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Salivary Alpha Amylase as a Marker to Predict Diabetes Mellitus in Children with Family History of Diabetes

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Abstract: ***The overall look of signs of adult serious diseases in children indicates that genetics are important, because the environment had only a short period of time to act. Genealogy of diabetic has been recognized as a significant threat factor of the disease. Family health background symbolizes valuable genomic information because its characterizes the combined connections between ecological, behavioural, and genetics. The aim of this research was to investigate the level of α-amylase in un-stimulated whole saliva of healthy children in relation to some oral health factors and to family history of diabetes; with the objective to compare salivary amylase stage in children with positive family history of diabetes and those with negative history. Questionnaires were filled by children families consisted of demography, health background and close relatives record of diabetes mellitus, with close relatives record categories were defined by 1st and second relative's. From 100 healthy primary school, saliva examples were gathered for 5- minutes between 9:00-11:00 AM. Flow-rate, Oral plaque and Gingival index were assessed using WHO criteria. Salivary amylase was analysed using EnzyChrom™ α-Amylase Analysis Kit (Quantitative Colorimetric Amylase Determination at 585nm). Considering family history, positive family history of diabetes mellitus was positively, associated with salivary amylase concentration. Children with positive family history of diabetes mellitus showed higher salivary amylase level compared to those with negative family history. According to the knowledge of the effect of diabetes mellitus on saliva, the basement membrane permeability of parotid gland is higher in diabetes mellitus and the components for example amylase, protein and glucose in blood raised , so raising their levels in saliva. This might be due to hormonal and metabolic changes related with disease. In this study Higher levels of alpha-amylase in saliva of children with a family history of diabetes mellitus might be important to recognize children at an increased risk for DM. Considering sex, males created greater salivary circulation amount in comparison to females, however the difference was non-significant. Several straight line regression research revealed that, being amales is expected to reduce salivary amylase by a mean of 11U/L in comparison to women, however non-significance, A good straight line connection was found between age of children and salivary amylase level (r=0.400 , P<0.001). Children with higher scores of gingivitis showed higher salivary amylase level (291.1 U/L) compared to those with lower score of gingival index (281.9 U/L).***

Keywords: children, unstimulated salivary flow rate, family history of diabetes

1. Introduction

The overall look of signs of mature serious diseases in children indicates that genetics are essential, because the environment had only a short period of time to work (1).

However, ecological risks are also at work, with severe deterioration in the patterns of diet and Physical activity as opposed to previous eras. Changes in exercising have been encouraged by modifications to environment and the technology we have come to depend on in our daily lives (1). In greater numbers of genetically susceptible people exposed early to risks, improved frequency and intensity of ecological risks, for serious illness as a result of both urbanization and industrialization (2).

Family history of diabetic issues has been identified as a significant risk factor of the disease. Family healthcare history symbolizes valuable genomic information because its characterizes the combined connections between ecological, behavioral and genetics.

First degree relatives of patients with kind 1 kind two diabetic issues obviously have an improved threat for the disease (3). children an improved diabetic issues threat can be identified prior to the overall look of islet autoimmunity by close relatives background inherited testing (3) Type 1 Diabetes outcomes from the destruction of insulin-producing beta cells in the islets of the pancreas by the body's immune system. Before the infection presents with symptoms, underlying autoimmunity to the insulin-producing cells can be detected by measuring antibodies in the blood stream vessels. These antibodies are known as islet autoantibodies and the condition is known as islet autoimmunity. children an increased diabetic issues threat can be identified prior to the overall look of islet autoimmunity by close relatives record for your body and inherited testing (3).

Alpha- amylase is one of the principal salivary proteins (4) has been proposed as a sensitive non-invasive biomarker with the main function is the enzymatic digestion of carbohydrates through hydrolysis of starch to sugar and maltose (5).

