Salivary and Serum Aspartate Aminotransferase and Alanine Aminotransferase in An Uncontrolled Diabetic Patients

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Abstract

Background: Diabetes mellitus (DM) is a metabolic disorder, characterized by a higher level of blood glucose resulted from either abnormality in insulin production (type1 DM) or resistance to insulin action (type 2 DM) or both. Long lasting elevated blood glucose is responsible for chronic damage, defect in function and impairment of various organs including salivary glands. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are cellular enzymes found in all animal tissues.

Aims of the Study: to estimate and compare the levels of salivary and serum AST and ALT between un controlled diabetic patients and normal control people.

Subjects, Materials and Method: The study comprised 90 adults of both gender. The patients were divided into 3 groups: 30 patients with type 1 diabetics (D1), 30 patients with type 2 diabetics (D2) and 30 healthy persons as a control group (C). Serum and un stimulated salivary samples were taking for the AST, ALT evaluating using standard spectrophotometer kits. The level of glycosylated hemoglobin (HbA1c) was measured using NycoCard kit to exclude the controlled diabetic patients. Data were analyzed using statistical analysis SPSS version 24.

Results: The results showed a significant increase of salivary enzymes in D1 and D2 compared to salivary enzymes of control group, while there was no significant difference in serum enzymes value among all studied groups.

Conclusions: An elevation in salivary AST and ALT in both types of diabetes compared to healthy group, with higher salivary level of AST and ALT in D1 compared to D2 and to healthy control group. This may suggest that autoimmunological activity in D1 responsible for more salivary gland injury in D1 group.

Key words: *alanine aminotransferase, aspartate aminotransferase, diabetes mellitus.*

Introduction

Diabetes mellitus is a group of metabolic diseases that are different clinically and genetically but with a common phenotype, defined by prolonged and abnormally high level of blood glucose with abnormal metabolism of carbohydrate, lipid and protein [1].

The most important feature of DM is a long standing hyperglycemia, as a result of failure of the pancreas to produce insulin or cellular resistance to the insulin action or both. According to American Diabetic Association, diabetes mellitus is divided into 4 types**:** type1which is due to damage of B-cells of the pancreas resulting in insulin reduction, type 2 is due to resistance of target organ to insulin cellular metabolic impact and the third type is the gestational DM that occur during pregnancy and another special type of DM due to other reasons [2]. Prolonged hyperglycemia in DM patients may lead to the formation of advanced glycation end products (AGEs) whose collection in blood and tissues is linked with DM complications [3]. Uncontrolled DM increases the danger of complications, with HbA1c is an important plasma marker used to estimate the level of glucose amount in the plasma during the previous 2-3 months [4-6]. Cytological enzymes such as AST and ALT

may be considered as potential markers of salivary gland in the patho-mechanism of DM [4]. Aminotransferases or transaminases are set of enzymes that stimulate the conversion of amino acid and oxalo acid by transmitting of amino groups to alpha-keto acid, so they are included in metabolism of protein. These enzymes are found in many body organs such as skeletal muscle, kidney, brain, pancreas, lungs, blood cells with more frequently found in the liver and heart, so any injury to these organs result in output of these enzymes into the extra cellular fluid [7].

Subjects, Materials and Methods

This cross-sectional study composed of 90 subjects. The study group consists of 60 diabetic patients who were un controlled depending on the level of HbA1c test which should be above 8 %. All participants were carefully informed about the aim and objective of the study. Patients or their parents were sign a consent form after their agreement.

Patients were recruited from the Endocrinology and Diabetic Centre of AL-Sadder Teaching Hospital in AL Najaf city in Iraq, from December -2018 to March -2019).

The study group was divided into 2 groups; the 1st was 30 Type1DM patients and the 2nd was 30 Type 2DM patients. Control group consist of 30 healthy subjects without any history of systemic diseases, with an age and gender matched with the study group.

Inclusion criteria:

1. Patient with type1 and type 2 DM who were diagnosed at least 6 months before the study.

2. Patient without any systemic diseases.

3. Patient who was not taking any medications at least 3 months prior the study.

4. Non-smoker individual.

5. HbA1c test should be >8 %.

Exclusion criteria

1 Patients were excluded if they were on medications other than taking for type1 diabetes or type 2.

2. Those with any other systemic disease apart from diabetic mellitus.

3. Patient who will not sign his consent form.

4. Smoking patient.

5. If HbA1c test is below 8%.

Saliva collection

The collection procedure of the saliva is by spitting method, with the subject was asked to set comfortably. Before collection of the saliva, patients were asked to rinse the mouth with distilled water.

