

Enzymatic Markers of Salivary Cell Injury in Saliva of Type 1 Diabetic Children

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Abstract:

Salivary components may suffer variations that can be detected by chemical determinations. The salivary glands may be additional target of the immunological attack mainly directed against pancreatic beta-cells resulting in type 1 diabetic children. Changes in enzymatic activity reflect metabolic changes in diabetic children and inflammation.

Aim of the study: The work is devoted to prove the hypothesis that changes in glucose concentration, aminotransferases, lactate dehydrogenase can be used as indices of cellular injury in the whole saliva of diabetic children, and to determine the physical and biochemical characteristics of saliva of a group of diabetic children compared to a control group in relation to oral health indices.

Subjects and methods: Twenty six diabetes children (5-19 years) and twenty control children (5-11 years) were included in this study. Glucose, aspartate and alanine aminotransferase (AST, ALT), and lactate dehydrogenase (LDH) were determined by enzymatic methods.

Results: Obtained results had shown statistically significant increases of saliva glucose level and increase in the activity of AST, ALT and LDH in saliva from diabetic patients and control group. The results also demonstrated that acidic pH diminishes salivary flow rate and excess foam is usually present in saliva of diabetic children.

Conclusion: These differences were confirmed by the discrimination test. Diabetic children have higher DFS (decayed filled surfaces) compared to those of control children. Some salivary components in addition to the diminished flow rate could be involved in the characterization of the oral health state of diabetic children.

Key words: Diabetes, children, saliva oral health.

Introduction:

Saliva has been discussed lately as an important biological material for the purpose of introducing new diagnostic tests which may contribute in the diagnosis and explaining the pathogenesis of many systematic diseases, such as : leukemia ,Sjogren´s syndrome , AIDS, systemic lupus erythematosus and diabetes mellitus⁽¹⁾.Among the important saliva components are various intracellular enzymes that are increasingly released from the damaged cells of periodontal tissues into the gingival fluid and saliva. These which are particularly relevant in this group of enzymes are the followings: aspartate and alanine aminotransferase (AST, ALT), and lactate dehydrogenase (LDH) ⁽²⁾.

Diabetic adults usually present altered salivary secretion rate that can cause disorders of hard and soft tissues of the mouth leading to cariostatic and gingival lesions. However, in children there is still no agreement in results that relate alteration of saliva chemical composition and oral health^(3,4) . Karjalainen ,etal.,⁽⁵⁾ reported that in poorly controlled children and adolescents the (DFS) (decayed filled surfaces) indices were significantly high and diabetes was associated with caries. However, the difference was not statistically significant if adjustments were made for age or age at the onset of diabetes and duration of the disease. Diabetes also increases the risk of periodontitis , which appeared at earlier ages ,and periodontal disease progression in adults ⁽⁶⁾ .

It is well known that oral disease can be caused by a number of factors of the oral cavity, with microorganisms being one of the main factors. However, diabetes may cause alterations of the salivary glands, which contribute to an increased pathogenic bacteria number. A slow flow rate also affects the oral flora and alters saliva composition ⁽⁷⁾ . So far not many salivary parameters have been used to characterize illness states, probably because of the great variability they usually present in whole saliva ⁽⁸⁾ .In the specific case of diabetes mellitus, there is no agreement on salivary parameters in children or adults. Many authors found higher glucose salivary levels in diabetic patients than in non-diabetics ^(3,9) .

While Sharon et al., ⁽¹⁰⁾ did not report any difference. The aim of this study is to describe physical, biochemical and dental characteristics of totals saliva of a group of children with diabetes mellitus, and to determine the most indicative salivary parameters of this illness.

Materials and methods:

Twenty six diabetic children (14 females and 12 males) admitted to the Endocrinology center at Al-Yarmok hospital were selected. Their chronologic ages ranged from (5-19 years) mean \pm SD: (12. 69 \pm 4.44) . On the day of saliva collection none of them were in metabolic acidotic state nor in a coma although several where in these states before. All diabetic children were treated with human insulin. The control group consisted of 20 clinically healthy children (12 females and 8 males) between (5 -11 years) of age (mean \pm SD) (8.7 \pm 1.2).

