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Case Report

Effect of Intravenous Adipose Tissue-Derived Mesenchymal Stem Cell Administration on Glycemic Index in a Cat with Diabetes Mellitus

Yasin PARLATIR *[1], **İbrahim SÜNER**[2], **Burak ANTAKYALIOĞLU**[2], **Mümin Alper ERDOĞAN**[2,3]

[1] Department of Internal Medicine, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Türkiye
[2] Mikrostem Company, İzmir, Türkiye
[3] Department of Physiology, Faculty of Medicine, İzmir Katip Çelebi University, İzmir, Türkiye

*Corresponding author e-mail: yasinparlatir@kku.edu.tr

A B S T R A C T

Diabetes mellitus is caused by the defect of the insulin mechanism and characterised by hyperglycemia. Diabetes mellitus is a common disease in humans and is seen in dogs and cats. The disease pathophysiology is the same as that of humans. For this reason, diabetes mellitus in dogs and cats is classified according to medical literature. There is not a certain treatment method for diabetes mellitus. Management of the disease is based on keeping blood glucose within normal limits. Different methods are applied to keep the glycemic index between reference parameters. In recent years, it has been reported that stem cell applications have the ability to regenerate pancreatic beta cells and that stem cell applications can be used in diabetes mellitus cases. Adipose tissue-derived mesenchymal stem cells are used in veterinary medicine as safe and non-complicated applications. When studies on humans and stem cells accessible in veterinary medicine are evaluated, adipose tissue-derived mesenchymal stem cells come to the fore in cases of diabetes. In this case report, we avaluated the results of stem cell application along with insulin and diet in controlling the glycemic index in a cat diagnosed with diabetes. In this case, no side effects were observed, and similar results were obtained with studies conducted on insulin and diet. As a result of this case, the existence of a larger number of patients was emphasized, and the potential reported features of stem cell applications in diabetes cases were emphasized.

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Ö Z E T

Diabetes mellitus, hiperglisemi ile seyreden, organizmadaki insulin mekanizmasının fonksiyon kaybından kaynaklanan bir hastalıktır. İnsanlarda yoğun olarak görüldüğü gibi kedi ve köpeklerde de görülmektedir. Patofizyolojisi insanlardakine benzemektedir. Bu sebeple kedi ve köpeklerdeki diabet olguları insanlardakine benzer şekilde sınıflandırılır. Diabetin kesin bir tedavisi bulunmamaktadır. Hastalığın yönetimi kan glukoz düzeyinin normal sınırlarda tutulmasına dayalıdır. Glisemik endeksi normal sınırlarda tutabilmek için farklı metotlar uygulanmaktadır. Son yıllarda yapılan çalışmalarda, kök hücre uygulamalarının bu yönde kullanılabileceği ve kök hücre uygulamalarının pankreas beta hücreleri üzerinde rejenerasyon kabiliyetinin olduğu bildirilmektedir. Yağ doku kökenli mezenşimal kök hücreler veteriner hekimlikte güvenli ve komplikasyona neden olmayan uygulamalar olarak yer almaktadır. İnsanlarda yapılan çalışmalar ve veteriner hekimlikte ulaşılabilen kök hücreler değerlendirildiğinde yağ doku kökenli mezenşimal kök hücreler diabet olgularında öne çıkmaktadır. Bu olgu sunumunda diabet tanısı koyulmuş bir kedide, glisemik indeksin kontrol altına alınmasında; insulin ve diyetin yanında kök hücre uygulamasının sonuçları değerlendirilmiştir. Bu vakada herhangi bir yan etki görülmediği gibi insulin ve diyet uygulanarak yapılan çalışmalar ile benzer sonuçlar elde edilmiştir. Bu klinik vaka çalışması sonucunda daha kapsamlı araştırmaların gerekliliği vurgulanarak, kök hücre uygulamalarının diabet olgularında potansiyel faydalar taşıdığı kanaatine varılmıştır.

