Asian Journal of Biochemistry

ISSN 1815-9923
Changes in Body Weight and Serum Biochemical Measures of German Shepherd Dogs following Alloxan Induced Diabetes Mellitus

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ABSTRACT

The body weight losses and serum biochemical indices are considered as major signs of diabetes mellitus. The aim of present research was to investigate possible changes in serum biochemical indices and live body weight in German shepherd dogs, as a laboratory model for diabetes studies. In this research, ten German shepherd dogs has been used for analysis; 5 of which was considered as experimental groups and 5 was considered as control group. The examinations were conducted to guarantee their health and absence of diabetes with Intra-venous Glucose Tolerance Test (IVGTT) has been approved. Alloxan monohydrate with 100 mg kg⁻¹ in was injected intravenously for experimental group. After weighting, the blood samples were taken from control and diabetic dogs following diabetes induction. Next, biochemical measures of serum measures include glucose, total cholesterol, triglyceride, creatinine, total protein concentrations, Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) activity were determined. Based on statistical analysis, regardless to dramatic increases in glucose level, ALT and AST activities are increased in diabetic groups (p<0.05) and observed increase in serum ALF activity was very significant (p<0.01). The body weight of control group had almost constant trend during experiment, but diabetic dogs had considerable weigh losses especially after second IVGTT and following diabetes induction. It can be concluded that the weight losing of diabetic individual may be largely Independent from serum total cholesterol or triglyceride concentrations and is related to protein metabolism, because of lack of glucose source for energy obtaining. Further studies with compleitive biochemical profile of plasma are needed to clarifying discussed correlations.

Key words: Alloxan, diabetes mellitus, German shepherd dog, serum biochemical measures, alanine transaminase

INTRODUCTION

Diabetes mellitus has high correlation with plasma biochemical condition. In a relative study, higher LDL- and lower HDL-cholesterol is most important plasma related signs of occurrence of diabetes (Wingard et al., 1983; Stamler et al., 1993). Cholesterol concentration has high relation with incidence of diabetic coronary artery disorders (Haffner et al., 1997). Furthermore in non-diabetic individuals, positive correlation between high triglyceride concentration and coronary artery disorder has been observed (Tatami et al., 1981). Increasing evidences can states that higher plasma triglyceride in diabetic condition can be a sign for incidence of coronary artery disorder (Laakso et al., 1993). The biochemical abnormalities due to diabetes are numerous and can
be noted as hyperglycemia and subsequent dyslipidemia and protein catabolism as energy source for body (Mooney, 1999; Ladan et al., 2007). So, diabetic individuals have abnormal glycemic and lipidemic status that may observe via simple biochemical analysis of plasma. In this regards, Naderi et al. (2006) had stated that diabetes can influence serum protease enzymes activity. Also, Heideri et al. (2008) had reported considerable effect of glycemic status on sleep and memory in diabetic individuals. Regardless to diabetic changes in blood biochemistry, change in body weight was considered by Vahedian Ardakani and Ghotbi (2009) and they had reported considerable weight losses in relation with higher glucose level following diabetes induction in rat model. Incidence of diabetic weight losing is reported by Akpaso et al. (2011) and Zafar and Naqvi (2010), too. Dog is one of the animals which has the most diabetes case among animals. In the other hand, dog can be a useful laboratory animal in studying diabetic deficiencies. In this regard, the effects of alloxan induced diabetes were studied on hematological indices (Valilou et al., 2011). The aim of present research was to investigate possible changes in serum biochemical indices and live body weight in German shepherd dogs as a laboratory model for diabetes studies.

MATERIALS AND METHODS
Experimental design: In this study, ten male and females German shepherd dogs with age of 1.5-2 years old were used. These animals were apparently healthy as monitored by clinical examinations and survival signs control and had no special disease in their history. Dogs were transferred to research institute of Shabestar branch-Islamic Azad University. All animals were numbered and weighed. Next To make sure, they were given Antiparasitic Levamisole® in dose of 10 mg kg⁻¹. Meanwhile, Rabies vaccination was injected under the supervision of local veterinary organization. A 32 m² space used to keep them in animal room of university which is equipped with ventilation system was provided. Dogs were kept in Animal room according to animal ethics, but they could easily access to water and food was available according to their requirements. The numbering was from 575 to 584 and 5 of them were considered as the experimental group and the other 5 as the control group. In order to make them adapted to the prevailing condition and to avoid stress, Dogs were not subjected to any experiment for one week but during this period, they were checked for clinical signs. After adaptation period and weighting, Intravenous Glucose Tolerance Test (IVGTT) experiment was applied to make sure of the absence of diabetes. Then after 5 days, 100 mg kg⁻¹ of Alloxan monohydrate (Sigma®) was injected (IV). A week later, the second IVGTT was done and the presence of diabetes was approved. A week later, the second IVGTT was done and the presence of diabetes was approved. During the whole time in the experiment, the dogs in both groups were examined carefully for clinical signs (rectal temperature, heart rate, respiratory rate etc.) When animals in the experimental group indicated lethal symptoms, they were studied rapidly. After weighting of experimental animals, the whole blood samples were collected from diabetic dogs into tubes (non-diabetic or control, before and after induction of diabetes). Biochemical measures (total cholesterol, triglyceride, glucose, creatinine, total protein, ALT, AST and ALP) at clinical pathology laboratory of Shabestar Branch, Islamic Azad University.