Saliva collection, physical characterization and conservation:

Total saliva collection from the diabetic and the control groups was performed in the morning. Stress situations of the children prior to and during saliva collection were avoided.

Saliva (5 min production) was collected with a sterile syringe. Avoiding contact with epithelia. No stimulation or spitting was practiced. Saliva was placed into chilled graduated tubes and brought immediately to the laboratory.

Flow rate was defined as the volume of saliva secreted per min. Viscosity, turbidity and foam were determined immediately in order to avoid variations and subjectivity of the observer, using a scale from + to +++. Foam formed was observed as soon as saliva was placed in the tube. Salivary turbidity was checked in the liquid collected, before ice-chilled, and viscosity was determined by the rupture of the mucus while taking the syringe off the tube. PH was also determined immediately using a Corning PS-30 PH meter. Once saliva was collected, it was centrifuge at 5000 rpm for 10 min , fractionated and frozen for further analyses.

Determinations of Saliva chemical constituents:

Glucose determination was performed using 100µl of saliva by the glucose-oxidase method. The activity of enzymes in saliva was determined spectrometrically ^(6, 7). The determination of enzymes activity was instant being aware that LDH activity decreases rapidly when frozen and we did not dispose other alternative method and device ⁽¹¹⁾. The applied statistical analyses were the followings: mean value, standard deviation, correlation coefficient and student's t-test.

Results:

Table 1 shows the mean \pm SD of saliva flow rate , pH, viscosity , turbidity , and foam formation for saliva of male and female diabetic and non diabetic children . Flow rate diminished significantly in diabetic children as reported by other authors ⁽³⁾ .Small sex differences were observed in both groups for pH. It can not be excluded that some of the differences observed between genders are due to differences in ages. Diabetic saliva was more viscous and had more foam than control saliva.

Table (1): Mean \pm SD of physical characteristic of saliva from diabetic and control children.

	Diabetics (n= 26)			Control (n= 20)		
	Male n=(12)	female (n=14)	Total	Male n=(8)	female (n=12)	Total
Flow rate (ml/min)	0.15 \pm 0.09	0.16 \pm .13	0.15 \pm 0.11	0.23 \pm 0.13	0.27 \pm 0.14	0.25 \pm 0.13
pH	6.81 \pm 0.51	6.93 \pm 0.64	6.89 \pm 0.58	6.98 \pm 0.33	7.16 \pm 0.27	7.08 \pm 0.3
viscosity	+++	+++	+++	++	++	++
Turbidity	+++	+++	+++	++	++	++
Foam	+++	+++	+++	++	++	++

Biochemical determinations showed important differences between both groups of children (table 2). Glucose, AST, ALT and LDH were greater in diabetic saliva, although showed a wide range of SD.

Table 2, Figure 1 show that saliva glucose levels in diabetic patients (Mean 24.45 gm/dl, SD 7.8) as compared to healthy controls (Mean 15.08 gm/dl, SD5.99). The difference was significant $p<0.0005$. also they show that saliva AST levels in diabetic patients (Mean 49.53IU/L, SD 17.4) as compared to healthy controls (Mean 11.87IU/L, SD4.8). The difference was also significant $p<0.0005$. The difference in ALT levels patients (Mean 58.07IU/L, SD 23.9) as compared to healthy controls (Mean 5.125IU/L, SD2.4), and LDH levels in patients (Mean 265IU/L, SD 49.39) as compared to healthy controls (Mean 227IU/L, SD24.16) were also significant $p<0.0005$.

Table (2): Difference between glucose (mg/dl), AST, ALT and LDH(IU/L) activity in saliva patients of type I diabetes and healthy control .