Salivary sample were collected, for all subjects between 8 -11 a.m. to decrease the diurnal differences. Patient was asked not eat 1 hour before the sample collection , then saliva was collected for 10 minute and after that, saliva was placed in the ice box and send for laboratory for centrifuge (3000 rpm for 15 min) and then transferred for biochemical analysis.

Blood collection

Two milliliters of venous blood was collected from antecubital vein, separated into 2 parts, one part in plan test tube for serum AST and ALT estimation, after centrifuging for 2 minutes, and in another test tube containing anticoagulant for HbA1c estimation. If the level of HbA1c was above 8%, then the collected samples in the plain tube (serum tube) were distributed in the appendrove tubes which then transferred for biochemical analysis.

Method for AST, ALT determination

Estimation of serum and salivary AST, ALT were done by standard spectrophotometer method (340 nm) with the aid of kinetic spectrophotometer kits from AGGAPE Diagnostic (Switzerland) according to international federation of clinical chemistry (IFCC).

Statistical Analysis

Descriptive statistic, mean, ANOVA test for variance analysis, post hoc (Dunnett T3) test, student T test and Pearson coefficient tests were used.

Results

Salivary and serum Aspartate aminotransferase

(AST)

Using ANOVA test, a highly significant difference was found in salivary AST value among type1, type 2 and control groups (p=0.000), table (1-1).

Table (1): Mean salivary AST among studied groups.

Parameters	Study groups	No.	Mean AST	SD	Std. Error	F	P- value
Salivary AST (IU/L)	Type 1 DM	30	94.17	22.38	4.08	184.9	0.000
	Type 2 DM	30	48.60	9.80	1.79		
	Control	30	21.53	7.04	1.05		

Using post hoc (Dunnett T3) test, the result revealed a higher significant difference in the salivary level of AST between type 1 and type 2 (p=0.000) and similarly between the two diabetic and control group (p=0.000), table (1-2).

Table (2): Post hoc (Dunnett T3) test of salivary AST between studied groups.

Enzyme	(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	p-value
Salivary AST (IU/L)	Type1DM	Type2DM	45.567*	4.460	0.000
		Control	72.033*	4.284	0.000
	Type 2DM	Control	26.467*	2.201	0.000

While no statistical difference was found (p=0.134) in serum AST among studied groups using ANOVA test, table (3).

Table (3): Mean AST in serum in type1, type 2 and healthy control group.

Parameters	Study groups	NO.	Mean AST	SD	Std. Error	F	P- value
AST serum (IU/L)	Type 1 DM	30	22.7	5.91	1.08	0.637	0.134
	Type 2 DM	30	19.41	5.31	0.97		
	Control	30	16.33	4.81	0.88		

Salivary and serum Alanine aminotransferase (ALT)

Using ANOVA test, a higher significant difference was seen in the level of salivary ALT among type1 DM, type 2DM and control group (p=0.000), Table (1-4).

Table (4): Mean salivary ALT level among studied groups.

Enzymes	Study groups	Mean ALT	SD	Std. Error	F	P- value
Salivary ALT	Type 1 DM	86.20	21.78	3.98	162.92	0.000
	Type 2 DM	55.17	8.48	1.55		
	Control	20.63	6.84	1.25		

Using post hoc (Dunnett T3) test, the result showed a highly significant difference between type 1 and control group, similarly between type 2 and control group (p=0.000).

Also, a significant difference in the salivary level of ALT between type1 and type 2 DM (P=0.000) was seen, Table (1-5).

Table (5) :Post hoc (Dunnett T3) test of salivary ALT between studied groups.

Enzyme	(I) Study groups	(J) Study groups	Mean Difference (I-J)	Std. Error	p-value
Salivary ALT (IU/L)	Type1DM	Type2DM	31.03	4.27	0.000
		Control	65.47	4.17	0.000
	Type 2 DM	Control	34.43	1.98	0.000

Considering serum level of ALT, there was no significant difference among the study groups (p=0.247) using ANOVA test, Table (1-6).

Table (6): Mean ALT level in serum of type1, type2 DM and healthy control group.

