ca levels in periodontic groups when compared to normal. Salivary TF/TP, PBF/TP, PBH/TP and FF/TP ratios and salivary zinc were significantly reduced in periodontic males.

Conclusion: On the basis of the above results, it can be concluded that these biochemical parameters, may be used as indicators for the extent of periodontal tissue damage, thus they can be used in the identification of periodontitis progresses and treatment follow up.

Keywords: Periodontitis, salivary fucose, salivary minerals, glycoconjugates, glycoproteins.

Introduction

Periodontitis is an oral infection induced by microorganisms that affect the supporting tissues of the teeth which include; the gums, the alveolar bone and the periodontal ligament fibers. Periodontitis is characterized by a progressive destruction of the periodontal ligament and alveolar bone with pocket formation [1,2]. The clinical parameters that commonly used to diagnose periodontitis are: periodontal pocket or probing depth, bleeding on probing, clinical attachment loss, mobility of tooth and bone loss which determined by radiography [3]. Saliva is a dilute fluid that is secreted from the major and minor salivary glands; it is bathing the teeth and oral soft tissues to preservative and maintains the oral health. Saliva is composed mainly of water, electrolytes, glycoprotein, and antimicrobial enzymes [4,5]. Several studies have been shown that there is a relationship between quantitative changes of salivary components and some oral disease [6,7]. Saliva can be used as a noninvasive diagnostic fluid to measure chemical biomarkers released during periodontal disease [6,8]. Saliva contains glycoproteins, which consist of short carbohydrate chains covalently attached to either serine/threonine or asparagine residues in the protein core [9]. The carbohydrates part of glycoprotein consists nearly of nine monosaccharides, these are: D-Glu, D-Gal, D-Man, GlcNAc,

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GalNAc, α-L-fucose, sialic acid, L-Arabinose and D-xylose [10]. α-L-Fucose is also a component of saliva and its level is correlate well with synthesis and secretion of glycoprotein [11]. α-L-Fucose is an important component of many N- and O- linked glycans and glycolipids produced by mammalian cells [12]. It is a methyl pentose sugar similar to galactose except for the loss of the alcohol group on carbon number 6 [13]. It is found on various cell membranes and play an important role in cell-cell adhesion [14]. Studies have been shown that, serum and salivary fucose and its related parameters are important in the detection of oral disease (gingivitis, periodontitis and oral cancer) [15,16]. The present work was designed to evaluate the salivary fucose and its related parameters in patients with periodontitis. Thus this work may be a primary step in investigating the possibility of using these salivary biochemical parameters as a diagnostic tool in periodontal diseases. Thus the aims of this study is to Assess the salivary glycoproteins in periodontitis, throughout evaluating salivary total α-L-Fucose (TF) and its related parameters including; protein bound fucose (PBF), lipid associated fucose (LAF), free fucose (FF), protein bound hexoses (PBH), total protein (TP), and some salivary electrolytes that related with glycoproteins, such as: total calcium, magnesium and zinc in periodontitis.

Methods

The present study was carried out in Erbil city. The data were collected through a personal interview and clinical periodontal examination in the dental teaching clinics at College of Dentistry, Hawler Medical University, and Khanzad Specialized Dental Center during the period of 10th November 2010 up to 1st April 2011.

Subjects (Study populations):

The subjects composed of two categories:
1. Thirty two healthy males (normal or control group), they represented individuals were free from all disease and they didn't take any medications at least 15 days Before sampling, their age rang was beween (20-54) years.
2. Forty seven male patients with periodontitis free of other diseases, their age rang was (20-60) years. These patients are classified into; advanced periodontitis (20) and moderate periodontitis (27), depending on the severity of the disease.

Clinical measurement:

The clinical periodontal examination measurement were carried out by the specialized dentists, to measure the bleeding on probing, pocket depth (PD), mobility of teeth and clinical attachment loss (CAL). Pocket depth (PD) was measured with calibrated periodontal probe (Williams probe) at four sides per tooth [3,17].

Saliva collection:

Unstimulated whole saliva samples were collected around 9 am to 12 pm, 2 hours after the subjects had breakfast [18]. The collected saliva was centrifuged for 10 minutes at (1000 g) to obtain a clear supernatant fluid [15], then each sample was divided into three portions and they were stored at -20^{0}C, until analysis.

Methods:

1- Salivary TP was determined by Biuret method described by Gornall et al[19,20], using a special kit.
2- Salivary TF level was estimated using Dische& Shettles method[21-23].
3- Salivary PBF level was determind using Dische& Shettles method[21-24].
4- Salivary LAF level was determined depending on Katopodis method[22,25,26].
5- Salivary PBHex level was estimated depending on orcinol reaction[24-26].
6- Salivary TCa, Mg and Zn were determined by atomic absorption spectropy.
7- The ratio of each of the above parameters to TP was calculated.

Statistical analysis: All data were expressed as mean ± standard error (SE). The statistical analysis was carried out using Statistical software (SSPS version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). The result were considered statistically significant(S) if the (P≤ 0.05) and highly

significant if the (P≤ 0.001).