Biochemical parameters	Diabetics (n= 26)	Healthy controls (n= 20)	T-test
Mean \pm SD			
Saliva glucose	24.4 \pm 7.8	15.08 \pm 5.99	
Saliva AST	49.53 \pm 17.4	11.87 \pm 4.8	8.73E -122
Saliva ALT	58.07 \pm 23.9	5.125 \pm 2.4	1.41758E-11
Saliva LDH	265 \pm 49.39	227. \pm 24.16	0.002537

* Significant difference between control and patients ($p<0.005$)

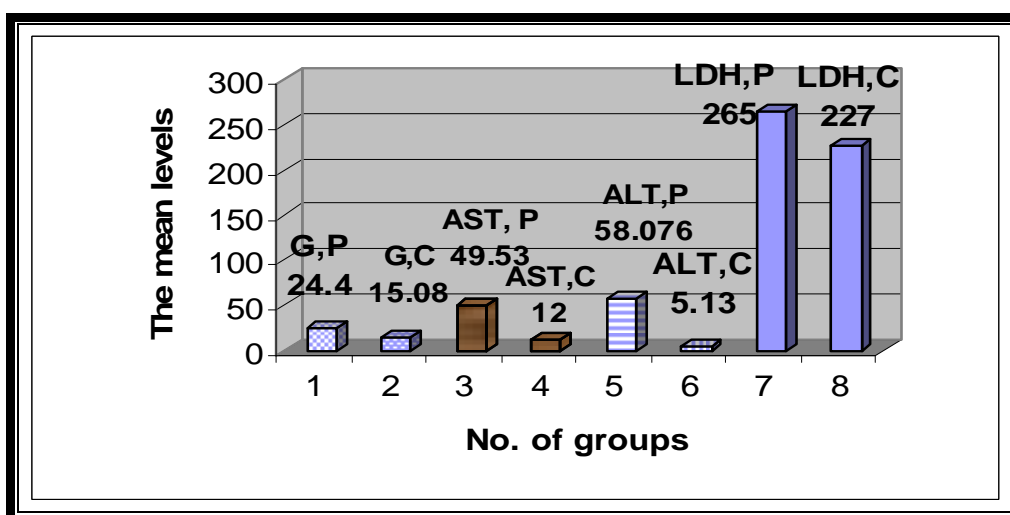


Figure (1): The mean levels of biochemical parameter (glucose=G, AST=Aspartate aminotransferase,ALT =Alanine aminotransferase and LDH = lactate dehydrogenase) in patients(p) and healthy controls (c).

Saliva glucose level was positively significantly correlated in diabetic patients ($r= 0.52$), $p<0.05$. (Figure 2)

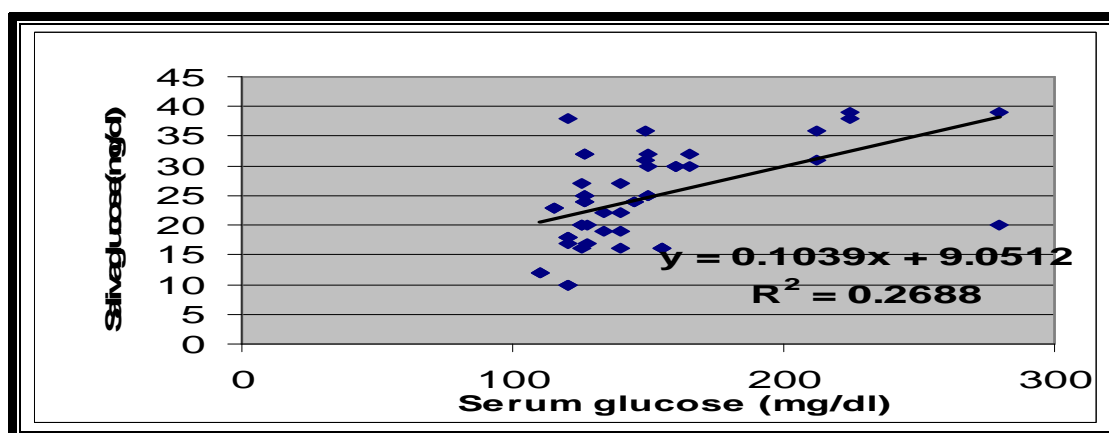
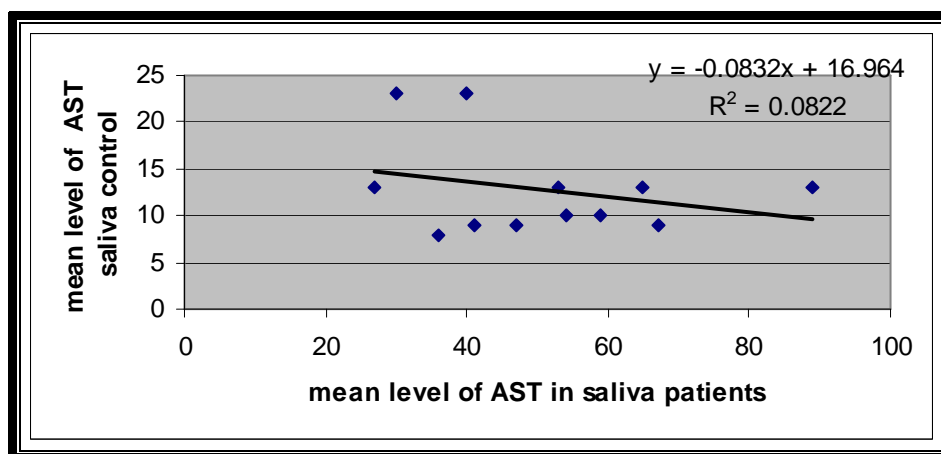


