Hypercholesterolemia and insulin resistance associated with ovarian remnant syndrome in a diabetic dog: case report

Hamidreza Moosavian[1]*, Mahsa Fazli[2]

[1] Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2] Department of Biology, Faculty of Basic Science, Islamic Azad University, Tehran, Iran.

Abstract

A 9-year-old spayed female Terrier dog was evaluated for lethargy, anorexia, polyuria, polydipsia and dysuria. The dog had been diagnosed with diabetes mellitus about 6 months ago and received subcutaneous doses of insulin. The patient showed insulin resistance and severe persistent fasting hyperglycemia in the face of high-dose insulin treatment, hyper-cholesterolemia and urinary tract infection. After a complete evaluation, the dog was diagnosed with a polycystic ovary and a cystic uterine remnant during an exploratory celiotomy. The polycystic ovary and cystic uterine remnant were removed and submitted for histopathological evaluation. Two weeks after surgery the blood glucose level and one month later serum cholesterol level were controlled, using a low level of insulin therapy (0.25 IU kg^{-1} Neutral Protamine Hagedorn (NPH) insulin, every 12 hr). In the present study, the clinical and laboratory results showed that ovarian remnant syndrome as an infrequently encountered condition in dog was related to some metabolic disorders such as insulin resistance, uncontrolled hyperglycemia, dyslipidemia and also recurrence urinary tract infection. To the best of authors' knowledge, no reports of hypercholesterolemia in dog have been made before as a complication of ovarian remnant syndrome.

Introduction

Ovarian remnant syndrome (ORS) is characterized by the presence of functional residual ovarian tissue in a female dog or cat having previously undergone ovariohysterectomy. It is often caused by a veterinary surgeon error or auto-transplantation of ovarian tissue and is responsible for 22.00% of all post-ovario-hysterectomy complications.[1] Behavioral and/or physical signs of estrus are seen in respective animals that usually occur months to years after ovariohysterectomy; however, they can begin within days after surgery.[2] The clinical signs being reported in ORS are vulvar swelling, vaginal discharge, mammary gland enlargement, pollakiuria and stranguria, dermal hyper-pigmentation and alopecia, attracting males, postural behavior indicative of estrus, vulvar or vaginal masses, polyuria and polydipsia (PU/PD), polyphagia, poor coat, weight loss and recurrent urinary tract infections (UTIs).[2] The most clinical and para-clinical findings are related to the ovarian hormones such estradiol and progesterone. Insulin resistance (IR) and hyperglycemia can be induced by progesterone and seen in female dogs with diestrus, gestation and ORS. Gestational diabetes mellitus (GDM) is a type of diabetes when hyperglycemia and glucose intolerance first appear during pregnancy. Transient IR and diabetes mellitus (DM) also could occur in bitches during the 2nd half of pregnancy or diestrus.[3] The end of pregnancy or diestrus phase can lead to improved GDM; however, in some dogs DM was permanent suggesting that chronic hyperglycemia can cause irreversible damage to the β-cells by means of glucotoxicity.[3] The etiopathology of GDM is associated with pregnancy hormones especially progesterone, progesterone-induced growth hormone (GH) originating from foci of hyperplastic ductular epithelium of the mammary gland, estrogens and cortisol. Furthermore, it seems that there is a genetic predisposition to develop GDM in some breeds of dogs.[3,4] Reportedly, transient diabetes and IR have been described in a dog with ORS being resolved after ovariohysterectomy.[5]

*Correspondence:

Hamidreza Moosavian. DVM, DVSc
Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: hrmoosavian@ut.ac.ir

Hyperlipidemia refers to an increased serum tri-glyceride and/or cholesterol concentrations and can be developed by primary and secondary causes. Primary hyperlipidemia in dogs is less common and is most often reported in certain breeds. Secondary hyperlipidemia is the most common form in dogs and it can be a result of hypothyroidism, DM, hyperadrenocorticism, obesity, cholestasis, pancreatitis and protein-losing nephropathy.[6] However, hyperlipidemia same as hypercholesterolemia has not been described previously in dogs with ORS. In the present study, clinical and para-clinical findings in a dog with DM and ORS are described.

