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Full Length Research Paper

Investigation of the ultrastructural and histopathological changes in coronary arteries of German shepherd dogs following alloxan induced diabetes mellitus

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Diabetes mellitus is one of the most common disease of the endocrine system in the body that is diagnosed by metabolic malfunction in the metabolism of carbohydrates, fat and proteins. This disease involves most tissues of the body and the consequent deficiencies reduce their efficiency, cause infections and diseases in the body. Alloxan is a chemical which is used in inducing experimental diabetes in animals. In this research, 9 German shepherd dogs were provided, 5 of which was considered as our experimental group and the remaining 4 was considered as the control group. The necessary examinations were conducted to guarantee their health and the absence of diabetes was ascertained with intravenous glucose tolerance test (IVGTT). 100 mg/kg of alloxan monohydrate were given to the experimental group. After the administration, animals were obsevered for clinical symptoms. If the symptoms indicate death, samples tissue of coronary arteries were taken. Microscopic and ultra structural deficiencies were checked for. The endothelial cells of the coronary arteries showed vacuolization of cytoplasm with decreased transitional vesicles and thickened basal membrane. It was concluded that diabetes mellitus is a potent risk factor for the development of coronary atherosclerosis.

Key words: Diabetes mellitus, ultra structural, coronary arteries, dog.

INTRODUCTION

Diabetes mellitus, a heterogeneous clinical syndrome of antiquity, is characterized by hyperglycemia. It is one disease that increases the risk of atherosclerosis. Researchers believe that high glucose level damage blood vessels, creating a greater likelihood of developing cardiovascular problems. Diabetes mellitus is one of the endocrine system diseases in human and animal which involves the blood circulatory system. About 6.3% of the world population live with diabetes. Diabetes creates symptoms such as thirst, polyurea, appetite increase and weight reduction, heart and coronary problems, kidney problems, sight problem, coma, shock, ketosis, blood glucose increase, blood pressure increase among others (Nelson, 1995; Daniel porte et al., 2005). The factors that contribute to diabetes include heritation, pancreas deficiencies (infections, immune deficiency and tumors), drug and chemicals, environmental factors (obesity, stress, high blood pressure, physical inactivity and age increase).

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 (Adock et al, 1983, Sima et al., 1991). Alloxan is a strong oxidizing agent. Its reduction product is dialuric acid. Alloxan and dialuric acid form a compound, alloxantin, which in water can dissociate into alloxan and dialuric acid. Claim were made and later disputed, that dialuric acid and alloxantin were diabeto-

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Table 1. Survival parameters in the start of test in all dogs.

Group	Control				Experimental				
Number	578	579	580	583	575	576	577	581	582
Temperature (°C)	38.6	38.7	38.3	38.5	38.7	38.5	38.4	38.2	38.7
Heart rate (per minute)	97	93	87	89	94	92	85	89	82
Respiratory rate (per min)	30	28	29	29	32	30	35	28	26

Table 2. Survival parameters in the end of test, control and experimental groups of dogs (after injection of alloxan to experimental groups).

Group	Control				Experimental				
Number	578	579	580	583	575	576	577	581	582
Temperature (°C)	38.5	38.3	38.1	38.6	38.9	37.7	38	38.4	37.8
Heart rate (per minute)	94	95	90	86	117	119	121	124	130
Respiratory rate (per min)	32	31	30	28	39	38	40	37	38

genic (Santilli et al., 2004).

Due to favourable conditions, diabetes has increased in animals. Dog is one of the animals which has the most diabetes case among animals. On the other hand, dog can be a useful laboratory animal in studying diabetic deficiencies and in this way help veterinary and medical researchers.

The effects of alloxan induced diabetes in ultra structural changes of kidney and testicles of German shepherd dogs were studied and observed detrimental effects of induced diabetes on these organs functions evaluated (Valilue et al., 2007; Valilue and Lotfi 2010). The aim of this project is to survey the microscopic and ultrastructural deficiencies of coronary arteries in German shepherd dogs which suffer diabetes via alloxan induction.

MATERIALS AND METHODS

In this study, nine male and female German shepherd dogs of age 1.5 - 2 years old were used. These animals were apparently healthy as monitored by clinical examinations and survival signs. Dogs were transferred to the research institute of Islamic Azad University - Shabestar branch. All animals were numbered and weighed. They were then given Antiparasitic *Levamisole*® in dose of 10 mg/kg. Rabies vaccination was also injected but under the supervision of a local veterinary organization.