Enzyme	Study groups	Mean	SD	Std. Error	F	p-value
ALT serum (IU/L)	Type 1 DM	25.23	5.69	1.04	0.431	0.247
	Type 2 DM	23.56	5.75	1.05		
	Control	18.77	6.40	1.17		

Regarding the duration of DM, patients were divided into 2 groups: more than 5 years duration and less than 5 years duration.

There was a statistically significant increase in the level of salivary enzymes in group less than 5 years compared to those of more than 5 years in type 1 DM. Considering the level of enzymes in the serum, no significant difference was found in relation to disease duration

Discussion

In the present study, there was statistically significant difference in the salivary AST and ALT levels in type1 and type2 DM as compared to salivary level of the control group. This is in agreement with other studies which found salivary AST and ALT levels was higher in type1 compared with type 2 DM and to healthy control group (3,7,9) as they were found three fold and four fold elevation in salivary level of AST and ALT of type1 DM group as compared to control group, respectively, similar to the result of present study.

Comparing type 1DM to type 2 DM, there was increasing in the salivary mean AST and ALT levels in type1 as compared to type2 DM was seen in the current study as there was twofold increasing in ALT and AST in type1 DM as compared to type2 DM group. This finding is consistent with what was reported in a study of Malicka et al. (2016) who reported twofold elevation in salivary AST and ALT levels as compared to type 2 diabetic group, also in agree with studies of (10,11,12) who found AST and ALT value were higher in type1DM as compared to type 2 DM. So the results of present and past studies support the hypothesis that an auto immunological process in type 1 DM will lead to salivary glands damage and increased level of these enzymes (3,7).

There was no significant difference was seen in serum levels of AST and ALT among study groups although there was slight rising in the value of AST and ALT in diabetic groups which is in parallel with a study that done by Vinod et al. (2018) who reported normal serum value of ALT and AST in diabetic and control groups but no statistically significant. Although the result of a study done by Vinod (2006) found normal value of serum AST and ALT levels in type 1 DM patients, but he was found significant difference regarding serum enzymatic value between type1 DM patients and control group.

Lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase are widely present in the body tissues and whole cells (cytological enzymes) especially in the cytoplasm of the cells in addition to that, AST is also located in the mitochondria. When there is damaging to the tissue, serum levels of these enzymes will be elevated as a result of their infiltration from the tissues that were damaged. In turn, elevated salivary level of AST and ALT may be attributed to damage to cells of the salivary gland by different way (3,10,13). Also the study that done by Cinquini et al. (2002) on type1 DM children reported damage in the salivary gland demonstrated by histopathological presentation of lymphocytic cells in the damaged glands cells. So, it was reported that there was an immunological activity in the salivary gland and causing cells damage similar to the action of antibodies to pancreatic B cells as there was particular antibodies against antigens of the salivary gland (10). This demonstrated why level of salivary AST and ALT were higher in type1 DM when compared to type2 DM and to control group.

It was found that inflammatory process of periodontal tissues in the same time of presence of DM causing elevation in the salivary value of AST and ALT (14). The present study reported different periodntal disease in two diabetic groups so this lead to a suggestion that autoimmunological activity plus periodontal inflammation will result in significantly elevated salivary level of AST and ALT in type1 DM as compared to type 2 and healthy groups; also in type 2 when compared to healthy control group.

In addition to that, this study reported a statistically significant difference in salivary levels of AST and ALT in type1 DM regarding different disease durations as their values were higher in 1-5 years duration than in more than 5 years duration of DM. This result consistent with the findings that were reported by (7,10, 11) who found higher levels of AST and ALT in patients discovered with diabetes since 4 years and 5 years clinical duration, respectively.

Although Vinod (2006) and Vinod et al. (2018) found high levels of salivary AST and ALT in 0-5

year clinical duration of diabetes than in more 5 years duration ,but was found no significant difference in serum and salivary AST and ALT values in different disease duration, but this study found statistically significant difference in salivary values of AST and ALT regarding different disease durations may be due to size of the sample and patient condition.

On the other hand, it was found no significant difference in serum and salivary enzymes level in different type2 DM duration. This finding is consistent with the study done by Al-Rubaee et al. (2010) (15) who reported no significant difference between salivary enzymes activities and various disease durations.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Dentist in department of oral diagnosis and all experiments were carried out in accordance with approved guidelines.

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