Case Description

A 9-year-old spayed female Terrier dog weighing 11.00 kg was evaluated for PU/PD. The dog had a history of ovariohysterectomy eight years ago. Hematology, bio-chemistry and urinalysis results revealed severe hyper-glycemia, glycosuria and mild neutrophilia. Based on the history, clinical manifestation and biochemical analysis, the dog was diagnosed with DM and insulin therapy was initiated. The insulin therapy was indicated as Neutral Protamine Hagedorn (NPH) insulin (Exir Pharma, Borujerd, Iran) 0.75 IU kg^{-1}, subcutaneously, q12h. Six months later, the patient was presented with lethargy, anorexia, PU/PD and dysuria. Remarkable conjunctival hyperemia was present in the clinical examination. Laboratory results showed neutrophilia, alkaline phosphatase elevation, hyper-cholesterolemia, hyperglycemia and mild hyperkalemia (Tables 1, 2 and 3). Also, IR (persistent hyperglycemia in the face of insulin dosage more than 3.00 IU kg^{-1}, NPH insulin, twice a day), glycosuria and UTI were confirmed in the patient (Table 4). For more evaluation, the effect of subcutaneous injection of regular insulin (1.50 IU kg^{-1}; Exir Pharma) on blood glucose levels 180 min after injection was studied (Fig. 1). During this time, the blood glucose concentration was between 428 and 471 mg dL^{-1}, indicating a decrease in insulin sensitivity. Similar results were observed in repeated sampling. Various causes of IR such as Cushing's syndrome and hypothyroidism were ruled out in the dog. Initially urine cortisol to creatinine ratio and then, low-dose dexamethasone suppression test as the screening tests for canine hyperadrenocorticism were studied. Furthermore, serum thyroid hormones were evaluated for thyroid function (Table 5). Furthermore, administration of gemfibrozil (Tolid Daru, Tehran, Iran) as a lipid-lowering agent (7.50 mg kg^{-1}, q12h)[7] for 3 weeks had no effect on the serum cholesterol level. In addition, Lovastatin (Osvah, Tehran, Iran) administration (15.00 mg, q24h)[8] for two weeks not only had no effect on serum cholesterol level but also increased serum alanine transaminase (237 IU L^{-1}) and creatine phosphokinase (518 U L^{-1}) and was discontinued. One week after stopping the medication, the activity of the enzymes decreased.

Table 1. Hematology results.

Parameters	Result	Reference range[19]
White blood cell (×10^3 μL^{-1})	15.50	5.00-14.10
Granulocyte (×10^3 μL^{-1})	14.10	2.90-12.00
Band cell (×10^3 μL^{-1})	0.31	0.00-0.45
Lymphocyte (×10^3 μL^{-1})	0.93	0.40-2.90
Monocyte (×10^3 μL^{-1})	0.16	0.10-1.40
Eosinophil (×10^3 μL^{-1})	0.00	0.00-1.30
Basophil (×10^3 μL^{-1})	0.00	0.00-0.14
Red blood cell (×10^6 μL^{-1})	6.29	4.95-7.87
Hemoglobin (g dL^{-1})	13.40	11.90-18.90
Hematocrit (%)	41.60	35.00-57.00
MCV (fL)	66.10	66.00-77.00
MCHC (g dL^{-1})	32.20	32.00-36.00
RDW	11.40	12.00-16.00
Platelet (×10^3 μL^{-1})	351	211-621

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distribution width.
The hematology analysis was performed by Celltac α cell counter (Nihon Kohden Co., Tokyo, Japan).

Table 2. Biochemistry results.