A 32 m^2 space was used to keep them in the research center of the University which is equipped with ventilation system. Dogs were kept in animal room where they could easily move in a limited space with water and food being provided according to requirements. The numbering was from 575 to 583 and 5 of them were considered as the experimental group and the other 4 as the control. Dogs were allowed to acclimatized for one week while checking for clinical signs.

After adaptation, intravenous glucose tolerance test (IVGTT) experiment was applied to ensure the absence of diabetes. Then after 5 days, 100mg /kg of alloxan monohydrate (Sigma®) was injected (IV). 3 days later, one of the dogs in this group died and autopsy was done quickly. A week later, the second IVGTT was done and the presence of diabetes was confirmed. During the whole time of the experiment, the dogs in both groups were carefully examined for clinical signs such as anal temperature, heart rate, respiratory rate among others.

When animals in the experimental group show dangerous symptoms, they were rapidly studied. If the symptoms indicate the death of the animal, to avoid any mortality of the animal at night and to avoid autolysis, autopsy was done and sample of the animal tissues taken.

The samples (include coronary arteries) for light microscope were fixed in formalin buffer 10% and the samples for electronic microscope were fixed in glutar-aldehyde buffer 3%. Autopsy and sample collection of coronary tissues in control group was also done. Light microscope sections were stained with H and E stain, while electronic microscope sections were stained based on standard methods using uranil acetate and lead citrate (Bozzola, 1992).

RESULTS

Clinical symptoms

Incidence of the disease appeared about 30 h after injection of alloxan. Probably due to diabetic acidosis, symtoms such as lack of appetite, vomiting, thirst, polyurea, increased rate of breathing, decreased consciousness, dehydration, slight hypovolemic shock signs and tachycardia, as well as decreased body temperature were observed. In addition, stomach ache due to acidosis, tiredness and lack of electrolyte balance were noted. Severe hyperglycemia, increase in plasma osmolarity, and decrease in liquid amount of the body contributed to decreased consciousness and coma in central nerves.

Survival parameters are shown in Tables 1 and 2, changes in dog's weight is shown in Table 3, duration of survival of dogs after alloxan injection is shown in Table 4 and comparison of water consumption mean to dog's weight in different times is shown in Table 5 . In all the experimental groups, the heart had hydropericard and

Table 3. Changes of weight in control and experimental groups of dogs during the research (Kg).

Group	Control				Experimental				
Number	578	579	580	583	575	576	577	581	582
First IVGTT	26.5	24	38.3	25.3	29.5	31	26	27.2	23
Injection of Alloxan in experimental group	27	24.6	38.5	26	30	32	27	28	23.5
Second IVGTT	27.5	25	39	27	29	30	-	26.5	22
End of Test	28.2	26	40	28.1	25	27	24	23.1	21.5

Table 4. Duration of survival in experimental groups of dogs after alloxan injection.

Parameters	Experimental group				
Number	577	582	576	575	581
Sex	M	F	M	M	F
Duration n (day)	3	14	18	38	43

Table 5. Comparison of water consumption based on dog's weight in different times.

Time	Control (water consumption mean to weight, L/Kg)	Experimental (water consumption mean to weight, L/Kg)
After IVGTT-1	0.0745	0.759
After IVGTT-2	0.0757	0.120

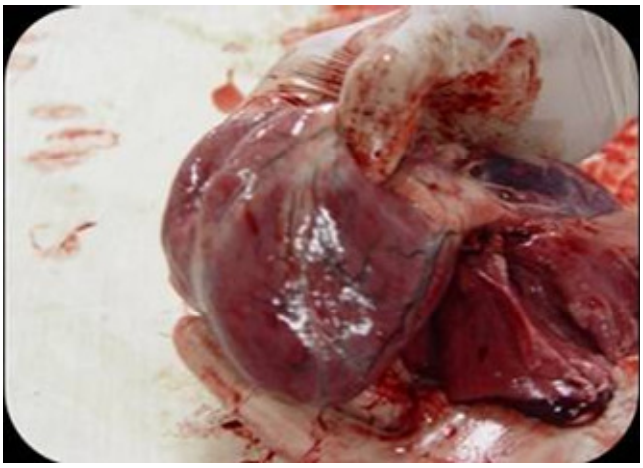

Figure 1. Heart of dog after injection of alloxan and incidence diabetes mellitus.

