Study of liver enzymes and phagocytosis activities in experimentally induced diabetes mellitus in puppies

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Abstract

This study was conducted to evaluate the activities of liver enzymes and phagocytic cell activity in puppies with experimentally induced diabetes mellitus. Ten puppies were included in this study which divided into two groups (control and diabetic groups). Diabetes mellitus was induced by single injection of alloxan monohydrate at dose of 100 mg/kg B.w. Into marginal ear vein. Blood samples were collected from animals of both groups at zero time (before alloxan injection), ten days, twenty days and thirty days after alloxan injection to measures the serum glucose levels, liver enzymes Alanin aminotransferase (ALT) & Aspartate aminotransferase (AST) and phagocytosis activity against Salmonella typhimurium. The results revealed that, the serum glucose concentration, Alanin aminotransferase and Aspartate aminotransferase activities were elevated starting from the first 10 days period after induction of diabetes mellitus, also there positive relation between the activities of liver enzymes (ALT & AST) and serum glucose level in the animals of diabetic groups. The results also revealed that the animals of diabetic group have deficient phagocytosis activity.

Introduction

Diabetes mellitus is a syndrome initially characterized by a loss of glucose homeostasis. The disease is progressive and associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness (7). Diabetes mellitus is a condition in which the pancreas no longer produce enough insulin or cell stop responding to the insulin that is produced, so that glucose in the blood can not be absorbed into the cell of the body (12). The disease is classified into the following types:

A-Type-I: also called insulin dependent diabetes mellitus (IDDM) is an autoimmune disease in which the pancreas produce little or no insulin (25). This type of disease sometimes called juvenile diabetes and begins most commonly in childhood and characterized by sudden onset and it is world wide in distribution (15).

B-Type-II diabetes mellitus:(formerly called non insulin dependent diabetes mellitus (NIDDIDM, obesity related diabetes or adult onset diabetes). The most common form of diabetes and a count for 90% of all cases (15). TypeII diabetes mellitus is a metabolic disorder resulting from body's inability to make enough or property used insulin (11).

C-Gestational diabetes: is defined as glucose intolerance that is first detected during pregnancy (18). Towards the end of pregnancy a pregnant women may has higher than normal levels of glucose in her blood stream. Although it is usually disappear after delivery, the mother is at increasing risk of developing type II diabetes later in life (17).

D-Secondary diabetes: hypersecretion of any of the hormones which tend to induce hyperglycemic effect may cause glucose intolerance, thus Cushing’s syndrome, phaeochromocytoma,acromegaly and glucaganomas may cause secondary diabetes. Generalized destruction of the pancreas by acute and chronic pancreatitis, haemochromatosis and occasionally carcinoma may cause insulin deficiency (27).

The typical signs and symptoms of all types of diabetes mellitus described in details by (6; 5; 21). There is an increased susceptibility to bacterial and fungal complications. Boils, carbuncles and urinary tract infection. Sometimes complicated by pyelonephritis and renal papillary necrosis are of frequent occurrence and may be precipitate diabetic comma. Diabetes have an increased risk of tuberculosis, especially of the lungs, and unless treated, the disease tends to progress
rapidly (28). Diabetes mellitus might interrupt the migration of neutrophils to the disease lung due to lack of useless glucose and energy. This will to increased tissue damage by bacteria and disturbed gas exchange process during pneumonia (2).

Materials and Methods

1- Study population:
Ten (10) local breed puppies aged between (2-4) months and weighted (3 - 4.5)kg were used in this study. All animals were prepared to experiment by treatment with ciprofloxacin (20 mg/kg B.w. daily for six days); Ivermectin (0.2 mg/kg B.w. single dose) and Niclosamind (50 mg/kg B.w.). The animals were divided into two groups each has 5 animals:

a- Control group, b-Diabetic group.

Diabetes mellitus was induced in overnight fasting puppies by single injection of alloxan monohydrate at dose 100 mg/kg into marginal ear vein. Each 100 mg of alloxan was diluted in 1 ml of 0.9% normal saline (1).

2-Samples:
Five (5) ml of blood were collected from animals of diabetic and control groups by venous puncture according to procedures described by (13). Each blood sample was divided into two portions, the first portion was kept without anticoagulant to obtain serum for biochemical analysis of glucose and liver enzymes and the second portion was kept in anticoagulant tube for phagocytosis test.

3- Laboratory examination:

a- Estimation of fasting serum glucose

Fasting serum glucose were estimated every ten days intervals from the beginning of experiment by using special kit from Randox Company.

b- Estimation of liver function:

Serum AST and ALT were estimated every ten days intervals from the beginning of experiment by using special kit from Randox Company.

c- Phagocytosis test: was done at 30 days after injection using Salmonella typhimurium as atarget antigen for leukocyte according to procedures described by (20) as the following:

1- Differential leukocytic count was done for any blood samples according to procedure described by (8).