Parameters	Result	Reference range[19]
Alanine transaminase (IU L^{-1})	88.00	10.00-109
Aspartate transaminase (IU L^{-1})	67.00	13.00-15.00
Alkaline phosphatase (IU L^{-1})	320	1.00-114.00
Gamma-glutamyl transferase (IU L^{-1})	8.50	0.00-10.00
Urea (mg dL^{-1})	42.80	17.00 -59.00
Creatinine (mg dL^{-1})	1.70	0.50-1.70
Serum total protein (mg dL^{-1})	8.50	5.40-7.50
Albumin (mg dL^{-1})	3.10	2.30-3.10
Globulin (mg dL^{-1})	5.40	2.70-4.40
Albumin/globulin ratio (mg dL^{-1})	0.57	0.60-1.10
Glucose (mg dL^{-1})	485	76.00-119
Total bilirubin (mg dL^{-1})	0.30	0.00-0.30
Direct bilirubin (mg dL^{-1})	< 0.10	0.00-0.30
Creatine phosphokinase (IU L^{-1})	319.00	52.00-368
Calcium (mg dL^{-1})	10.80	9.10-11.70
Phosphorous (mg dL^{-1})	5.50	2.90-5.30
Cholesterol (mg dL^{-1})	790	135-278
Triglyceride (mg dL^{-1})	121	40.00-169
Amylase (IU L^{-1})	1,050	226-1,063
Lipase (IU L^{-1})	365	60.00-330

The biochemistry analysis was performed by Global 240/720 autoAnalyzer (Global Co., Roma, Italy).

Table 3. Venous blood gas (VBG) and electrolyte analysis.

Parameters	Result	Reference range[19]
pH	7.35	7.31-7.42
pCO$_2$ (mm Hg)	33.40	29.00-42.00
pO$_2$ (mm Hg)	61.00	49.90-54.20 (at sea level)
Na (mmol L^{-1})	143	142-152
K (mmol L^{-1})	5.70	3.90-5.10
Ca^{2+} (mmol L^{-1})	1.22	1.21-1.42
Cl (mmol L^{-1})	123	110-124
HCO$_3$ (mmol L^{-1})	18.1	17.00-24.00
Base excess (mmol L^{-1})	-6.8 0	+/-4 (For Atrial blood gas)
Anion gap (mmol L^{-1})	7.60	5.00-17.00
mOsm (mmol L^{-1})	323	288-305

The VBG and electrolyte analysis was performed by blood gas analyzer (Edan Co. Shenzhen, China).
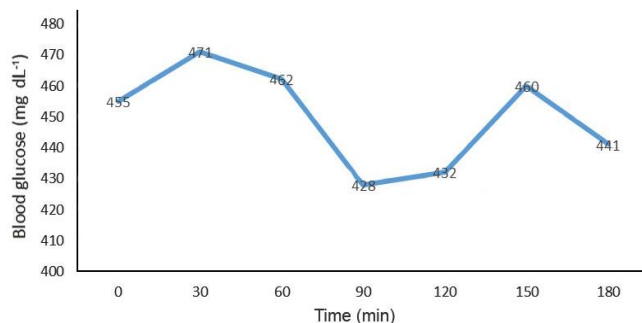
Fig. 1. Blood glucose level profile 180 min after administration of subcutaneous injection of 1.50 IU kg $^{-1}$ regular insulin. During this time, the blood glucose concentration was between 428 and 471 mg dL $^{-1}$.

In the patient history, there was evidence of vulva swelling in the recent weeks. A cytology sample from the vagina was taken and studied. In the cytology study, a remarkable number of intermediate epithelial cells (60.00%), superficial epithelial cells (35.00%) and para-basal epithelial cells (5.00%) with few neutrophils and no bacteria and erythrocytes were seen (Fig. 2).