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INTRODUCTION

Diabetes Mellitus is a common disease that is characterized by chronic hyperglycemia as a result of a malfunction in the insulin mechanism and production (Rand and Gottlieb, 2000). It is reported to be seen in 1 in 100 cats or 1 in 500 cats (Baral et al, 2003; McCann et al, 2007; Sallander et al, 2012: Ackerman et al, 2018). Diabetes mellitus is classified according to the pathophysiology in humans. It was stated that there was no clear classification system in cats and dogs (Ackerman et al, 2018). If a classification is to be made, the same classification system as in humans is accepted and the disease is divided into 4 types. The types are 1, 2, gestation and other specific types (Rand, 2020). Type 1, gestational type and other specific types are rarely encountered in cats, but Type II accounts for 90% of diabetes cases in cats (Rand et al, 2004; Marshall et al, 2009).

The most common clinical findings in diabetus mellitus are polyuria, polydipsia, polyphagia and weight loss (Reusch, 2015). The hair coat may appear dull, dirty and dry, along with dandruff. Peripheral neuropathy, marked by weakness, especially in the hind legs, may develop in 10% of cases. A clinical picture of chronic complications that develop as a result of diabetes may be encountered. Renal failure may develop as a result of diabetes mellitus and a clinical picture of renal failure may be seen. If pancreatitis is accompanied, symptoms related to pancreatitis may be observed (Williams and Heath, 2006). The first markers in the diagnosis of diabetes mellitus are polyuria, polydipsia, polyphagia and weight loss (Reusch, 2015). In addition to clinical findings, hyperglycemia in the blood and glycosuria in the urine are other parameters included in the diagnosis (Kruth and Cowgill, 1982; Reusch, 2010). Serum fructosamine level is also a useful marker in diagnosis (Link and Rand 2008). The upper limit of blood glucose level in cats is 113-117 mg/dl (6.3-6.5 µmol/l). In the diagnosis of diabetes mellitus, a blood glucose level between 117-180 mg/dl (6.5-10 µmol/l) is considered preDM. If the glucose level is between 180-288 mg/dl (≥10-16 µmol/l), hyperglycemia is defined as an effective value for the presence of diabetes mellitus. At the same time, the presence of glucose in the urine is used as another parameter for the presence of diabetes along with the blood glucose level (Gottlieb et al, 2015; Reeve and Johnson et al, 2016).

Diabetes Mellitus is a disease that is affected by many environmental factors, may be genetically based, and is directly related to living conditions. There are many different approaches to the treatment and management of the disease. The common goal of all treatment protocols is to improve quality of life through successful glycemic control and to eliminate clinical findings associated with diabetes. For this purpose, medical therapy (insulin therapy), special diet applications, oral hypoglycemic agents and management of concomitant conditions are included in the treatment practices to successfully control the glucose level (Reusch 2015). In addition to these applications, pancreas transplants and islet transplants are also performed in humans (Zhang et al, 2020). However, these applications have major disadvantages due to donor limitation, immunosuppression potential, and short lifespan in the transplanted organism (Teramura and Iwata, 2010; Gaba et al, 2012). Studies conducted in recent years indicate that stem cell applications, obtained from different origins, have great potential in controlling diabetes. It has been reported that stem cells have immunomodulatory properties and high regeneration capabilities on insulin-producing cells (Hess et al, 2003; Pera and Tam, 2010; Chhabra et al, 2013). In a study conducted on dogs, due to the potential of stem cells, allogeneic adipose tissue-derived mesenchymal stem cells were applied to diabetic dogs, and it was observed that the diabetes and its complications were successfully controlled (Rhew et al, 2021). It is also reported that the use of mesenchymal stem cells derived from bone marrow or produced from adipose tissue is effective and safe (Dang et al, 2017).

In this case report, the effect of adipose mesenchymal stem cell application in addition to conventional treatment was examined in a cat with diabetes mellitus. While there are studies in this direction in humans and dogs, similar studies in cats are lacking. The effectiveness of stem cell application on the clinical picture and blood glucose level was determined in the case of diabetes mellitus in a cat.