Figure (2): The correlation of saliva glucose levels (mg/ml) in patients of diabetes and control. ($r= 0.52$)

Saliva AST level was significantly negatively correlated in diabetic patients and control ($r= 0.3$), $p<0.05$. (Figure 3)



Figure(3): The correlation of saliva AST levels (IU/L) in patients and control. ($r= 0.3$)

Saliva ALT level was significantly negatively correlated in diabetic patients and control ($r=0.56$), $p<0.05$. (Figure 4)

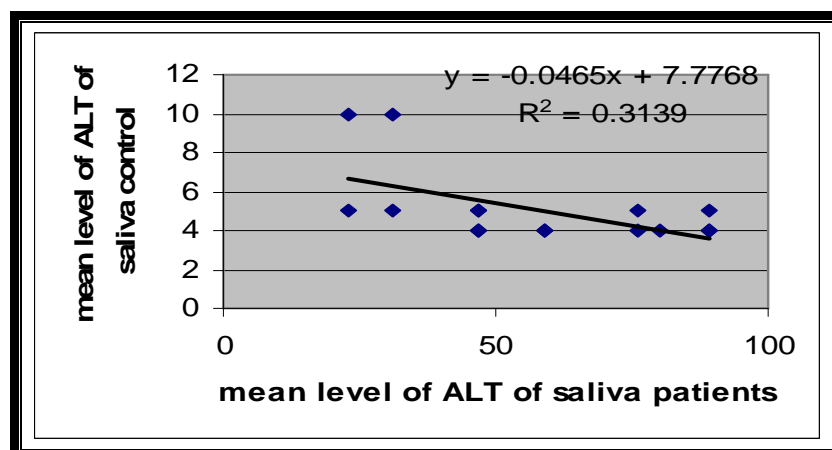


Figure (4): The correlation of saliva ALT levels (IU/L) in patients and control. ($r=0.56$)

Saliva LDH level was significantly negatively correlated in diabetic and control ($r=0.45$), $p<0.05$. (Figure 5)

Discussion

In this study, the biochemical aspects of saliva and oral health bereaved were compared between diabetic and non diabetic control children with similar characteristics.

Whole saliva was unstimulated on collection. Flow rate, which was significantly diminished ($p<0.001$) in diabetics, was associated with saliva viscosity and foam. Foam is reflected by the higher level of proteins in diabetic patients and salivary turbidity is related to mucus ,epithelial cells and especially to oral bacterial presence⁽⁶⁾. There are much works relating diminished flow rate to diabetic adults and children as if the overall dehydration could cause irreversible changes in the glands⁽⁵⁾.

The low pH in diabetic children may be associated either to microbial activity or to a decrease of the bicarbonate level with flow rate.