2- The blood samples were diluted by Hanks cell solution to obtain the concentration of leukocytes about 1×10^3 cell/ml of suspension.

3- Salmonella typhimurium from recent clinical isolated were cultured on trypticase soya broth (Diffco) and incubated at 37ºC for 24 hours, then diluted by Hanks cell solution to obtain the concentration of bacteria about 1×10^3 cell/ml.

4- Phagocytosis test was done by using screw caped tubes. 0.5 of blood suspension was added to 0.5ml of bacterial suspension; the tubes were rotated and incubated at 37ºC for 24 hours, then bacterial colony count raised from 0.1 ml of mixtures was done for each tube.

Results and Discussion

1- Fasting serum glucose concentration:
At zero time there was no significant differences in the means of serum glucose concentration between diabetic and control groups (5.3 ±0.6 Vs 5.2 ±0.52 mmol/L) (P>0.05). On day ten after alloxan injection, the serum glucose concentration increased significantly (P<0.01) in the diabetic group compared with control group and the same results were reported on 20th and 30th days (Table 1). This result is similar to the result of (2 and 4). Alloxan acts on the insulin producing pancreatic B.cells within islets of Langerhans which are selectively destroyed by oxidant production (24). Current evidence suggests that the selective cytotoxicity of alloxan is due to the function of three factors: efficient uptake, oxidant production by coupling of drug with intracellular reluctant (ascorbat and thiols) and coupled with low levels of glutathione peroxidase in the islets (7).

2- Serum Alanin aminotransferase (ALT) concentration:
On zero time the results revealed no significant difference between diabetic and control group (P>0.05). On day ten after
alloxan injection the serum ALT concentration increased significantly in diabetic group compared with control group (P<0.01). The same results were reported at twenty and thirty days after alloxan injection (Table 2).

3- Serum Aspartate aminotransferase (AST) concentration:
On zero time there was no significant difference in serum AST concentration between diabetic and control groups (P>0.05). On day ten after alloxan injection the serum AST concentration increased significantly in diabetic group compared with control group (P<0.01). The same results were reported at twenty and thirty days after alloxan injection (Table 2). Kechrid and Bauzernav (16) found that serum AST and ALT of diabetic animals were significantly higher than those of non diabetic animals. Torbenson et al. (26) indicated that the concentrations of the transaminases were dramatically elevated to greater than 10 times the upper limit of normal. Some investigators have suggested that the increase in enzyme levels in patients with diabetes mellitus resulted from the influence of insulin on liver and muscle tissue (23). Muscles and liver destruction is frequently associated with diabetes mellitus so that serum activities derived from the muscles and liver such as creatinin phosphokinase (CPK), lactate dehydrogenase (LDH) and AST are elevated (9). Liver tissue contains three times the amount of ALT as heart muscles or kidney so that rise in ALT activity is almost always due to hepatocellular damage and usually accompanied by rise of AST (23). The levels of ALT and AST of patients with secondary complication were significantly higher than normal which may be due to hepatocellular damage followed by cardiac tissue damage (22). Species with high alanin aminotransferase (ALT) activity in the cytoplasm of the hepatocytes include dogs, cats and primate whereas the liver of equine, bovine, birds and marmoset donot (3). Hepatocellular injury or metabolic disturbances cause alter hepatocellular membrane permeability which results in release of this soluble enzyme. Subsequent to an acute, diffuse injury, the magnitude of ALT increase in the plasma crudely reflects the number of affected hepatocytes. In chronic inflammatory liver disease the magnitude of ALT rise dose not related to the degree of pathology. A variety of tissues such as liver and skeletal muscle contain high AST activity. Because myositis and myocarditis are uncommon disease in dogs arise of plasma AST activity generally indicate hepatic pathology (19).

5- Phagocytosis activity:
The number of bacterial raised from 0.1ml of mixture at different periods of incubation was showed in table (4). At zero time there was no statistical differences in number of colonies between the diabetic and control groups (P>0.05). After half an hour period of incubation the number of colonies raised from 0.1ml of mixture obtained from the diabetic group was significantly higher than those obtained from the control group (P<0.01), and the same results were obtained at 1 hour and two hours after incubation. This result can be explained by several points:
1. The function of polymorphonuclear leukocyte may be impaired this point require further study to establish or exclude it.
2. Phagocytosis promoting peptide (tuftsen) may be deficient (10).
3. Antibody mediated serum opsonization activity may be deficient due to decrease of serum immunoglobulin level in diabetes mellitus (14).
4. Complement mediated serum opsonization activity may be deficient (20).