CASE IDENTIFICATION

A 13-year-old, neutered, female, mixed-breed cat presented with respiratory symptoms. A mucopurulent discharge from the nose, respiratory distress, and cough upon tracheal palpation were detected. Whole blood and biochemistry panel results were obtained for routine diagnostic purposes (Table 1). Friction sounds were detected on lung auscultation. Tram tracks and donut images were identified in the thorax radiography. In addition, it was observed that the walls of the trachea were thickened along the entire line and the tracheal rings were prominent. Whole blood results were found to be within normal limits. In the biochemistry results, the glucose value was seen to be 270 mg/dl. When the patient's owner was asked whether she had complaints of polyuria and polydipsia, the answer was that they could not follow them. The patient was hospitalized for 5 days. Amoxicillin (15 mg/kg, SC, SID), non-steroidal anti-inflammatory drugs (1 mg/kg, SC, SID), vitamins B1 (50 mg/kg, SC, SID) and B12 (1 mg/kg, SC, SID) and vitamin C (500 mg/kg, IV, SID) were administered for complaints related to the respiratory system. Glucose monitoring was performed throughout the hospitalization period (Table 2). A gradual transition to diabetic food was made during the hospitalization. The patient's oral intake has never been interrupted since the time of admission. A glucometer (On Call® Plus, Acon, USA) device was used for glucose monitoring for 5 days. The values were determined as 270, 165, 268, 203, and 267 mg/dl, respectively. At the end of the 5th day, the patient's respiratory system problems disappeared. The patient was discharged with a recommendation for home glucose monitoring for 15 days. Glucose monitoring was organized at least 3 times a day with an interval of 6-8 hours, and recommendations were made to the patient owner by creating a schedule. In the data obtained, it was seen that the patient owner could not comply with the created schedule, but the measurements obtained were still above the reference ranges.

Table 1. *First examination whole blood and biochemistry results*

Parameters	Results	Parameters	Results
WBC (10^9/L)	12.0	ALB (g/L)	30.9
LYM (10^9/L)	4.1	TP (g/L)	86.2
MID (10^9/L)	0.9	GLOB (g/L)	55.3
GRAN (10^9/L)	7.0	A/G	0.56
LYM (%)	34.2	TB (µmol/L)	0.9
MID (%)	7.9	GGT (U/L)	<2
GRAN (%)	57.9	AST (U/L)	19
RBC (10^{12}/L)	8.91	ALT (U/L)	51
HGB (g/dL)	12.9	ALP (U/L)	58
HCT (%)	40.5	LDH (U/L)	48
MCV (fL)	45.5	CK (U/L)	196
MCH (pg)	14.4	Crea (µmol/L)	81.2
MCHC (g/dL)	31.8	BUN (µmol/L)	8.70
RDW_CV (%)	15.4	GLU (mg/dL)	270
RDW_SD (fL)	27.6	TC (µmol/L)	5.06
PLT (10^9/L)	175	TG (µmol/L)	0.94
MPV (fL)	11.0	tCO2 (µmol/L)	17.6
PDW (fL)	12.0	Ca (µmol/L)	2.58
PCT (%)	0.19	PHOS (µmol/L)	1.93

Table 2. *5 days glucose results during hospitalization.*

Day	Glucose Result (mg/dL)
1	270
2	165
3	268
4	203
5	267

As a result of the measurements taken by the patient's owner, the patient's full blood and biochemistry results were obtained. The results are shown in table 3. A fructosamine sample was taken on the same day and the fructosamine value was determined as 263,2 µmol/l (Table 3). It was also determined that complaints of polyuria and polydipsia started during the follow-up period. In line with the data obtained and clinical complaints, the patient was approached regarding diabetes mellitus. It was decided to administer insulin in order to control the glycemic index. Initially, subcutaneous insulin glargine (Lantus) was administered BID at a dose of 1.0 IU/cat. It was decided to continue the diabetic formula during the patient's follow-up. In addition to the treatment protocol, intravenous allogeneic mesenchymal adipose tissue-derived stem cells (AT-MSC) were administered and the patient was monitored at home.

Table 3: *Second whole blood and biochemistry results*

Parameters	Results	Parameters	Results
WBC (10^9/L)	15.7	ALB (g/L)	29.1
LYM (10^9/L)	5.3	TP (g/L)	83.8
MID (10^9/L)	1.2	GLOB (g/L)	54.5
GRAN (10^9/L)	9.2	A/G	0.53
LYM (%)	34.2	TB (µmol/L)	0.6
MID (%)	8.1	GGT (U/L)	<2
GRAN (%)	57.7	AST (U/L)	21
RBC (10^{12}/L)	8.26	ALT (U/L)	52
HGB (g/dL)	12.3	ALP (U/L)	33
HCT (%)	38.9	LDH (U/L)	44
MCV (fL)	47.2	CK (U/L)	113
MCH (pg)	14.8	Crea (µmol/L)	65.1
MCHC (g/dL)	31.6	BUN (µmol/L)	5.89
RDW_CV (%)	14.8	GLU (mg/dL)	291
RDW_SD (fL)	27.6	TC (µmol/L)	2.85
PLT (10^9/L)	148	TG (µmol/L)	1.13
MPV (fL)	8.9	tCO2 (µmol/L)	24.7
PDW (fL)	9.5	Ca (µmol/L)	2.52
PCT (%)	0.13	PHOS (µmol/L)	1.50
		Fructosamine (µmol/L)	263.2

