Hematopoietic measures in German shepherd dogs following Alloxan induced diabetes mellitus

Mohammadreza Valilou, Jalal Shayegh, Behrad Eshratkhah, Alireza Lotfi

Department of Veterinary medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran
Department of Animal science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Mohammadreza Valilou, Jalal Shayegh, Behrad Eshratkhah, Alireza Lotfi; Hematopoietic measures in German shepherd dogs following Alloxan induced diabetes mellitus

ABSTRACT

Diabetes mellitus involves most physiological functions include hematopoiesis. In this research, ten German shepherd dogs were provided; 5 of which was considered as experimental groups (pre- and post- diabetes induction) 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 in was injected intravenously for experimental group. The blood samples were taken from control and diabetic dogs before and after diabetes induction. Next, hematopoietic measures (Red blood cells (RBC), hematocrit percentage, and hemoglobin, MCHC, MCH and MCV) were determined. RBC number hadn’t change after Alloxan injection (6.8 in compare with 6.93 or 6.77 million/micrometer before diabetes induction and control groups). Also Hemoglobin (Hb) concentration hadn’t significant difference between experimental groups (14.18, 15.28 and 13.92 mg/dl). Hematocrit percentage, MCH, MCV and MCHC hadn’t significant changes after diabetes induction. Data shows that Alloxan induced diabetes mellitus can slight (no significant) reduce Hb concentration but it hasn’t significant effect on RBC number. It is concluded Alloxan induced diabetes mellitus in German shepherd dogs didn’t have negative effect on Hematopoietic activity after short-term alloxan induced diabetes.

Key words: Diabetes mellitus, hematopoiesis, Red blood cells, dog.

Introduction

Diabetes mellitus is one of the endocrine system diseases in human and animal which involves the blood circulatory system. About 6.3% of world populations live with diabetes. Diabetes creates the following common symptoms during its chronic length: thirst, poly urea, appetite increase and weight reduction, heart and coronary problems, kidney problems, sight problem, coma, shock, ketosis, blood glucose increase, blood pressure increase and so on [10].

Alloxan has been widely used as a diabetogenic agent. It has been suggested that the selective destruction of pancreatic beta cells is mediated by free radicals of oxygen formed by redox cycling [2, 12]. Alloxan is a strong oxidizing agent. Its reduction product is dialuric acid. Alloxan and dialuric acid form a compound, allooxinant, which in water can dissociate into alloxan and dialuric acid. Claim were made and later disputed, that dialuric acid and allooxinant were diabetogenic [11].

Due to formation of suitable condition for diabetes it has increased in animals. Dog is one of the animals which has the most diabetes case among animals. In the other hand, dog can be a applicable laboratory animal for studying diabetic deficiencies and in this way helps veterinary and medical
The pathological damages of alloxan induced diabetes in dogs have been reported on kidney [16, 17], Testis [14], coronary arteries [15], Liver [17] and etc.

About hematopoietic effects of alloxan induced diabetes, published limited findings show partially and no significant [1] or significant [4] decreasing hematocrit in rat following diabetes induction. Azeez et al., [4] showed that alloxan induced diabetes caused significant decrease in hematopoietic measures include RBC, Hb, PCV, MCV, MCH and WBC. Findings In patients with diabetic retinopathy show no significant decrease in hematopoiesis activity or any significant correlation between hematological parameters and diabetic retinopathy [7].

With attention to Watanada et al., [17] reports for hematological changes (specially in platelet count) and different or opposite reports about hematopoietic effects of induced diabetes in mammalian [1, 4, 7], the aim of this study was determination of Hematopoietic measures in German shepherd dogs (as an animal model) following Alloxan induced diabetes mellitus for evaluation of possible diabetic effects on hematopoiesis.

Materials and Methods

In this study, nine 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 10mg/kg. Meanwhile, Rabies vaccination was injected under the supervision of local veterinary organization. A 32 m² space used to keep them in research center of university which is equipped with ventilation system was provided. Dogs were kept in Animal room according to animal ethics, but they could easily move in a limited space, water and food were 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, Intravenous glucose tolerance test (IVGTT) experiment was applied to make sure of the absence of diabetes. Then after 5 days, 100mg/kg 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 (anal temperature, heart rate, respiratory rate...)