Based on the cytology results, the possibility of the presence of an active ovary and ORS was raised. The blood hormone analysis (Table 5) showed serum progesterone level as 63.00 ng mL^{-1} (progesterone < 0.50 ng mL^{-1} shows no functional corpus luteum).[9] In exploratory celiotomy, the presence of right ovary as a polycystic ovary and a cystic uterine remnant were confirmed.
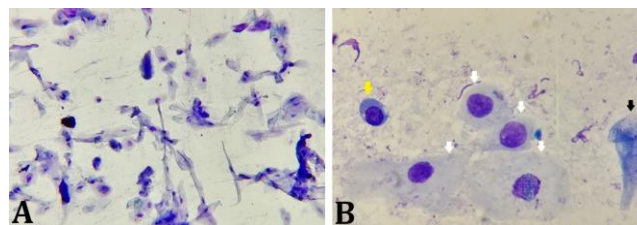

Fig. 2. Vaginal smear. **A)** Many intermediate epithelial cells with a remarkable number of superficial epithelial cells were seen in the vaginal smear (Giemsa staining, 200×). **B)** The vaginal smear in higher magnification. Few parabasal epithelial cells (yellow arrow), many intermediate epithelial cells (white arrows) and some superficial epithelial cells (black arrow shows an anucleated cornified superficial epithelial cell) were present in the vaginal smear (Giemsa staining, 1000×).

Table 4. Urinalysis and urine culture results (urine collection type: cystocentesis).

Component	Result	Reference range[19]
Physical examination		
Color	Yellow	Pale to dark yellow
Appearance	Slightly cloudy	Clear
Specific gravity	1.032	1.000 > 1.050
Chemical examination		
pH	6.00	5.00-7.00
Protein	Negative	Negative
Glucose	Positive	Negative
Ketone *	Positive	Negative
Bilirubin	Negative	Negative
Urobilinogen	Negative	Negative
Ascorbate	Positive	-
Blood	Negative	Negative
Microscopic examination		
Red blood cell	1-2 / High-power field	<5/ High-power field
White blood cell	10-12 pus cell/ High-power field	<5/ High-power field
Squamous epithelial cells	0-1/ High-power field	<5/ High-power field
Cast	None	-
Crystal	None	-
Bacteria	Seen	None
Culture		
Bacterial culture	Positive: Gram-negative bacteria (*E. coli*)	

* Ketonuria was not consistently seen in all urinalysis results.

Table 5. Hormonal assays using ELISA method.

Parameters	Result	Reference[9]
Total thyroxine (µg dL^{-1})	1.80	1.20-5.20
Free thyroxine (ng dL^{-1})	0.90	0.80-2.50
Progesterone (ng mL^{-1})	63.00	< 0.50: No corpus luteum function as during anestrus or in neutered animals
Urine cortisol/creatinine ratio	16.00 ×10^{-6}	< 13.00 × 10^{-6}
Low dose dexamethasone suppression test		
Serum cortisol (0 hr), (µg dL^{-1})	7.30	1.00-4.00
Serum cortisol (4 hr), (µg dL^{-1})	0.80	< 1.50
Serum cortisol (8 hr), (µg dL^{-1})	1.20	< 1.50

Macroscopically, multiple corpora lutea with different sizes (2.00 - 8.00 mm) in the ovary and enlarged portion of the right uterine horn with brownish contents were seen. In histopathology study, the presence of multiple corpora lutea in the ovary and mild endometrial hyperplasia without inflammation in the uterine wall were confirmed (Fig. 3). Two weeks after surgery, the blood glucose (random blood glucose: 80.00 - 150 mg dL^{-1}) and one month later, serum cholesterol level (110 mg dL^{-1}) were controlled, using a low level of insulin administration (0.25 IU kg^{-1} NPH insulin, q12h).
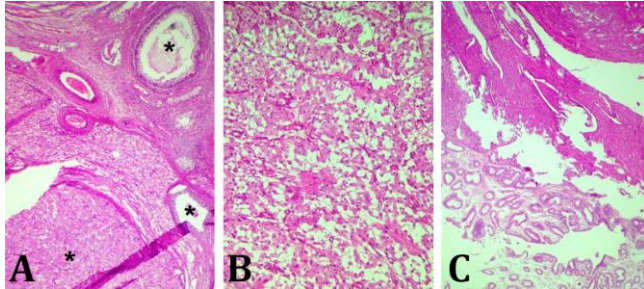