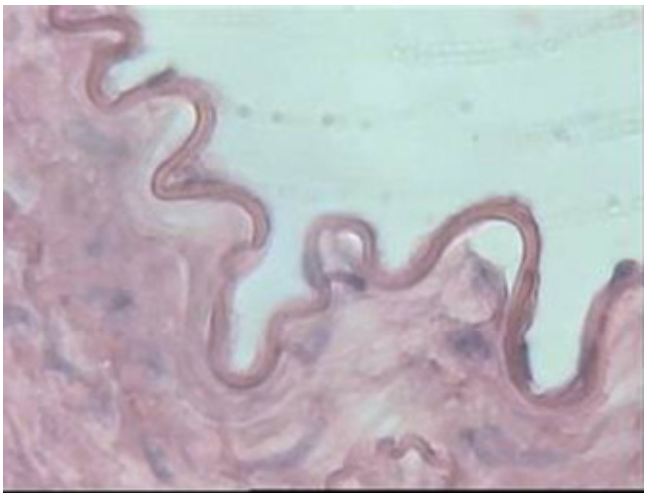

Figure 2. Coronary artery in control group. Endothelial cells are normal (H&E X400).

hyperemia features (Figure 1)

Light microscopic lesions

Normal coronary arteries were observed in the control group (Figure 2). Cases 575 and 581 showed vacuoles in the endothelial cells of coronary arteries (Figure 3).

Ultra structural lesions

Normal endothelial cells were observed in coronary arteries of control group (Figure 4). Abnormality of cells, vacuolization of cytoplasm, demolition of nucleus, cell degeneration, thickened basal membrane and decreased transitional vesicles in the endothelial cells of coronary

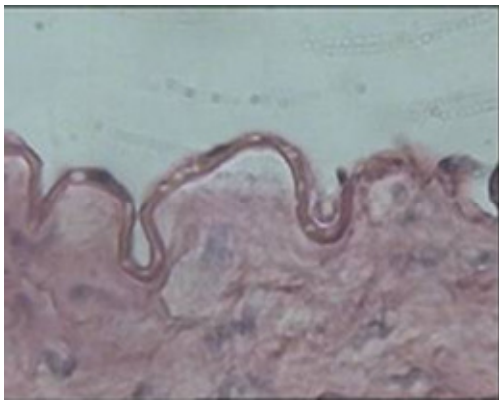

Figure 3. Coronary in dog after injection of alloxan and incidence diabetes mellitus vacuoles was seen in the endothelial cells (H&EX400).

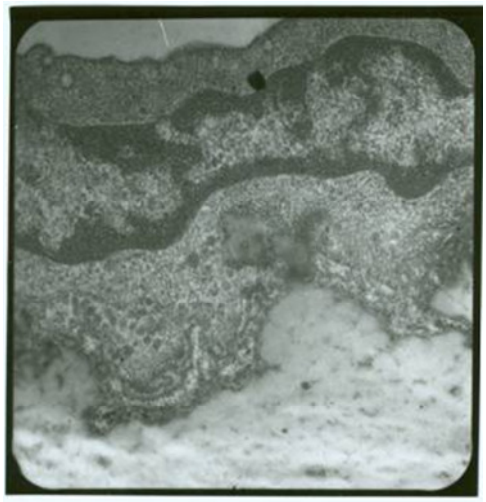

Figure 4. Electron micrograph of normal endothelial cell in coronary in control group of dog (X27000).

arteries in the experimental group were also observred (Figure 5).

DISCUSSION

Diabetes is one of the major risk factors for heart attack, stroke and other cardiovascular - related diseases. Majority of diabetic patients have high blood pressure, which contributes to heart disease and other diabetic complications (Horiuchi et al., 2005, Jacoby and Nesto, 1992). Atherosclerosis is a disease in which fatty plaque gradually forms on the inner walls of the arteries. This disorder, also known as hardening of the arteries, causes the arteries to become narrow and restricts the blood flow to organs and tissues (Horiuchi et al., 2002; Widlansky et al., 2003; Fukuda 2005). Diabetes increases the risk for

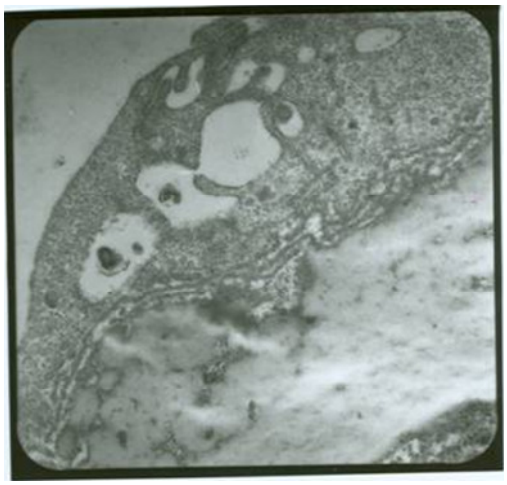

Figure 5. Electron micrograph of endothelial cell in coronary after injection of alloxan and induce diabetes mellitus in dog including vacuolization of cytoplasm, demolition of nucleus, cell degene-ration, thickened basal membrane was and decreased transitional vesicles (X27000).