Results

The mean and standard error (±SE) of salivary TF, PBF, FF, LAF, PBH and TP in moderate periodontitis, advanced periodontitis and normal groups are given in Table 1. The levels of TF in moderate and advanced periodontitis were significantly (P≤0.05) higher comparing to normal group . Salivary PBF was significantly higher in advanced periodontitis when compared to normal group, whereas no significant difference was observed between moderate periodontitis and normal group. The levels of LAF in moderate and advanced periodontitis groups were significantly (P≤0.05) higher comparing to normal group. The patients with moderate and advanced periodontitis had significantly (P≤0.05) higher levels of free fucose when compared to normal group . Salivary TP levels in moderate and advanced periodontitis groups were significant (P≤0.001) high comparing to the normal group, whereas PBH levels showed no significant difference in patients with periodontitis comparing to normal males. Table 2 and figure 1 show the mean and standard error (± S.E) values of salivary TF/TP, PBF/TP, FF/TP and PBH/TP ratios. The values of these ratios were significantly decreased in patients with advanced and moderate periodontitis groups when compared to normal group. Table 3, shows the comparison of the levels of calcium, magnesium, Mg/Ca ratio and zinc among the patients with moderate and advanced periodontitis and normal males. A significance increase (P≤0.05) in the levels of calcium in patients with advanced periodontitis was observed comparing to normal group. Salivary zinc levels significantly decreased (P≤0.05) in the periodontitis compared to the healthy subjects, whereas non-significant difference was observed in the levels of salivary magnesium and Mg/Ca ratio among patients with moderate, advanced periodontitis and normal groups.

Table 1: The mean, and standard error (±SE) of salivary total fucose and fucose related parameters in periodontitis and normal groups.

Chemical parameters	Groups	N	Mean	±S.E
*TF (mg/dL)	Moderate periodontitis	27	15.64	1.11[b]
	Advanced periodontitis	20	18.19	1.36[b]
	Normal male	32	10.73	0.57[a]
*PBF (mg/dL)	Moderate periodontitis	27	2.89	0.23[a]
	Advanced periodontitis	20	3.85	0.58[b]
	Normal male	32	2.58	0.24[a]
*LAF (mg/dL)	Moderate periodontitis	27	6.61	0.54[b]
	Advanced periodontitis	20	6.66	0.45[b]
	Normal male	32	4.70	0.34[a]
*FF (mg/dL)	Moderate periodontitis	27	6.16	0.69[b]
	Advanced periodontitis	20	7.69	0.91[b]
	Normal male	32	3.46	0.42[a]
*PBH (mg/dL)	Moderate periodontitis	27	11.86	0.99[a]
	Advanced periodontitis	20	12.69	1.03[a]
	Normal male	32	13.26	1.14[a]
**TP (gm/dL)	Moderate periodontitis	27	1.65	0.14[b]
	Advanced periodontitis	20	1.89	0.19[b]
	Normal male	32	0.47	0.04[a]

Different letters in the same column refer to significant changes, while similar letters refer to non-significant changes. ** (P≤0.001), *(P≤0.05) .

Table 2: Salivary total fucose and fucose related parameters to total protein ratio in periodontitis and normal males.

Chemical parameters	Groups	N	Mean	±S.E
TF/TP (mg/gm)	Moderate periodontitis	27	10.60	0.79[a]
	Advanced periodontitis	20	11.33	1.28[a]
	Normal male	32	26.40	1.94[b]
PBF/TP (mg/gm)	Moderate periodontitis	27	2.05	0.20[a]
	Advanced periodontitis	20	2.28	0.38[a]
	Normal male	32	6.31	0.64[b]
FF/TP (mg/gm)	Moderate periodontitis	32	4.36	0.51[a]
	Advanced periodontitis	27	4.98	0.80[a]
	Normal male	20	8.03	1.01[b]
PBH/TP (mg/gm)	Moderate periodontitis	32	8.42	0.94[a]
	Advanced periodontitis	27	8.00	1.01[a]
	Normal male	20	33.21	3.54[b]

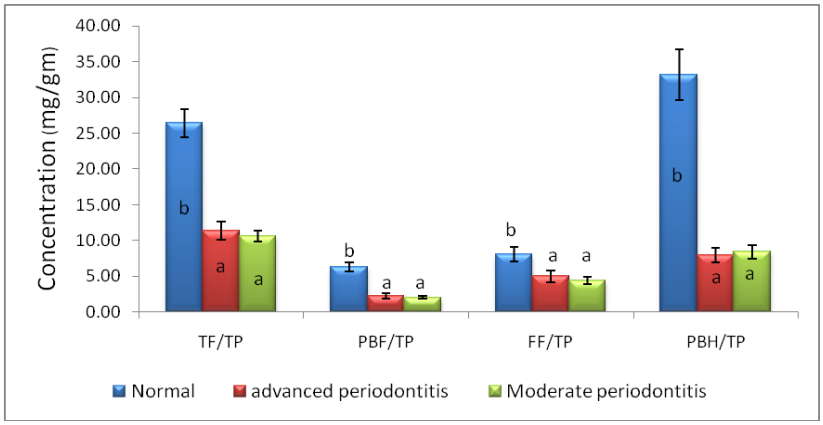

Figure 1: Comparison of salivary total fucose, protein bound fucose, free fucose, and protein bound hexose to total proteins ratio between periodontitis and normal groups.