Fig. 3. Histopathological sections from ovary and uterine remnant using Hematoxylin and Eosin staining. **A)** Multiple corpora lutea (asterisks) with different sizes (2.00 - 8.00 mm) were seen in the ovary (100×). **B)** The corpora lutea were composed of luteinized granulosa cells (400×). **C)** Mild endometrial hyperplasia without inflammation in the uterine wall (100×).

Discussion

Insulin resistance is defined differently in human and veterinary medicine. In human, IR is defined as a peripheral insulin antagonism and can be caused by insulin receptor, post-receptor or glucose transport defects.[10] However, in veterinary medicine, IR is described as a persistent hyperglycemia in the face of insulin dosage more than 1.50 IU kg^{-1}.[11] Variety of agents and disorders are mentioned that can cause IR in human and veterinary medicine. The most causes of IR are glucocorticosteroid hormones, hypothyroidism, GH excess due to acromegaly (especially in cats) or increased progesterone (in dogs), infections and inflammatory diseases, GDM during diestrus due to pregnancy associated hormones especially progesterone and progesterone-induced GH in dogs, obesity, dyslipidemia, neoplasia, glucagonoma and pheochromocytoma.[11]

The IR secondary to diestrus should always be considered in any intact female dog.[12] In the present study, multiple corpora lutea and the high serum progesterone levels caused by the ORS have played essential role in IR development and hyperglycemia. Progesterone reaches peak concentration of 20.00 - 90.00 ng mL^{-1} within 30 days after ovulation and slowly declines thereafter throughout diestrus[9]. Dyslipidemia as hypertriglyceridemia is a relatively common biochemical finding in dogs which can be caused as either primary (idiopathic) or secondary to other diseases such as hypothyroidism, DM or hyperadrenocorticism.[6] However, hypercholesterolemia especially severe hypercholesterolemia without hypertriglyceridemia is less common.[5,13] Primary hypercholesterolemia without hypertriglyceridemia has been described in a family of rough Collies and in 15 Briards from the United Kingdom.[5] Common causes of secondary hypercholesterolemia without hypertriglyceridemia in dogs are nephrotic syndrome, hypothyroidism and cholestasis. Furthermore, hypercholesterolemia can be caused by DM in dogs.[14] However, in the present study, it appears that hypercholesterolemia was caused by the polycystic ovary, because hypercholesterolemia was controlled only after polycystic ovary removal. Hypercholesterolemia can be seen in pseudo-pregnant females and also in dogs with daily subcutaneous injection of progesterone or prolactin.[15] On the other hand, hyperlipidemia is an important cause of IR.[11] So, in the present study, in addition to the fact that hypercholesterolemia could be caused by IR, it can be a cause of IR.

In the present study, chronic UTI was another clinical complication seen in the dog. The IR and DM are associated with an increased susceptibility to infection and sepsis.[16] The growth rate of bacteria in urine is stimulated by glycosuria. There are some evidences that abnormalities in native and specific immunities such as neutrophil chemotaxis, adhesion and intracellular killing as well as disorder in humoral immunity are effective in pathophysiology of diabetes-induced sepsis; so, susceptibility to infection and sepsis is increased in diabetic patients.[17] Furthermore, infections and inflammatory diseases with different pathways such as hyperglucagonemia, adrenergic stimulation and inflammatory cytokine production can lead to IR. Inhibition of insulin signaling in hepatocytes and subsequently hepatic IR can be caused by the inflammatory cytokines. In hepatic IR, suppression of glucose production by insulin in hepatocytes is impaired leading to hyperglycemia.[18] These cytokines could also affect other organs and reduce insulin sensitivity.

