Clinical Studies to Evaluate Pancreatic Functions in the Patients of Type 2 Diabetes Mellitus

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ABSTRACT: Diabetes mellitus is a chronic metabolic disorder which is associated with hyperglycemia. It is caused by a derangement in the secretion or function of the endocrinial portion of the pancreas. The aim of the present study was to determine the blood glucose, MDA, amylase levels in 50 cases of type 2 Diabetes Mellitus and to compare and correlate these parameters with those 50 of age and sex matched healthy controls. Glucose and amylase concentrations were analyzed spectrophotometrically by kits in all patients with DM as well as in the control subjects but MDA concentrations was measured by manual methods. The results of type 2 DM were compared with control group using One way ANOVA-test to compare parameters in different studied groups. The results show a significant elevation (P ≤ 0.05) in levels of glucose, MDA, amylase of type 2 diabetic patients in comparison healthy subjects.

KEYWORDS: Type 2 diabetes mellitus, Pancreas, Glucose, Malondialdehyde (MDA), Amylase.

1 INTRODUCTION

Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Long-term vascular complications represent a major cause of morbidity and mortality in patients with diabetes mellitus. In addition, various biochemical disorders associated with vascular complications, such as hyperlipidemia and oxidative stress which frequently co-exist with diabetes mellitus (1), appear inadequate to explain the increased risk of vascular diseases. The observations suggest that additional factors may be involved in the acceleration of diabetic vascular disease.

The islets of Langerhans make up a small but critical mass of the pancreas that is composed of key endocrine cells. The main cell types within the islets are b, a, d, F and e cells that secrete hormones such as insulin, glucagon, somatostatin, pancreatic polypeptide and grehlin, respectively. Together, these cells help regulate energy metabolism by hormonal control and in particular glucose homeostasis. The majority of the islets consist of the pancreatic b-cells, making up about 70% of total islet volume (2).

As it known that Diabetes mellitus is one of the world’s major diseases, with an estimation of 347 million adults affected in 2011 (3). Type 2 diabetes mellitus, by far the most common type, is a metabolic disorder of multiple etiology characterized by carbohydrate, lipid and protein metabolic disorders that includes defects in insulin secretion, almost always with a major contribution of insulin resistance (4). In this context, the inhibition of carbohydrate digestive enzymes is considered a therapeutic tool for the treatment of type 2 diabetes (5). The most important digestive enzyme is pancreatic alpha-amylase. Amylase are hydrolysis enzymes catalyses the hydrolysis of complex carbohydrate molecules into smaller components. Human amylase is termed (α-amylase) or endoamylose because of its ability to split poly saccharide α(1-4) linkage in a random manner. The end product of α-amylase action on poly saccharide is the formation of dextrans maltose and some glucose molecules(6).
Lipid peroxidation, owing to free-radical activity, plays an important role in the development of complications of diabetes. Although increased levels of lipid peroxidation, as a consequence of free radical activity, have been reported in type 2 diabetes with vascular complications (7)(8) other studies failed to detect any significant elevation in lipid peroxidation in diabetic patients,(9) probably owing to heterogeneity of the patient population. Hence, in this study, we have evaluated the levels of Malondialdehyde (MDA) (index of lipid peroxidation) in patients of type 2 diabetes without complications and related it with amylase levels with a Possible Correlation between them and compared to healthy subjects.

2 MATERIALS AND METHODS

This study was conducted in AL-Hussein Education Hospital and the Special Center of the Endocrine Glands and Diabetes in Thi-Qar for a period of seven months. 50 normal healthy subjects with fasting blood sugar (FBS) < 6.20 mmol/L were selected as control group. The ages ranged from 20 to 65 years. 50 patients of type 2 Diabetes mellitus whose (FBS) > 17.58 mmol/L were included in this study. About (10 mL) of fasting venous sample all included subjects were taken and allowed to clot to get serum by putting it in empty disposable tube’s centrifuge to separate it in the centrifuge at 3000 (rpm) for 10 min, the serum samples were separated, stored at (-20˚C) for later measurement biochemical parameters, unless used immediately.