Table 3: The mean and standard error (SE) for salivary calcium, magnesium, Mg/Ca ratio, and zinc in periodontitis and normal groups.

Chemical parameters	**Groups**	**N**	**Mean**	**±S.E**
Magnesium (ppm)	Moderate periodontitis	27	2.52	0.31[a]
	Advanced periodontitis	20	2.98	0.42[a]
	Normal male	21	2.60	0.26[a]
Zinc(ppm)	Moderate periodontitis	27	0.70	0.04[a]
	Advanced periodontitis	20	0.81	0.05[a]
	Normal male	21	1.04	0.06[b]
Calcium (mg/dL)	Moderate periodontitis	27	3.80	0.37[a,b]
	Advanced periodontitis	20	4.71	0.54[b]
	Normal male	32	3.37	0.23[a]
Mg/Ca	Moderate periodontitis	27	0.78	0.095[a]
	Advanced periodontitis	19	0.89	0.28[a]
	Normal male	20	0.73	0.09[a]

Discussion

Glycoproteins are compounds composed of protein and carbohydrate, in which the sugars are hardly linked to the peptide. Changes in the levels of different glycoproteins have been associated with different types of disease. Among the nine sugars present in the structure of GPs, fucose can be used reliably and conveniently for the investigations of glycoprotein synthesis and secretion [10]. The fucose levels elevation in these pathological conditions was interpreted by the alteration in both Glycoproteins metabolism and fucosylation of Glycoproteins. In the present study, salivary TF, LAF, and FF levels were significantly elevated in both moderate and advanced periodontitis compared to normal group, while a significant increase in salivary PBF was observed only in advanced periodontitis patients. This increase in salivary fucose parameters in periodontitis might represent the breakdown of plasma and tissue glycoproteins which may occur as a result of inflammation [27]. There is indirect evidence to indicate that, increased salivary glycoprotein level is offset by increased fucosidase activity that causes the breakdown of saliva and tissue glycoproteins [15]. Thus periodontitis may cause an increase in fucosidase activity. L-fucosidase,which is a lysosomal enzyme present in all mammalian cells, and an important enzyme for fucose catabolism. It is also responsible for the alteration of fucosylation status of glycoproteins [16]. The results of salivary total protein showed a significant increase in moderate and advanced periodontitis subgroups when compared to normal group. The increase in salivary total protein concentration in periodontitis patients may be related to the salivary glands, which had responded to inflammatory disease (periodontitis) by enhancing synthesis of acinar proteins. The synthesis of protein or glycoprotein increased with progression severity of periodontal disease [28]. In addition, the rise in salivary albumin also plays a role in the rise in the total proteins. High salivary albumin levels that found in subjects with gingivitis and periodontitis, may be due to the leakage of plasma proteins as a results of the inflammation [29]. Thus, the results of the present study indicated that salivary total protein concentration can be used as a biochemical marker for periodontal disease. The results also indicated that salivary total protein levels increased with the degree of gingival inflammation and probing pocket depth. In general, the ratios of salivary TF, PBF, FF and PBH to total protein in advanced and moderate periodontitis were significantly reduced when compared to normal individuals. Thus decrease these appears to be a characteristic feature of oral diseases. It is possible that, the decrease of these parameters may be due to increase in glycosidases [30], and Fucosidase [16], and may be due to increase in utilization of the released oligosaccharide units by microorganisms. Salivary total calcium concentrations were increased significantly in advanced periodontitis. A high level of salivary calcium is closely related to the rapid demineralization of plaque in periodontitis [31]. Increasing in salivary calcium was also correlated with increasing in salivary glycoprotein or protein in patient with periodontitis. Elevation of salivary total calcium in periodontitis, may be associated with bone resorption, which occurs when host cells respond to periodontal pathogenic bacteria, by releasing of cytokines [32]. Salivary zinc concentration significantly reduced in advanced and moderate periodontitis subgroups when compared to the normals. Several studies showed that patients with periodontitis had decreased serum Zn levels when compared to normal group [33,34]. The decrease in salivary zinc in periodontitis, may be related to increase in rate of zinc as salt deposition in cementum and plaque of involved teeth. These elevations in zinc in cementum and plaque, and decreasing in saliva, may play roles in the progress of periodontitis [35]. In general, the salivary magnesium level and Mg/Ca.

ratio did not change in periodontitic when compared to normal group. Yoshihara *et al*. [36] indicated that, the levels of calcium and magnesium in serum are associated with periodontal disease. They published that lower level in serum Ca^{2+}/Mg^{2+} ratio was associated with periodontal disease progression in Japanese elderly smokers. On the basis of the results of the present study, it can be concluded that these biochemical parameters, may be used as indicators for the extent of periodontal tissue damage. Thus they can be used in the identification of periodontitis progresses and treatment follow up.

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