For allogenic mesenchymal stem cell application, mesenchymal stem cells (MSCs) were isolated with a minimal invasive procedure from adipose tissue. The stem cells were expanded in a sterile environment and cultured, with characterization performed at passage 3. MSCs were identified based on their adherence ability to plastic surfaces, positive expression of specific markers (CD90, CD44, and CD105) via flow cytometry, and fibroblast-like morphology. A dose of 1 × 10^6 cells/kg body weight was administered intravenously via the cephalic vein.

Following the application, the patient was followed for 4 months. The patient owner monitored her glucose level with the glucometer (On Call® Plus, Acon, USA) device for 3 months. For the first month after the application, the patient was called for a check-up every week, and complete blood and biochemistry values were checked in our center once in this month. The average glycemic index values by first month are shown in Figure 1. In the 2nd month after the application, glucose values were observed to be within normal reference ranges, and insulin use was terminated by applying decreasing doses in the last 15 days of the 2nd month (Figure 2). In the third and fourth months of monitoring the glycemic index, glucose values were found to be controlled and the values were found to be within or near the normal reference ranges. (Figure 3, 4) . During this period, diabetic food was continued and lifelong use was recommended. After glucose levels were brought under control, home glucose monitoring was discontinued. At the end of this follow-up period, the patient was kept under control for a year, and the patient was examined regularly; complete blood and biochemistry parameters were taken every month. During this one year, glucose values were detected within normal reference ranges and no abnormalities were found in other blood results (Table 4).