Serum glucose was determinate by an enzymatic colorimetric test on basis of Trinder-Reaction (10)(11). MDA in plasma was performed as described by Fong et al (12). The amylase activity was measured by an enzymatic method (13).

The results were analyzed statistically expressed as mean ± standard deviations (mean ± SD) by using SPSS version 10.0. and correlation coefficients on the suitable software. P values of < 0.05 were considered as statistically significant.

3 RESULTS AND DISCUSSION

3.1 IN GENERAL

Clinical characteristics of the study subjects are shown in Table 1. Serum glucose concentrations were significantly higher (P≤0.05) in type2 diabetic group in comparison with control group. The MDA concentration in type2 diabetic were a significantly higher in comparison with control group (P≤0.05). However, an apparent increase was also observed in concentrations of serum amylase in type2 diabetic in comparison with control group (P≤0.05) as shown in figures (1)(2)(3). There was a significant (p value <0.05) positive correlation between the fasting blood glucose levels and serum MDA (Pearson’s correlation coefficient, r= 0.95) also a positive correlation between the fasting blood glucose levels and serum amylase activity (Pearson’s correlation coefficient, r= 0.89) in the diabetic patients as shown in figures (4)(5).

Table 1. Clinical characteristics of the study subjects.

<table>
<thead>
<tr>
<th>Clinical characteristics of the subjects</th>
<th>Control group</th>
<th>Type2 diabetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20-60</td>
<td>20-60</td>
</tr>
<tr>
<td>Fasting Blood Glucose(mmol/L)</td>
<td>4.99 ± 1.09(^a)</td>
<td>17.37± 2.01(^b)</td>
</tr>
<tr>
<td>Serum MDA (nmol/L)</td>
<td>6.58 ± 4.36(^a)</td>
<td>70.92 ± 2.03(^b)</td>
</tr>
<tr>
<td>Serum Amylase(U/L)</td>
<td>40.19 ± 10.50(^a)</td>
<td>175.35 ± 21.74(^b)</td>
</tr>
</tbody>
</table>

* Each value represents mean ± SD values with non identical superscript (a, b ...etc.) were considered significantly differences (P ≤ 0.05).
Fig. 1. Comparison of fasting blood glucose levels in control group and type 2 diabetic group

Fig. 2. Comparison of MDA levels in control group and type 2 diabetic group
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Fig. 3. Comparison of serum amylase levels in control group and type 2 diabetic group

Fig. 4. Correlation of serum MDA with serum glucose levels in diabetic patients showing a positive correlation \((r=0.95)\).

Fig. 5. Correlation of serum MDA with serum amylase levels in diabetic patients showing a positive correlation \((r=0.89)\).
It will be known that diabetes is a group of endocrine diseases developing as a result of relative or real lack of insulin hormone, or disturbances of its interaction will be the cells of an organism, therefore the steady increase of sugar (glucose) content in blood develops hyperglycemia (14).

Glucose is the major source of energy used by the cells. However, glucose cannot enter the cell unless the presence of insulin is there. In a normal working of pancreas, the right amount of insulin is produced to move glucose into the cells. In an abnormal pancreas, little or no insulin is produced, or the body cells do not respond to the insulin that is produced, it leads to accumulate glucose in the blood and elevation of its levels and causes Diabetes Mellitus (15).

Table (1) shows a significant elevation in concentration of serum glucose levels in type 2 diabetic group in comparison with control group (P≤0.05).

This finding is matched with the result of Tan et al (2001) (16) and Annette et al (2003) (17) and the reason of this state is due to the weakness of β-cell, modicums of insulin production as well as responding and increasing of its resistance. Where all this appears by aging so glucose levels increase with this type.

Also, a positive correlation relationship between MDA and glucose levels in Diabetes Mellitus patients we found. Sinha R., et al (2004) (18) also found this positive correlation relationship between MDA and glucose in Diabetes Mellitus patients. Figure (3) the positive correlation relationship between MDA and glucose in Diabetes Mellitus patients) correlation coefficient of this relation is (r=0.95).