Knowledge of the effects of diabetic issues on salivary composition and function remains equivocal. Basement tissue layer leaks in the structure of the parotid gland is reported to be greater in kind two diabetic issues,and this

result in raised percolation of components such as sugar, amylase and proteins from blood stream,

thus raising their stages in saliva [6-7].Additionally, salivary alpha-amylase has been suggested to inhibit the adherence and growth of bacteria to prevent microbial attachment to oral surfaces and to enable microbial clearance from the oral cavity (8), so it is important for the mucosal immunity in the oral cavity (9- 10). Finally, the fact that leader -amylase binds to teeth as a constituent of enamel pellicle play a role in modulating the adhesion of microbial species to the teeth (11).

It is well known that dental plaque is closely related to the most common oral illnesses, dental caries and periodontal illness (12). Formation of dental plaque is a complex process includes the connections between streptococci and the salivary proteins alpha-amylase (13-14).

Considering age and sex, similar to other body organs, it has been clearly established that synthesis and release of enzymes in un stimulated spit decrease with age (15-16). This has been supported by a study conducted by Kalipatnapu et al., (1983) who revealed that surge in salivary proteins and amylase in both individuals up through middle age and then the focus of these constituents remain unaffected through the rest of life (16).

Considering sex, previous research revealed that men discharge more spit than women, accordingly, men synthesize and discharge larger amounts of proteins and amylase into spit in comparison to women (16).

2. Materials and Methods

100 primary school children 6-13 years took part in this research A structured questionnaire that was distributed in Arabic language and sent to close relatives members of each student, child health background and family health background, focusing on close relatives reputation of kind two diabetic issues, with diabetic issues close relatives record categories was defined by 1st and second relative's. The questions were mainly closed –ended rather than open questions, thus to avoid the misunderstanding clinical oral examination that carried out under natural light using disposable plane dental mirrors and probes. Plaque and gingival health status was determined by plaque index (PI), gingival index (GI) depending on Silness and Loe, (1964), Loe and Silness, (1963) respectively.

After clinical oral examination, saliva was gathered from all members under the same conditions. Your kids were supervised and instructed to be comfortably seated with their head tilted slightly forward. Saliva was allowed to accumulate in the floor of the oral cavity and to expectorate all spit formed over 5- moments period into the graduated sterile test tube. The spit examples of all the members were identified by a code number during the period of sample collection and processing. After the disappearance of the salivary froth, the salivary flow- amount was estimated in milliliters per moments. Samples were stored at -80°C, until analyzed. Sampling sessions were limited to the hours between 9:00 and 11:00 AM to minimize the impact of diurnal variations.

Saliva research Quantitative Colorimetric Amylase Dedication at 585nm was used to estimate the focus of salivary amylase. Enzy Chrom TM α-Amylase Analysis Kit (ECAM-100) from BioAssay Systems (USA).

Statistical research was performed with SPSS version 19.0 (SPSS Inc; Chicago, IL, USA). Descriptive mathematical analysis, student T-test, research of variance (ANOVA) and straight line and multiple straight line connection were used. A p-value of less than 0.05 was considered to indicate mathematical importance.

3. Aims of the Research

1) To see if alpha-amylase level in children with a positive family history of diabetes mellitus is different from individuals with a negative family history of diabetes.
2) To investigate the level of salivary alpha-amylase in children aged 6-13 years and to analyze its relation with selective oral health status factors.

4. Results

In this study 100 primary school students were registered, the age ranged from 6-13 years.45% were males and55% were females.

Family history of diabetes and Salivary Amylase Level

Considering family history of diabetes mellitus (DM), the results showed that children with positive family history of DM observed with higher salivary amylase level compared to those with a negative family history (293.06 U/L vs 282.58 U/L); figure 1, however the difference was not significant. Taking in consideration each parent history of DM, mean salivary amylase level was higher in children with positive father history than those with mother history of DM (287.35 U/L vs 268.94 U/L), but the difference was unable to reach statistical Significant.

The correlation between age and salivary flow-rate was observed significant($r=0.400$, $P<0.001$); the higher salivary secretion produced together with increased age figure (2).

Females' students produced lower mean salivary flow- rate than Male, but the correlation was not-significant.

Figure (3) showed that female students indicated higher mean salivary amylase level compared to male students; in spite of this the difference was non-significant. Using multiple linear regression analysis presented that being a male is expected to reduce salivary amylase by a mean of 11 U/L compared to female after adjusting for the possible confounder effect of the remaining explanatory variable contained in the model. The result of gender, still failed to reach the level of statistical significance, possibly because of small sample size.