The prevalence of DM was found to be higher in female children than in males. This is in agreement with the work of Levitt, N.S.1993 and Abdul-Ridha , F.,M,1998^(12,13) who reported lower prevalence of DM in males than in females and this discrepancy increase with age. They explain these results on the basis of female saliva glands being smaller than those of males, and probably may be also due to estrogen hormone.

Salivary glucose was significantly higher ($p<0.0005$) in diabetic patients than in the control group. The increase in saliva glucose in diabetic patients may be due to the fact that diabetic patients have usually gingival inflammation causing increased capillary fragility in the reticular tissue. This condition allows more glucose to pass from the hyperglycemic blood. In addition to that diabetic children have increased membrane permeability⁽¹⁴⁾. Salivary glands act as filters of blood glucose that would be altered by hormonal or neural regulation⁽¹⁵⁾

Diabetic patients have usually periodontal disease and numerous markers in saliva have been proposed as a diagnostic test for periodontal disease such as intercellular enzymes (CK, LDH, AST, ALT, GGT, ALP and ACP). Their activity can be proved in saliva, within some normal limits, as these enzymes are determined even in blood of healthy persons. However, if a periodontal tissue becomes diseased, or its cells are damaged, due to edema or destruction of a cellular membrane, intracellular enzymes are increasingly released into the gingival crevicular fluid and saliva and their activity can then be measured⁽¹⁶⁾.

Lactate dehydrogenase, AST,ALT are intracellular enzymes included in metabolic processes of cells and they are mostly present in soft tissues.

These enzymes are indicators of a higher level of cellular damage and their increased activity in gingival crevicular fluid and saliva is a consequence of their increased release from the damage cells of soft tissues of periodontium and reflection of metabolic changes in the inflamed gingival^(2,16). Other studies concluded similar observations, although most of them were related to testing the activities of these enzymes in gingival crevicular fluid but not in saliva of oral cavity⁽¹⁵⁾. The major number of studies were focused on the activity of these enzymes in saliva in relation to gingivitis and periodontal diseases and shown similar results with our study⁽¹⁶⁾. This paper is a study which has shown that the increased activity of certain tissue enzymes in periodontal disease can be proved in saliva as a reflection of the pathological changes in the cell of periodontal tissues. The value of their activity can reflect the depth of pathological process and damages in soft tissues. That is to say, this study shows a good correlation between the activities of AST, ALT and LDH in saliva and the value of gingival index. i.e. by increasing the value of gingival index, the activity of the above mentioned enzymes was linearly increasing⁽¹⁷⁾. This could be also stated on the basis of the typical enzyme profile in periodontal disease in relation to the healthy persons. The increased activity of AST, ALT and LDH indicates the pathological changes located in soft tissues only. The activity of these enzymes in saliva can be of useful for the assessment of efficiency of changing the therapy in curing periodontal disease^(2, 16).

A negative correlation was found between enzymatic activities and duration, the highest values being detected in the diabetic subgroup diagnosed for less than a year. This suggests that some cell damage could be present in salivary glands of recently diagnosed diabetic children, likely as a result of immune-mediated alterations. This result is in agreement with those of Cinquini⁽¹⁸⁾

Conclusion:

On the basis of results obtained it can be concluded that the activities of ALT,AST and LDH enzymes were significantly increased in the saliva of diabetic patients in relation to healthy subjects. This is probably a consequence of pathological process in periodontal tissues where from these intercellular enzymes are increasingly released into the secretion which surrounds them saliva. It was also established the correlation between the enzyme activity and the value of gingival index. On the basis of the results of this study the salivary enzymes can be considered as a biochemical markers of the functional condition of tissue damage that provide new opportunities in making diagnosis and following the efficiency of curing periodontal disease.

A negative correlation was found between enzymatic activities and duration, the highest values being detected in the diabetic subgroup diagnosed for less than a year.

This suggests that some cell damage could be present in salivary glands of recently diagnosed diabetic children, likely as a result of immune-mediated alterations. These results may support the hypothesis that, the salivary glands could be an additional target of the immunological attack mainly directed against pancreatic cells and resulting in type 1 diabetes.

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