Table (1) shows a significant increase in concentration of serum MDA levels in type 2 diabetic group in comparison with control group (P≤0.05)

Because of hyperglycemia is associated with the increase of the oxidative stress and free radicals production (19) so it’s effected on oxidant-antioxidant balance where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. However, excessive ROS accumulation will lead to cellular injury, such as damage to proteins, DNA and lipid membranes.

We found that lipid peroxidation marker (MDA) were higher in patients with type 2 diabetes compared with control groups, and this may be due to the increasing of oxidative stress in type2 diabetes because of the exposure to prolonged periods of hyperglycemia, which causes glucose to be in its highest levels. Our study confers with the study conducted by Jain et al(1999)(20) and West et al(2000)(21) and similar finding was reported by Dierckx et al(2003)(22).

A significant increase was found in concentration of serum amylase levels in type 2 diabetic group in comparison with control group (P≤0.05) as shown in table(1). The result also showed a positive correlation relationship between MDA, the marker of lipid peroxidation and serum amylase in patient groups (r=0.89).These results was shown in Figure (4).

According to the results of this study there are an increase in amylase levels was related with the insulin deficiency and the glucagon excess in diabetes affect the normal milieu of the pancreas (23). Our results suggest that the low serum amylase levels in diabetes are associated with an impaired insulin action due to insulin resistance and/or inadequate insulin secretion, as was indicated by the raised blood glucose levels in our study (24).

Clinical characteristics of the study subjects are shown in Table 2. Serum glucose concentrations were significantly higher (P≤0.05) in T2DMA group in comparison T2DMY group. The MDA concentration in T2DMA were a significantly higher in comparison with T2DMY group (P≤0.05). However, an apparent increase was also observed in concentrations of serum amylase in T2DMY in comparison with T2DMA (P≤0.05) as shown in figures (4)(5)(6).

| Table 2. Clinical characteristics of the study subjects according to the age |
|-------------------------|-------------------------|-------------------------|
| Clinical characteristics of the subjects | T2DMY | T2DMA |
| N | 25 | 25 |
| Age (years) | 20-40 | 41-60 |
| Fasting Blood Glucose(mmol/L) | 15.39±2.14^a | 18.14±4.01^b |
| Serum MDA (nmol/L) | 68.50±1.95^a | 71.37±3.67^b |
| Serum Amylase(U/L) | 190.85±6.99^a | 180.83±4.55^b |

* Each value represents mean ± SD values with non identical superscript (a, b ...etc.) were considered significantly differences (P ≤ 0.05).
**T2DMA**: Type 2 Diabetes Mellitus age (20-40)

**T2DMY**: Type 2 Diabetes Mellitus age (41-60)

*Fig. 6.* Comparison of fasting blood glucose levels in T2DMA and T2DMY groups

*Fig. 7.* Comparison of serum MDA levels in T2DMA and T2DMY groups
The results of present study indicates that fasting glucose levels in T2DMA increased in comparison T2DMY groups were statistically significant (p<0.05). The reason of this state is that type 2 diabetes mellitus appears after age 40 and the increasing in glucose concentration in this type and diabetes mellitus is due to the weakness of β-cell, medications of insulin production as well as responding and increasing of its resistance. Where all this appears by aging so glucose levels increase with this type(16)(17) as shown in table(2) and figure(4), also it may be result of the weakness of the work in pancreas and other organs by aging (25).

In table(2) and figure(5) it can be observed that the level of serum MDA in T2DMY is decreased in comparison with T2DMA group because MDA play a role as cofactors in the pathogenesis and complications of type 2 DM and this role be influential greatly by getting older and Advancing age(26).

Also we can obviously seen in table (2) and figure(6) the increasing of serum amylase in T2DMY in comparison with T2DMA. Our results suggest that the low serum amylase levels in diabetes are associated with an impaired insulin action due to insulin resistance and/or inadequate insulin secretion, as was indicated by the raised blood glucose levels in our study and this may be caused by the insulin deficiency in diabetes affect the normal milieu of the pancreas, thereby decreasing the total volume, the amylase secretions(27).

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