In table(2), Children with higher scores of gingivitis observed with higher mean of salivary amylase levels

compared to those with lower score of gingival index was not significant.

The gingival index was classified into mild and moderate score, with the majority of study groups were within the moderate scores 61%. There was a significant positive linear correlation among salivary amylase concentration and gingival index (P= 0.04). As shown in table(1) and figure(4) children with higher scores of gingivitis observed with higher mean of salivary amylase levels compared to those with lower scores, though the mean differences were not significant.

As per shown in table (2), the explanatory variables include: gingival index, salivary flow rate, plaque index, age and gender. among the tested explanatory variables, only the age showed a statistically significant positive association with salivary amylase level after controlling for the remaining explanatory variables included in the model. For each one year increase in age figure (5), the salivary amylase concentration is expected to increase by an average of 5.2 units.

5. Discussion

100 primary school students were participated in our study, with age ranged from 6 - 13 years.

Unstimulated whole saliva was used and salivary samples were collected at the same time of the day (9:00 -11:00 AM) to reduce the effect of diurnal variations. The students were from the same economic status, presented with any systemic disease and none of the children were taking any medicine.

Positive family history of DM was positively, although not significantly associated with salivary amylase concentration. In this study, children with positive family history observed with higher salivary amylase level compared to those with negative family history. This may be explained on the base of the knowledge of the effect of DM on saliva; the basement membrane permeability of parotid gland is higher in DM and the components such as glucose, protein and amylase in blood raised, thus raising their levels in saliva which may be due to hormonal and metabolic changes associated with disease (17-18). This concept agrees with other studies which found a significant increases in salivary amylase levels in diabetics (19-20).

In 2006, Hariri et al found that a positive family history identified about 73% of all participants with diabetes (21). Family history would be made as a part of mass-awareness strategies and prevention campaigns aimed to reduce the burden of diabetes and their risk factors.

Thus parents may be more motivated to engage their children in healthy behaviors if they are aware of the familial risk of disease.

Much research needs to be done, however, on the most effective ways to incorporate family history in those strategies and campaigns, particularly for children and young adults.

Family-based lifestyle interventions with parents as coaches may be more effective than individual approaches.(22)

The observations derived from this study require more comprehensive evaluation with emphasis on broader representation. We believe that these new perspectives will provide insight in reaching a clear consensus regarding changes in salivary composition and function in diabetes mellitus.

Salivary amylase:

Regarding DM, the composition of saliva reflects both the activity of exocrine organs and the composition of plasma with proteins and amylase being synthesize also diffuse into saliva from plasma (23).

The salivary amylase levels were significantly higher in controlled diabetics when compared with healthy non-diabetics. A dramatic important of salivary amylase activity has been found in diabetic patients compared to healthy controls. The present study has shown that the alpha-amylase levels in un stimulated saliva from type 2 diabetic patients are higher than in control groups. There is considerable disagreement in the literature about salivary amylase activity in diabetic patients; different rescales have reported that salivary amylase concentrations from diabetics are higher (24), lower (25) or the same (26).with regard to salivary total protein, the present study results are consistent with most previous studies, higher (25) lower or the same. A significantly positive correlation was observed between total protein levels of uncontrolled diabetic group and controlled diabetic group (16).

The presence of salivary biomarkers specific for diabetes in edentulous subjects mimics those in serum, especially those related to inflammatory/lipid metabolism. While this exploratory study requires further validation with a larger population, it provides proof-of-principle for salivary proteomics for edentulous subjects with diabetes. (27)

Studying the flow rate, the correlation was a significant among salivary amylase level and both salivary flow-rate and age. Salivary flow- rate increases through age in adolescent populations and children (25, 28-30) while other students, have described controversial findings (31-32).

In our study, females' students produced lower mean salivary flow- rate than male, though the correlation was not significant. Our results agree with earlier clinical research that indicated a lower saliva output of females than males similar in children populace. (25, 28-30)

This might be described on a base of hormonal changes that must be recommended to influence salivary flow- rate (30).