The possible mechanism for complement deficiency observed in diabetes mellitus would include decrease synthesis of complement component and/or increase utilization of these factors (3). The fact that liver and spleen are the major sites of complement synthesis (20). In addition to demonstration of sever histopathological changes in diabetes mellitus (3) suggest that; the complement system deficiency in
diabetes mellitus due to decrease synthesis of complement component by severely affected macrophages or hepatocytes is the main possible cause of phagocytosis deficiency in diabetes mellitus.

Table (1): Serum glucose concentration in diabetic and control groups by mmol/L.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zero time</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.3 ± 0.6</td>
<td>5.4 ± 0.5</td>
<td>5.6 ± 0.43</td>
<td>5.72 ± 0.56</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5.2 ± 0.52</td>
<td>10.2 ± 0.36</td>
<td>15.62 ± 0.42</td>
<td>18.21 ± 0.36</td>
</tr>
<tr>
<td>P value</td>
<td>P&gt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Table (2): Serum ALT concentration in diabetic and control groups by IU/L.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zero time</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.21 ± 1.2</td>
<td>17.32 ± 0.9</td>
<td>16.81 ± 0.83</td>
<td>16.12 ± 1.12</td>
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<tr>
<td>Diabetic</td>
<td>16.22 ± 1.32</td>
<td>136.23 ± 4.33</td>
<td>184.26 ± 5.32</td>
<td>205.3 ± 5.62</td>
</tr>
<tr>
<td>P value</td>
<td>P&gt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Table (3): Serum AST concentration in diabetic and control group by IU/L.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zero time</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.5 ± 1.6</td>
<td>25.6 ± 1.3</td>
<td>23.3 ± 1.7</td>
<td>22.3 ± 1.6</td>
</tr>
<tr>
<td>Diabetic</td>
<td>23.6 ± 2.6</td>
<td>68.18 ± 2.3</td>
<td>98.12 ± 3.2</td>
<td>112.4 ± 2.8</td>
</tr>
<tr>
<td>P value</td>
<td>P&gt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Table (4): Number of bacterial colonies raised from 0.1ml of suspensions in diabetic and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Zero time</th>
<th>1/2 hrs</th>
<th>1 hr</th>
<th>2 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.18 ± 2.23</td>
<td>45.3 ± 1.82</td>
<td>40.2 ± 1.32</td>
<td>25.3 ± 1.61</td>
</tr>
<tr>
<td>Diabetic</td>
<td>50.2 ± 02.16</td>
<td>56.2 ± 1.33</td>
<td>61.3 ± 3.52</td>
<td>72.5 ± 2.91</td>
</tr>
<tr>
<td>P value</td>
<td>P&gt;0.05</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

References


22. Sekar, N.; William, S.; Balasubramaniyam, N.; Kamarjan, P. and
دراسة انزيمات الكبد وكفاءة عملية البلعمة في داء السكر المستحدث
تجريبياً في الجراء

رحمن كاظم محسن عباس يعقوب جاسم طهور عبدالله علوان
كلية الطب البيطرية/جامعة البصرة

الخلاصة
أجريت هذه الدراسة لتقييم بعض التغييرات التي تحدث في فعالية إنزيمات الكبد وكفاءة عملية البلعمة أثناء الإصابـة
بداء السكري التجريبي. استخدمت في هذه الدراسة عشرة (10) جراء قسمت إلى مجموعتين (مجموعة السيطرة ومجموعة
داء السكري). تم استخدام داء السكري في إحدى المجموعتين عند طريق الحقن الوريدي لمادة الألوكسان وبجرعة
100ملجم/كلغ. جمعت نماذج الدم من حيوانات المجموعة في زمن الصرفر (قبل حقن مادة الألوكسان) 10 و30 يوماً
بعد حقن الألوكسان وذلك لقياس تركيز الكولوكوز في المصل وفعالية انزيمات الكبد (AST&ALT) وكذلك لدراسة
كفاءة عملية البلعمة ضد جراثيم Salmonella typhimurium. أظهرت النتائج أن هناك ارتفاعاً معنويًا في مستوى كولوكوز
الدم في حيوانات مرجعية داء السكري وقد بدأت هذه المعايير بالإرتفاع ابتداءً من الأيام
العشرة الأولى حتى نهاية التجربة وكذلك لوحظ ان هناك تناوبًا طفيفًا بين فعالية إنزيم الكبد (AST&ALT) ومستوى
كولوكوز الدم في حيوانات مجموعة داء السكري. كذلك أظهرت النتائج إن الحيوانات المصابة بداء السكر التجريبي تعاني
من نقص في كفاءة عملية البلعمة.