Statistical analysis: Obtained data were analyzed by SAS software Ver.9.1 and Duncan’s multiple range tests were done for detection of significance difference between group means (n: 5, p<0.01).

RESULTS
Serum indices: According to Table 1, the dramatic elevation of glucose in diabetic group was so predictable and is almost important sign of diabetes. Total cholesterol, triglyceride, creatinine and
Fig. 1: Live body weight of German shepherd dogs during experiment

Table 1: Serum biochemical indices in German shepherd dogs following alloxan induced diabetes

<table>
<thead>
<tr>
<th>Serum biochemical measures</th>
<th>Pre-allocan injection</th>
<th>Post-allocan injection (diabetic)</th>
<th>p-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg dL⁻¹)</td>
<td>78.40</td>
<td>41.60</td>
<td>&lt;0.0010**</td>
<td>8.72</td>
</tr>
<tr>
<td>Total cholesterol (mg dL⁻¹)</td>
<td>232.60</td>
<td>240.00</td>
<td>0.7437**</td>
<td>15.46</td>
</tr>
<tr>
<td>Triglyceride (mg dL⁻¹)</td>
<td>27.40</td>
<td>29.00</td>
<td>0.5637**</td>
<td>1.88</td>
</tr>
<tr>
<td>Creatinine (mg dL⁻¹)</td>
<td>0.77</td>
<td>0.78</td>
<td>0.9256**</td>
<td>0.10</td>
</tr>
<tr>
<td>Total protein (mg dL⁻¹)</td>
<td>6.06</td>
<td>6.88</td>
<td>0.8062**</td>
<td>0.42</td>
</tr>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>57.40</td>
<td>82.60</td>
<td>0.01370*</td>
<td>5.67</td>
</tr>
<tr>
<td>AST (IU L⁻¹)</td>
<td>68.20</td>
<td>82.40</td>
<td>0.02850*</td>
<td>5.00</td>
</tr>
<tr>
<td>ALP (IU L⁻¹)</td>
<td>40.80</td>
<td>98.80</td>
<td>&lt;0.00100**</td>
<td>5.19</td>
</tr>
</tbody>
</table>

ns: Not significant, *Significant, **Very significant

total protein have non-significant changes in diabetic animals. ALT and AST activities are increased in diabetic groups (p<0.05) and increases in serum ALP activity was very significant (p<0.01).

**Body weight:** The body weight of control group was almost constant trend during experiment, but diabetic dogs had considerable weight losses especially after second IVGTT and following diabetes induction. The control group had normal weight gain trend during experimental period, where at the onset of experiment, both of groups had similar body weight (Fig. 1).

**DISCUSSION**

**Biochemical measures:** In present study, induced diabetes couldn't cause any considerable changes in plasma lipids. Our observation in this regard is in agreement with Firoozrai and Abasian (2002) report on serum lipid level in patient with type 2 diabetes mellitus. Also, similar survey of Alambay et al. (2011) show constant total cholesterol level for diabetic groups when it compared with healthy condition. But in some studies induced diabetes caused hyperlipidemia due to higher cholesterol level (Subash-Babu et al., 2008; Chigozie and Chidinma, 2012; Kannan et al., 2012).

It seems that induced diabetes couldn't cause considerable shift to hyperlipidemia condition. The creatinine and total protein levels didn't have considerable changes following diabetes induction. In Senthilkumar and Subramanian (2007) study, the serum AST, ALT and ALP had significant elevation in diabetic animals. Also in present study diabetes induction in dogs caused increase transaminase enzymes activity (p<0.05) and also ALP enzyme (p<0.01) (Table 1). Leakage of this enzyme from liver cytosol to blood circulation due to diabetes is mentioned by Senthilkumar and
Subramanian (2007) as a reason for increased serum measures of these enzymes (AST, ALT and ALP), but the another suggested reason for increase in AST activity following diabetes is greater need for gluconeogenic substrate due to diabetic condition (Tanaka et al., 1988), although elevation of both enzymes (ALT, AST) may also reflect the damage of the hepatic cells via injected alloxan (Kim et al., 2006). The elevated ALP activity in diabetic dogs (present study) is in agreement with Maxwell et al. (1986) suggestion that observed mean fasting serum glucose was significantly higher in the group with elevated ALP and suggested association between the severity of diabetes and diabetic bone disease.

**Body weight:** In Akpaso et al. (2011) report, diabetes caused 10.5 g weight losses in experimental rats whereas healthy (control) animals had 6.8 g weight gains during experimental period. Also, in Ahmida (2011) report, alloxan induced diabetes caused considerable weight losses (-12.5%), when compared with control or healthy rat (+8.9%). In present experiment, induced diabetes caused 11.7% weight losses in diabetic dogs, where control group had 7.3% weight gain. Present data for weight losses is in agreement with diabetes signs. The diabetic animal has considerable energy losses via glycosuria that it can be major source of energy and weight maintenance in a healthy animal. This event is coupled with loss of muscle and adipose tissue due to excessive breakdown of protein, as a metabolic disorder. So, an untreated diabetic animal will have negative body weight trend (Ahmed et al., 2005).

**CONCLUSION**
With attention to biochemical profile and weight trends of diabetic dogs it can be concluded that the weight losing of diabetic individual may be largely Indendent from serum total cholesterol or triglyceride concentrations and is related to protein metabolism. Further studies with complete biochemical profile of plasma are needed to clarify discussed correlations.

**REFERENCES**