The results of analysis indicated that female student produced salivary amylase by more than ten- times matched to male after adjust additional confounder, though not significant.

The not significant relation might be as a result of the small sample size. This was in contrast to the preceding study

which discovered a higher level of salivary alpha amylase in male participants (33).The controversial discovery might be due to socioeconomic status and different age group of the study populace.

Concerning age, the salivary amylase concentration is expected to increase by 5.2 units for each one- year increase in age, This was maintained by preceding studies that discovered the level of salivary alpha amylase activity increased along with age (33-34).

In this study, students with higher scores of gingivitis presented the highest levels of salivary amylase. This discovering was in agreement with other studies which indicated an increased concentration of salivary amylase in patients with gingivitis (26, 35-36).

In adding to it was α- amylase exhibits inhibitory activity against various microorganisms, digestive action, (35) and thus might contribute in the oral defense mechanism (37).Therefore, the higher levels of amylase could be due to the response of salivary glands to inflammatory diseases like gingivitis leading to increase synthesis and secretion of certain acinar proteins (α-amylase) to enhance the oral defense mechanism (37-38). This might indicated the essential role of salivary amylase as a defense molecule for the innate immunity in the oral cavity. Furthermore, it has been indicated that the increased levels of amylase may be due to plasma proteins leakage into saliva due to inflammation (39).

Gingivitis and periodontitis induces an increase in the output of total proteins, mucin and α- amylase, as a result of responding of salivary glands to inflammatory diseases thereby increasing the protective potential of saliva and this is accompanied by a decrease in flow rate (40-41).

6. Conclusion

1) Higher levels of alpha-amylase in saliva of children with a family history of DM may be important to identify children at an increased risk for DM.
2) The information obtained may serve as a reference values for the growing interest in saliva a diagnostic tool for future research
3) Future research with larger samples, in multicentre and longitudinal studies are required
4) Detection of these alterations in hormonal and metabolic parameters in children with a positive family history suggests that at least some of the determinants are genetic/heritable.
5) Detection of alterations in salivary parameters in children with a positive family history of DM may suggest that at least some hormonal and/ or metabolic changes may act as determinants of genetic/heritable diseases.

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Tables and figures

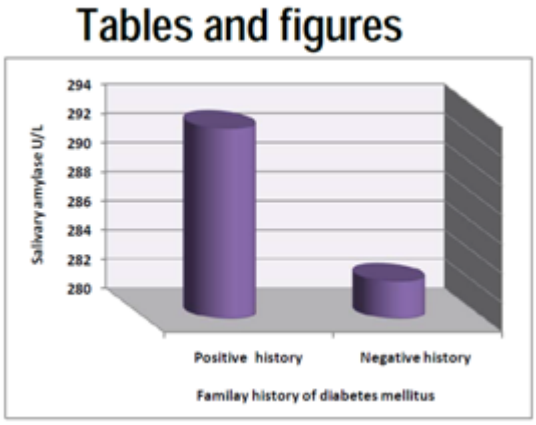

Figure 1: Family history of Diabetes mellitus and salivary amylase level

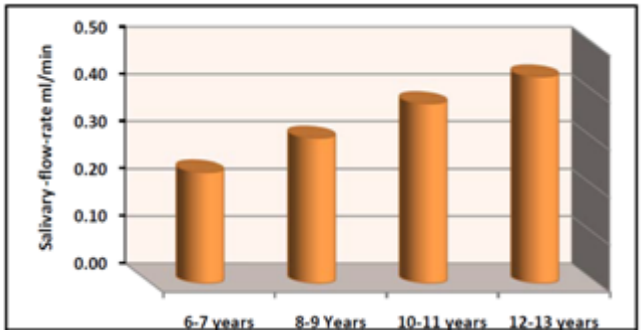

Figure 2: Age group in relation to salivary flow-rate (milliliter/minutes)