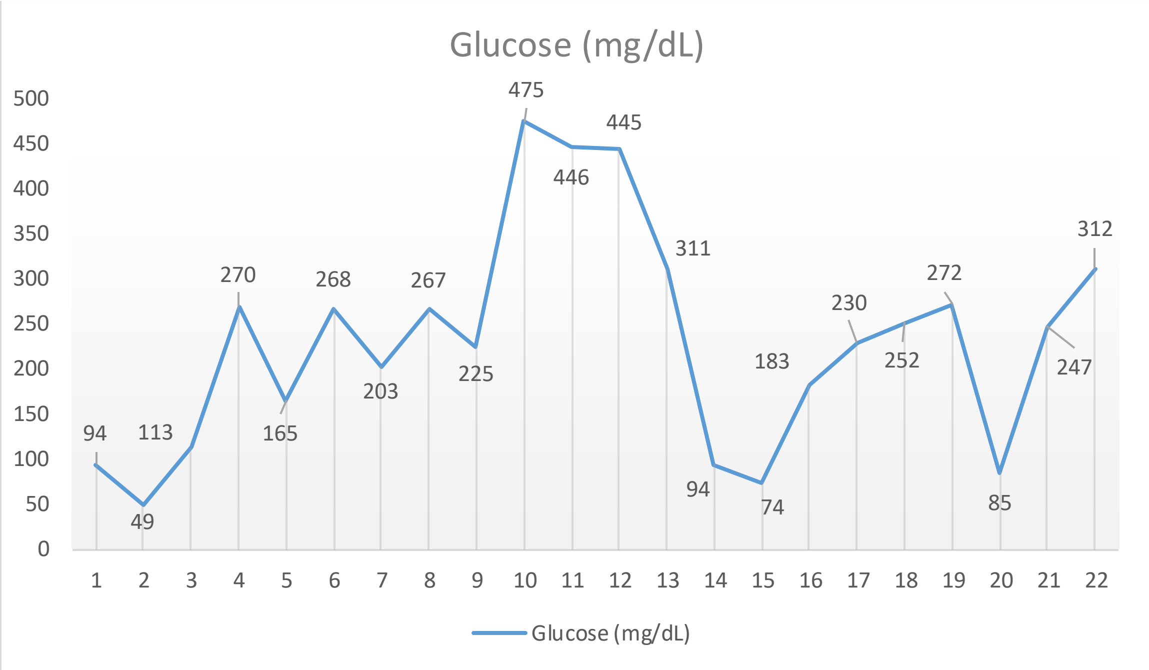

Figure 1: *First month glucose results after application of AT-MSC*

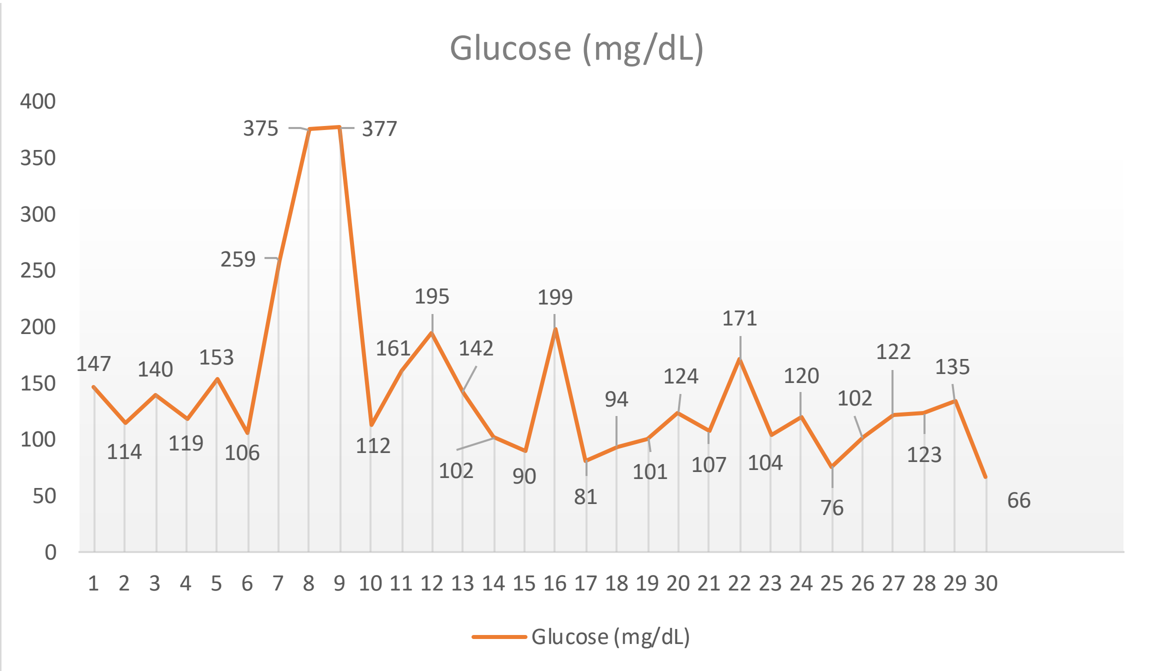

Figure 2: *Second month glucose results after application of AT-MSC*

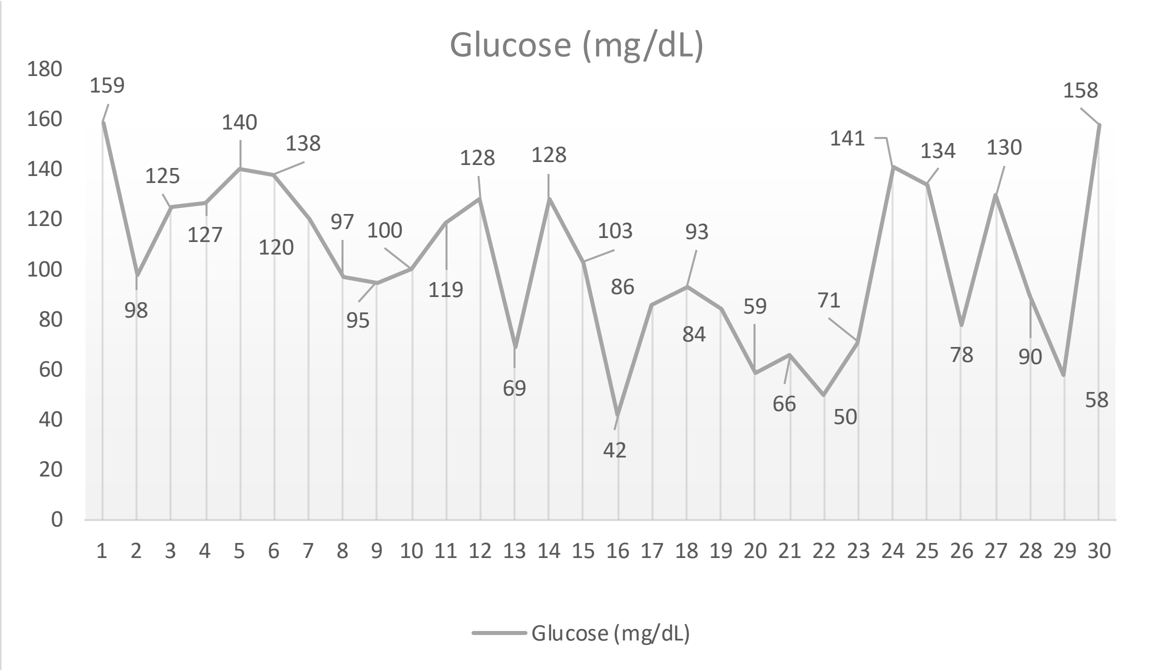