The results of the present study showed the role of ORS in some metabolic disorders such as IR, uncontrolled hyperglycemia, hypercholesterolemia and also recurrence UTI in female dogs.

Acknowledgments

Conflict of interest

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

References

1. Pearson H. The complications of ovariohysterectomy in the bitch. J Small Anim Pract 1973; 14(5): 257-266.
2. Ball RL, Birchard SJ, May LR, et al. Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007). J Am Vet Med Assoc 2010; 236(5): 548-553.
3. Fall T, Johansson Kreuger S, Juberget A, et al. Gestational diabetes mellitus in 13 dogs. J Vet Intern Med 2008; 22(6): 1296-1300.
4. Eigenmann JE, Eigenmann RY, Rijnberk A, et al. Progesterone-controlled growth hormone overproduction and naturally occurring canine diabetes and acromegaly. Acta Endocrinol (Copenh) 1983; 104(2): 167-176.
5. Pöppl AG, Mottin TS, González FH. Diabetes mellitus remission after resolution of inflammatory and progesterone-related conditions in bitches. Res Vet Sci 2013; 94(3): 471-473.
6. Comazzi S, Pieralisi C, Bertazzolo W. Haematological and biochemical abnormalities in canine blood: frequency and associations in 1022 samples. J Small Anim Pract 2004; 45(7): 343-349.
7. Tilley LP. Smith Jr FWK. Blackwell's five-minute veterinary consult: canine and feline. 4th ed. Victoria, Australia: Blackwell 2008; 1492.
8. Nelson RW, Couto Guillermo C. Small animal internal medicine. 6th ed. Amsterdam, Netherlands: Elsevier Health Sciences 2020; 908-914.
9. Vaden SL, Knoll JS, Smith Jr FWK, et al. Blackwell's five-minute veterinary consult: laboratory tests and diagnostic procedures: canine and feline. 1st ed. Iowa, USA: Wiley-Blackwell 2009; 495-497.
10. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol 2014; 6(1): a009191. doi: 10.1101/cshperspect.a009191.
11. Ettinger SJ, Feldman EC, Cote E. Textbook of veterinary internal medicine. 8th ed. Philadelphia, USA: WB Saunders 2017; 4280-4282.
12. Feldman EC, Nelson RW. Canine and feline endocrinology and reproduction, 3rd ed. Toronto, Canada: WB Saunders Company 2003: 526-527.
13. Xenoulis PG, Suchodolski JS, Levinski MD, et al. Investigation of hypertriglyceridemia in healthy Miniature Schnauzers. J Vet Intern Med 2007; 21(6): 1224-1230.
14. Gans JH, Cater MR. Hypercholesterolemia of streptozotocin-induced diabetes mellitus in dogs. Life Sci II 1971; 10(6): 301-308.
15. Zanetti ME, Tennent DM. Hormonal hypercholesterolemia in the dog: influence of niacin, niacinamide, benzmalecene, and cholestyramine resin. Proc Soc Exp Biol Med 1963; 112: 991-995.
16. Kelly CC, Lyall H, Petrie JR, et al. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 2001; 86(6): 2453-2455.
17. Koh GC, Peacock SJ, van der Poll T, et al. The impact of diabetes on the pathogenesis of sepsis. Eur J Clin Microbiol Infect Dis 2012; 31(4): 379-388.
18. Meshkani R, Adeli K. Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. Clin Biochem 2009; 42(13-14): 1331-1346.
19. Duncan KSLJR. Generating and interpreting test results: test validity, quality control, reference values, and basic epidemiology. In: Latimer KS (Ed.). Duncan and Prasse's veterinary laboratory medicine: clinical pathology. 5th ed. Iowa, USA: Wiley-Blackwell 2011; 365-382.