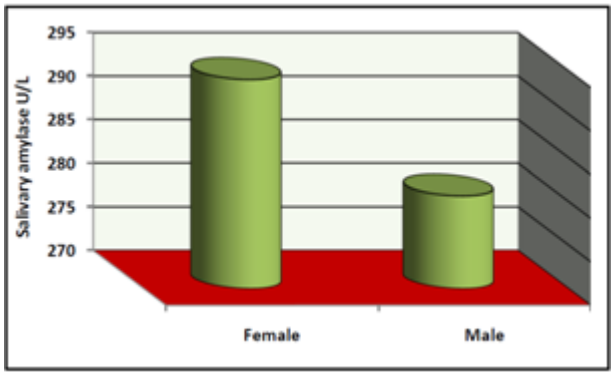

Figure 3: Gender and mean salivary amylase level

Table 1: Age and oral health status parameters in relation to salivary alpha-amylase

	Salivary Amylase (U/L)					
	Range	Mean	SD	SE	N	P
Plaque index						
Mild (0.1 -1	(287.9 - 334.9)	305.8	25.4	14.7	3	0.72[NS]
Moderate (1.1-2)	(187.2 - 373)	287.2	32.8	4.1	63	
Severe (2.1-3)	(133.5 - 371.5)	287.5	45.6	6.6	47	
Gingival index						
Mild (0.1 -1)	(150.1 - 373)	281.9	41.3	6.5	40	0.22[NS]
Moderate (1.1-2)	(133.5 - 371.5)	291.1	36.5	4.3	73	
Age group (years)						
6-7	(133.5 - 358.7)	270.6	41.0	7.6	29	<0.001
8-9	(150.1 - 373)	281.2	35.9	6.1	35	
10-11	(212.5 - 371.5)	300.6	34.1	5.8	34	
12-13	(272.3 - 367.1)	309.7	27.4	7.1	15	
Family history (DM)						
Negative first degree	(133.5 - 358.7)	282.7	35.8	4.7	57	0.25[NS]
Positive first degree		286.6	36.8	8.2	20	

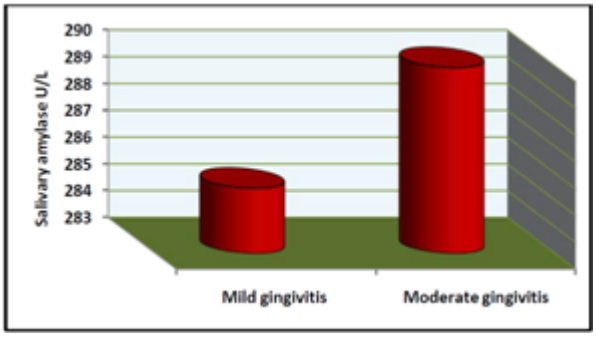

Figure 4: Gingivitis and mean salivary amylase level

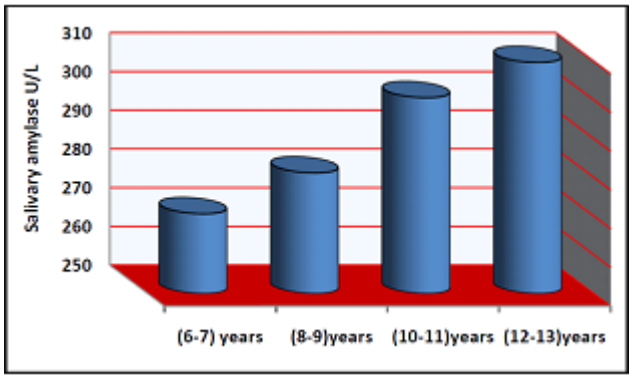

Figure 5: Age group in relation to mean salivary amylase concentration.

Table 2: Multiple linear regression model with salivary Amylase concentration as the dependent (response) variable and selected explanatory variables

	partial regression coefficient	P	Standardized regression coefficient
(Constant)	224.5	<0.001	
Age (years)	5.2	0.02	0.27
Male gender compared to female	-10.6	0.15[NS]	-0.14
Gingival index	36.5	0.19[NS]	0.13
Positive Family history (DM)	8.1	0.28[NS]	0.11
Plaque index	-10.0	0.35[NS]	-0.09
Salivary flow rate	0.27	0.94[NS]	0.01

R^2=0.21
P (Model)=0.005