Figure 3: *Third month glucose results after application of AT-MSC*

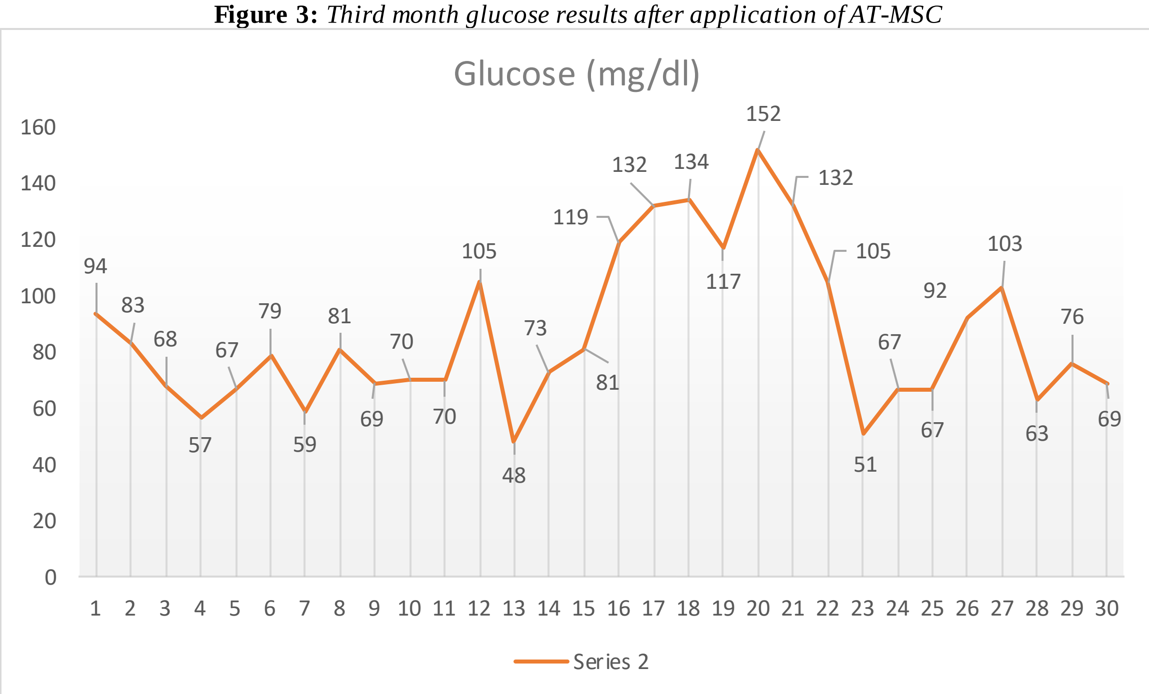

Figure 4: *Fourth month glucose results after application of AT-MSC*

Table 4:*12-month glucose results*

Month	Glucose Result (mg/dL)
1	100
2	115
3	98
4	107
5	85
6	112
7	80
8	116
9	113
10	102
11	98
12	105

DISCUSSION

The basis of stem cell applications in patients with diabetes mellitus lies in the effects of stem cells on the beta cells of the pancreas. It is reported that in stem cell therapies, stem cells can transform into new beta cells and ensure the preservation and continuity of functions of active, undamaged beta cells (Rhew et al, 2021). It is also reported that stem cells contribute to the regeneration of beta cells and have immunomodulatory effects (Hussain and Theise 2004). Studies conducted in humans include mesenchymal stem cell applications in diabetes mellitus cases. The studies indicated that successful results have been achieved in mesenchymal stem cell applications in patients with diabetes and that 20% of the patients no longer need insulin. Stem cell applications from different origins resulted in a 59.9% insulin independence rate (Zhang et al, 2020). Adipose-derived stem cell applications are also used in diabetic patients, and in studies conducted in humans and diabetic model rats, it has been reported that venous or intrasplenic channel applications and AT-MSC applications increase insulin secretory capacity (Xiao et al, 2013; Dang et al, 2017). In veterinary medicine, there are a limited number of studies on this subject. While there are studies in this direction in dogs, no studies have been found on the use of cat stem cells in patients with diabetes mellitus.

Rhew et al. (2021) used AT-MSC on 4 dogs with diabetes mellitus in their study. They reported that increased secretion capacity was detected in 3 dogs and they thought AT-MSC could be used as a potential and alternative option in the complications of diabetes mellitus and in controlling the glycemic index (Rhew et al, 2021). Similarly, Will et al. (2012) reported that stem cell applications have promising restoration capacity in pancreatic islet cells (Will et al, 2012). Both studies emphasized that stem cell therapies are promising, but all stem cell treatment protocols need to be developed and supported by more advanced studies. Many factors come into play in the stem cells reaching the target tissue and performing regeneration and restoration processes in the target area. Although the mechanisms in the differentiation capacity of stem cells are still not fully understood, they may vary depending on extracellular space signaling pathways, effects of growth factors, hormones, and cell-cell interactions (Georgia et al, 2004; Fadini et al, 2006, Will et al, 2012). In this case report, the evaluation of stem cell application, which is considered safe, in terms of clinical picture in a cat with diabetes mellitus in the light of literature information was discussed. Although no complications were encountered, the glycemic index was successfully controlled and insulin was terminated in a controlled manner during the post-application follow-up. It is reported that diabetic remission may occur in 25% to 50% of cases within the first 3 months of treatment (Reusch 2015). Remission includes a controlled glycemic index with a glucose level that does not fall below 80-120 mg/dL. In this case, results were obtained in accordance with the literature and no complications were encountered regarding stem cell application.

This case report has deficiencies in several aspects. While checking only the glucose value following stem cell application is the main indicator of potential improvement in insulin secretion, the lack of C-peptide and triglyceride values constitutes the weakness of the evaluation of the case. In veterinary medicine, only studies conducted on dogs have been encountered. In these studies, insufficient sample numbers are a major handicap in the evaluation of stem cell activity. In cats, no studies have been found on stem cell applications in cases of diabetes. In this case report, AT-MSC application was performed, glucose level was clinically controlled and insulin administration was discontinued. In addition, the patient was monitored for one year after insulin was discontinued, and no evidence of diabetes or increased glucose levels was found. We believe that the findings obtained and the case report will contribute to the literature. It is aimed to emphasize the need for more comprehensive studies on this subject and at being a source for further studies to be conducted.

In this case report, the effect of AT-MSC administration together with insulin and diet food on glycemic control was evaluated in a diabetic cat. Glycemic control was achieved, and insulin therapy was successfully discontinued. For this reason, we believe that stem cell application in diabetic cats can be considered as a useful alternative in line with literature information and that more comprehensive studies in this direction may have potential in diabetic cases.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interests.

AUTHOR CONTRIBUTION

YP and **MAE** conceived and designed the case. **BA** and **İS** acquired the data and made the application of stem cells. **YP** and **MAE** analysed the interpreted the data. **YP, MAE, BA** and **İS** wrote the case.

CONSENT FOR PUBLICATION

All authors have read and agreed to the published version of the manuscript.

ETHICAL APPROVAL

This case report was constituted with an informed consent form obtained from the cat's owner.

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