When animals in the experimental group indicated dangerous symptoms, they were studied rapidly. The whole blood samples were collected from diabetic dogs into EDTA included tubes (cnon-diabetic or control, before and after induction of diabetes). RBC, hematocrit, Hb, MCHC, MCH and MCV were measured via hemocytometer method at pathology laboratory of Shabestar Branch, Islamic Azad University.

Results and Discussion

In this experiment, data obtained from experimental groups did not show any significant difference between all of determined measures (RBC, Hb, Hematocrit, MCHC, MCH and MCV) [table 1].

Diabetes mellitus may cause anemia as secondary disorder, for example after occurrence of diabetic nephropathy [13]. Diabetes-related anemia has been observed in the advanced uremia of diabetic nephropathy; however, diabetes affects the hematologic system in several ways [8]. It was suggested anemia occurrence in diabetic cases may be because of insufficient androgen releasing function of adrenal glands or less erythropoietin concentration [3]. In our previous study [16] histo-pathological changes of dogs kidney include cell degeneration, vacuolization of endothelial cells and thickness of basal membrane were seen at glumerolar filtration surface.

Bonakdaran et al., [5] suggested that Anemia has a high prevalence in type 2 diabetic patients and it has high correlation with kidney disorders. Li Vecchi et al., [9] reported that diabetes in corporation with kidney nephropathy can cause anemia. In present study alloxan injection caused diabetes in experimental dogs but hadn’t any significant effects on RBC, Hb, Hematocrit, MCHC, MCH and MCV rates (table 1), that are in agreement with Li Vecchi et al., [9] and Erkin et al., [7] in human and Abu-Samak et al., [1] findings in animal model, but is apposite to Watanabe et al., [17] in dogs or Azeez et al., [4] in rat. Our findings show that hematopoiesis activity in diabetic dogs in almost constant after short-term (42day) induction of diabetes. Difference between our findings and Watanabe et al., [17] can
Table 1: Hematopoietic measures in germen shepherd dogs at control, pre- and post-Alloxan injection (diabetes induction) condition

<table>
<thead>
<tr>
<th>No. Measures</th>
<th>Control</th>
<th>Pre-alloxan injection</th>
<th>Post-alloxan injection (diabetic)</th>
<th>Significance p value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (million/microliter)</td>
<td>6.77</td>
<td>6.93</td>
<td>6.8</td>
<td>0.871 ns</td>
<td>0.220</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.18</td>
<td>15.28</td>
<td>13.92</td>
<td>0.097 ns</td>
<td>0.428</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.2</td>
<td>45.6</td>
<td>47.22</td>
<td>0.443 ns</td>
<td>1.146</td>
</tr>
<tr>
<td>MCHC (%) (g/dl)</td>
<td>29.35</td>
<td>29.64</td>
<td>28.86</td>
<td>0.951 ns</td>
<td>1.759</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.83</td>
<td>22.06</td>
<td>20.7</td>
<td>0.012 ns</td>
<td>2.173</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>66.86</td>
<td>68.79</td>
<td>71.98</td>
<td>0.244 ns</td>
<td>2.236</td>
</tr>
</tbody>
</table>

be because of longer diabetic period in their study (fourteen months), in other words induced diabetes could has anemia-related effects after longer experimental period, but in present study 42day post-diabetic condition (shorter than Watanabe et al., [17] experiment) may couldn’t sufficient for anemia or nephropathy-related anemia occurrence in dogs. It was concluded that alloxan induced diabetes mellitus hasn’t significant effect on hematopoietic indicators include RBC, Hb, Hematocrit, MCHC, MCH and MCV, also can’t affect hematopoiesis in short-term post-diabetes in German shepherd dogs as animal model. Investigations on hematopoiesis in diabetic condition in both of short or long time duration can be necessary for clarifying that when induced diabetes can destroy hematopoiesis activity?

References:

