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Histopathological and Ultra Structural Lesions Study of Kidney of Alloxan Induced Diabetes Mellitus in German Shepherd's Dog

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Abstract: Diabetes mellitus is one of the most common disease of endocrine glands in body that is diagnosed by malfunction in natural metabolism of carbohydrate, fat and protein. This disease involves most tissues of the body and the consequent deficiencies reduce their efficiency, cause infections and disease in body. Alloxan is a chemical which is used in creating 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 our control group. The necessary examinations were conducted to guarantee their health and we approved the absence of diabetes with Intravenous Tolerance Test (IVGTT).We injected intravenously Alloxan mono hydrate with 100 mg kg^{-1} in experimental group. After approving the presence of diabetes by IVGTT, we reported clinical signs. We scrutinized, macroscopic, microscopic and ultra structural deficiencies of kidney and then we stated the result.

Key words: Diabetes mellitus, alloxan, dog, ultra structural, kidney

INTRODUCTION

Diabetes mellitus is one of the endocrine glands disease in human and animal which involves the blood circulatory system. About 6.3% of world population 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 (Silvio *et al*., 2005).

The factors that contribute to diabetes may be as following: heritation, pancreas deficiencies (infections, immune deficiency and tumors), drug and chemicals, environmental factors (obesity, stress, high blood pressure, physical inactivity, age increase and high cholestrol).

Due to formation of suitable condition diabetes 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 useful laboratory animal in studying diabetic deficiencies and in this way help veterinary and medical researchers.

The goal of this project is surveying macroscopic, microscopic and ultra structural deficiencies of kidney in German shepherd dogs which suffer diabetes via Alloxan.

MATERIALS AND METHODS

To do this research, 9 German shepherd dogs which have been apparently healthy through clinical examinations and survival signs control and had no special disease in their history were provided.Their age range between 1-2 and both male and female. They were transferred to research institute of Islamic Azad University of Shabestar. All animals were numbered and weighed. To make sure, they were given anti-parasite (levamisole) with the dose of 10 mg kg^{-1}. Meanwhile, Rabies vaccination was injected under the supervision of veterinary organization of the city.

We devoted a 32 m square space to keep them in research center of university which is equipped with ventilation system. Dogs were tied through special things. So that they could easily move in a limited space, water and food were available but they couldn't bother each

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other. The numbers were from 575-583 and 5 of them were considered as our experimental group and the other 4 as our control group.

The dogs diet was defined according to the consultation of scholars and people who are involved in breeding and keeping dogs and then was given to the dogs. This diet was given based upon dog's weight, twice a day (morning and afternoon) and water was freely available.

In order to make them get used to the condition and to avoid stress, we didn't do any experiment for one week but during this period we checked clinical signs of the animals daily and took a note of them.

With doing IVGTT experiment, we made sure of the absence of diabetes. Then after 5 days, we Injected (IV) 100 mg kg^{-1} of Alloxan mono hydrate purchased from Sigma Company within a minute at 8:00 in the morning to the dogs of our experimental group.

Three days later, one of the dogs in this group died and we quickly did autopsy. A week later, the second IVGTT was done and the presence of diabetes was approved.

During the whole time in the project, the dogs in both groups were examined carefully from the view point of clinical signs in away that anal temperature, heart rate, respiratory rate, sight evaluation by threat reflex and approaching finger to eye, mouth inspection, skin, movement organs behavior and movement of animal, eating condition and water were scrutinized.

When animals in experimental group indicated dangerous symptoms, we studied the cases rapidly. If the symptoms were indicating the death of the animal, to avoid dying of the animal during night and to remove autolysis, we did autopsy and took sample of the animal.

The samples for light microscope were put in fixative formalin buffer 10% and the samples for Electronic microscope were put in fixative glutar-aldehyde buffer 3%. After that, the next stages of tissue passage, was done. After finishing the experimental group, we started autopsy and taking samples of kidney tissues in control group. Light microscope sections were colored based on standard methods (uranil acetate and lead citrate) (Bozzola, 1992).

RESULTS

Clinical symptoms: At the beginning of the disease which appeared about 30 h after injection of Alloxan, due to diabetic acidosis, we had symptoms like lack of appetite, vomiting, thirst, poly urea, breathing increase, consciousness decrease, dehydration, slight hypovolemic shock signs and tachycardia. Temperature of body decreased a little.

We had stomach ache due to acidosis, tiredness and lack of electrolyte balance. Sever hyperglycemia, increase in plasma osmolarity and decrease in liquid amount of the body contributed to decrease of consciousness and coma in central nerves.

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

	Group								
	Control				Experimental				
Parameters	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 min)	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				
Parameters	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 min)	94	95	90	86	117	119	121	124	130
Respiratory rate (per min)	32	31	30	28	39	38	40	37	38

Table 3: Changes of weight in control and experimental groups of dogs in duration of research (kg)

	Group								
	Control				Experimental				
Time	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 injection of alloxan

No.	577	582	576	575	581
Sex	M	F	M	M	F
Duratio n(day)	3	14	18	38	43

Table 5: comparison of water consumption mean to dog's weight in different times

	Group	
	Control	Experimental
Time	Water consumption mean to weight	Water consumption mean to weight
After IVGTT-1	0.0745	0.759
After IVGTT-2	0.0757	0.120

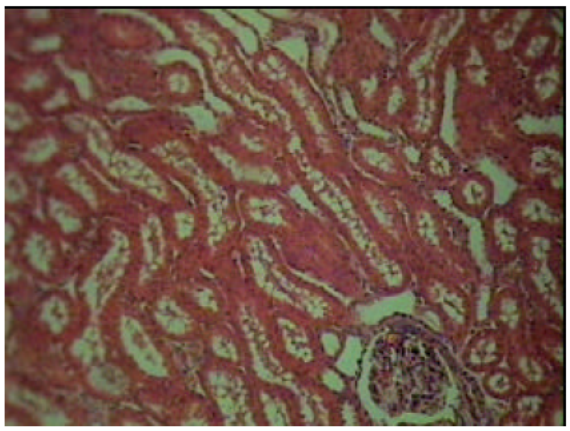

Fig. 1: Kidney in dog after injection of Alloxan and incidence diabetes mellitus hallow vacuoles, cloudy swelling and coagulative necrosis in epithelial cells of tubules, hyaline casts in tubules (X100)

Alopecia specially in case number 581 was observed. Survival parameters are shown in Table 1 and 2, changes in dog's weight are shown in Table 3, duration of living 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.

Macroscopic deficiencies: Hyperemia in kidney was observed in case 577. Kidney inflation symptoms and its capsule and hyperemia were observed in case 575. Kidney inflation and a little paleness were seen in case 575 and 581. Kidney inflation and hyperemia were present in case 582.

Microscopic deficiencies: Some vacuoles were seen in epithelial tissue of tubules in case number 575. There were hyaline casts in tubules. Hyperemia and coagulative necrosis were seen in epithelial cells of tubules.

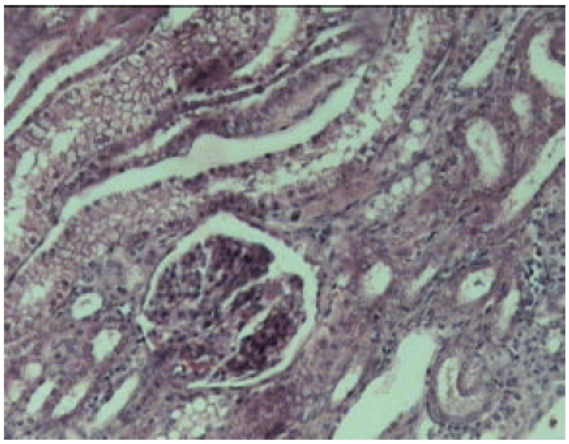

Fig. 2: Kidney in dog after injection of Alloxan and incidence diabetes mellitus hallow vacuoles, cloudy swelling and coagulative necrosis in epithelial cells of tubules, inflammation cells were seen intertubules (X100)

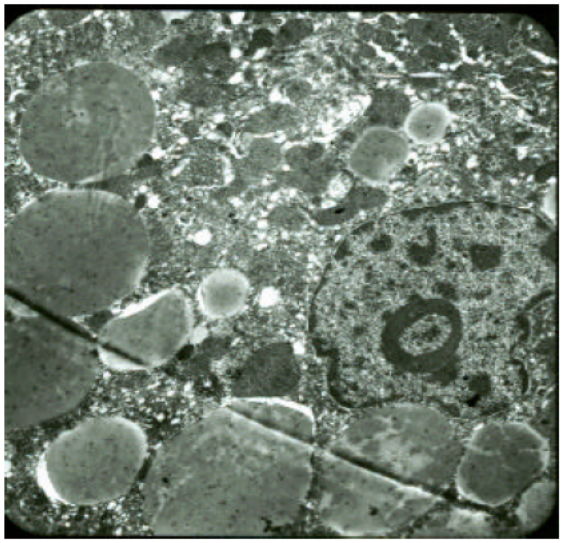

Fig. 3: Electron micrograph of epithelial cell of tubule in kidney after injection of Alloxan and incidence diabetes mellitus in dog including vacuolization of cytoplasm, cell degeneration, demolition of mitochondria (X6000)

In case number 581, glycogen precipitate occurred in epithelial cells of tubules and they appeared light. Inflammation cells were among tubules.

In case 576 and 582, there was hyperemia in kidney. There were cloudy swelling and vacuoles in the epithelial cells of tubules. Inflammation cells were seen between the tubules. There was coagulative necrosis (Fig. 1 and 2). In case 577 we could see hyperemia.

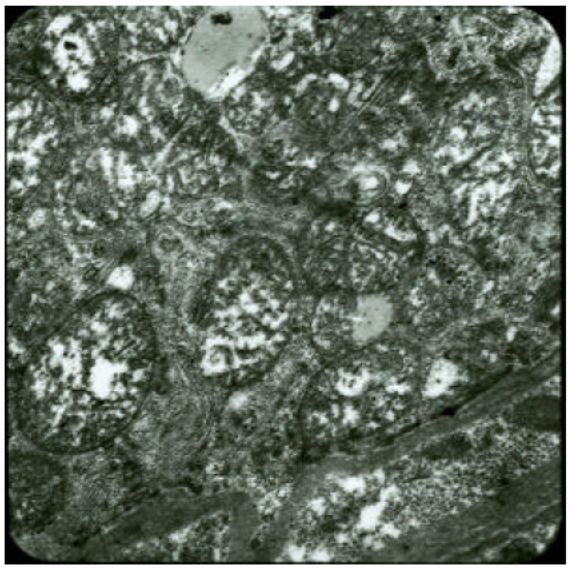

Fig. 4: Electron micrograph of epithelial cell of tubule in kidney after injection of Alloxan and incidence diabetes mellitus in dog including vacuolization of cytoplasm, cell necrosis, demolition of mitochondria and rupture of its crista (X27000)

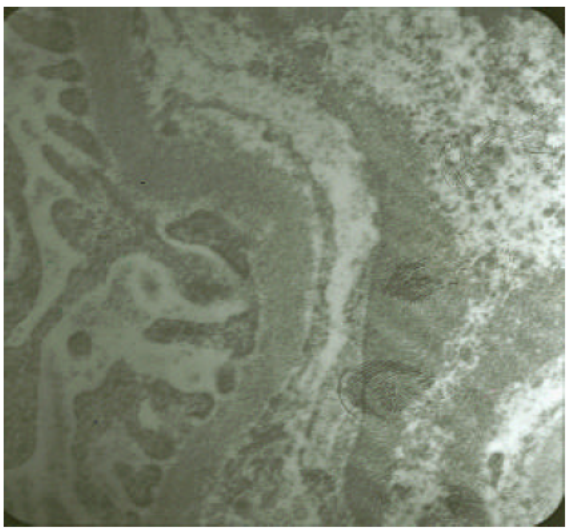

Fig. 5: Electron micrograph of glumerolar filtration surface in kidney after injection of Alloxan and incidence diabetes mellitus in dog including vacuolization of endothelial cells, transitional vesicles decrease, basal membrane is thicken, microangiopathy (X46500)

Electronic microscope deficiencies: In the epithelial cell of tubule in kidney, we could see vacuolization of cytoplasm, cell degeneration, demolition of mitochondria and rupture of its cristas.

In glumerolar filtration surface of kidney we could see vacuolization of endothelial cells, transitional vesicles decrease, basal membrane is thicken and microangiopathy (Fig. 3-5).

DISCUSSION

The distribution of diabetes is reported normally one in 200. Incidence of diabetes mellitus by itself in dog either elementary or secondary is due to pancreas atrophy because of beating, by itself atrophy, pancreas hypoplasia, aplasia, dysplasia, sexual cycle (estrus stage) pancreatitis, influence of amyloid, glycogen, collagen and connective tissue in langerhans islets, treatment with prednisolone, acidophil cell's adenoma in hypophysis, obesity, infection, hyperadrenocorticism is reported. Of course pregnancy diabetes is reported in dog too (Prathaban, 1994).

Diabetes among 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 and so on (Gunduz *et al*., 1993).

The normal dose of creating diabetes in dog with Alloxan is 65-200 mg kg^{-1} in form of IV (Nicholas *et al*., 1988). The injection speed is very effective and the drug should be provided recently (Adock *et al*., 1983).

Clinical signs in humans and animals are almost the same which include: Over thirst, severe thinness, increase of urine, increase of appetite, hyperglycemia, glycosuria and ketonuria. In this project, the above mentioned symptoms were also seen. Also,we observed abnormality in respiratory system, loss of hair and diarrhea (Taniyama *et al*., 1995)

Researchers have found out that glomerulo-sclerosis is the most common histologic problem.The kidney of patients with diabetic nephropathy show arteriosclerosis and arteriolosclerosis. In arteries atheromatosis changes usually improved and may play a role in kidney insufficiency with ischemia and paranchyma atrophy. In kidney arterioles, thickening of the hyaline is the index of arterioles and it is believed that it has an important role in increasing the blood pressure (Anderson *et al*., 1993).

In more advanced cases, tubular atrophy is seen. In diabetic people, kidney deficiencies can be in the form of Glomerular, tubular, vessles and pyelonephritis. Glycogen precipitation occurs inside the last parts of proximal and distal convoluted tubules and descending limb loop of

henle. It is possible that fat, mucopolysaccharide, calcium, albumin and other proteins precipitate in diabetic kidney tubules (Silvio *et al*., 2005).

CONCLUSION

In experimental group, kidney was seen paling. In microscopic study, vacuoles were seen in epithelial cells of tubules. Inside the tubules, there were hyaline casts and in some parts, inflammation cells were seen between tubules and there was coagulative necrosis. In ultra structural study, in the epithelial cell of tubule in kidney, we could see vacuolization of cytoplasm, cell degeneration, demolition of mitochondria and rupture of its cristas. In glumerolar filtration surface of kidney we could see vacuolization of endothelial cells, transitional vesicles decrease, basal membrane is thicken and microangiopathy.

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