atherosclerosis and other cardiovascular complications partly because of excess glucose (blood sugar). This hyperglycemia damages arteries by making the walls thicker and less elastic. The change makes it more difficult for the blood to pass through the vessels. Atherosclerosis increases the risk of heart attack in which blockages within the coronary arteries prevent blood from reaching the heart. Similarly, atherosclerosis can cause cerebrovascular disease, which restricts blood flow to the brain and increases the risk of stroke. Atherosclerosis can affect other parts of the body such as the legs, and contributes to the development of peripheral arterial disease. However, there are a number of things that people with diabetes can do to prevent atherosclerosis.

Diabetes in dogs is usually seen in middle age and old dogs. In order to create experimental diabetes in animals, we can make use of pancreatectomy or prescribe chemical drugs such as alloxan, streptozotocine, etc (Gunduz et al., 1993). The normal dose of creating diabetes in dog with alloxan is 65 - 200 mg/kg intravenously. Clinical signs signs in humans and animals are almost the same which include over thirst, severe thinness, increase rate of urination, increase of appetite, hyperglycemia, glycosuria and ketonuria. In this project, the above mentioned symptoms were seen. Also, we observed abnormality in respiratory system, loss of hair and diarrhea (Taniyama et al., 1995). People who have type 1 diabetes tend to develop disease that affects small arteries, such as those in the eyes, nerves, and kidneys, leading to vision loss, nerve damage, and kidney failure. Some people with type1 diabetes and most people with type2 diabetes tend to develop atherosclerosis in the large arteries. These people also tend to develop atherosclerosis at an earlier age and more extensively than do people who do not

have diabetes. The risk of developing atherosclerosis is 2 to 6 times higher for people with diabetes, particularly women. People who have diabetes have the same risk of death as someone who has had a prior heart attack, and doctors usually try to help these people keep other risk factors (such as high cholesterol levels and high blood pressure) under careful control. (Nelson, 1995, Inzucchi, 2004, Thalhammer et al., 1999). Atherosclerosis is a narrowing of the arteries caused by a buildup of fatty substance (cholesterol) that can eventually partially or completely block blood flow. Coronary artery disease (CAD) occurs when these blockages develop in the arteries that feed the heart. CAD can cause chronic hypertension, chest pain, and heart attack. A heart attack or myocardial infarction occurs when a nearby blood vessel becomes blocked. Blood flow (and the oxygen the blood carries) is reduced and the affected area of heart muscle suffers damage or tissue death (Perez et al., 1992).

A hypothesis for the initial event of atherosclerosis is endothelial dysfunction associated with changes in the concentration of the chemical messengers produced by the endothelial cells or by blunting of the nitric oxide - dependent vasodilatory response to acetylcholine (Santilli et al., 2004). The endothelial abnormalities observed in patients with diabetes are poorly understood, but the loss of normal endothelial function could be involved in the pathogenesis of diabetic long-term complications such as angiopathy, as endothelial dysfunction associated with diabetic micro- and macro-angiopathy. Some authors reported activation of protein kinase C, overexpression of growth factors, cytokines and oxidative stress in endothelial cells of arteries in patients with diabetes (Lusis, 2000; Matner et al., 1992; Nelson, 1995).

This study show microscopic degeneration of coronary arteries with electron micrographs for the first time. Cardiovascular disorders following induced diabetes were reported by Mautner et al (1992) and Horiuchi et al. (2002). Results of the present electron microscopic study are in accordance with their studies. On the other hand, in our previous studies (Valilou et al., 2007; Valilou and Lotfi, 2010), micrographs of pathological degeneration in kidney, testis and disorder in spermatogenesis following induced diabetes were published and reported.

In the present work, relative microscopical degeneration in coronary arteries were observed too.

Conclusion

The macroscopic study showed hydropericard and hyperemia in all the experimental group. Endothelial cells were seen in the histopathological study of coronary arteries in the experimental group after injection of alloxan and incidence diabetes mellitus, vacuoles were seen. In the ultra structural study in the coronary arteries, vacuolization of cytoplasm, demolition of nucleus, cell degeneration, thickened basal membrane and decreased transitional vesicles were seen. Conclusively, it could be said that diabetes mellitus is a potent risk factor for the development of coronary atherosclerosis.

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