Journal of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2017;31:1403–1405

Relationship between Total Homocysteine, Folic Acid, and Thyroid Hormones in Hypothyroid Dogs

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Background: Both elevated homocysteine and decreased folic acid concentrations are observed in human patients with hypothyroidism and can influence the development of numerous secondary disorders.

Objectives: The aim of the study was to assess total homocysteine concentration in serum and to examine its relationship with the concentration of folic acid and thyroid hormones (tT4 and fT4).

Animals: Ten healthy and 19 hypothyroid client-owned dogs.

Methods: Dogs with clinical signs of hypothyroidism had the diagnosis confirmed by additional tests. Total homocysteine, folic acid, total thyroxine, and free thyroxine concentrations in serum were evaluated.

Results: Hypothyroid dogs were diagnosed with increased homocysteine (median 22.20 μmol/L; range, 16.50–37.75) and decreased folic acid (median 20.62 nmol/L; range, 10.54–26.35) concentrations, as compared to healthy dogs (11.52 μmol/L; range, 10.00–16.65 and 30.68 nmol/L; range, 22.84–38.52, respectively). In sick dogs, total homocysteine was inversely correlated with folic acid ($\rho = -0.47$, $P < 0.001$), total thyroxine ($\rho = -0.69$, $P = 0.0092$), and free thyroxine ($\rho = -0.56$, $P = 0.0302$).

Conclusions: Hypothyroidism in dogs causes hyperhomocysteinemia. Concomitant mild folic acid decrease in hypothyroid dogs might be as a result of hyperhomocysteinemia.

Key words: Canine hypothyroidism; Folic acid; Hyperhomocysteinemia.

Homocysteine is an endogenous amino acid containing sulfur, that is created as a result of systemic conversion of methionine, an exogenous amino acid present in animal proteins. Most homocysteine is remethylated to methionine or transsulfurated to cysteine. N5-methyl TH4, a derivative of folic acid, is a necessary substrate of the methylation reaction.[1,2] Folic acid itself, administered as a dietary supplement, lowers toxic homocysteine levels, although in dogs the correlation between the concentration of the two substances is not clear.[3,4] In humans, elevated concentrations of homocysteine in blood, resulting from metabolic aberrations, are a sensitive marker and a risk factor for blood clotting, cardiovascular, and neurodegenerative disorders, as well as folic acid deficiency.[5–7]

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The study was conducted at the Endocrine Office of the Faculty of Veterinary Medicine of University of Life Sciences in Lublin, Poland. The study was not supported by the grant.

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Submitted March 27, 2017; Revised June 5, 2017; Accepted July 13, 2017.

DOI: 10.1111/jvim.14804

Abbreviations:

FA	folic acid
fT4	free thyroxine
GFR	glomerular filtration rate
MTHFR	methylenetetrahydrofolate reductase
PABA	p-aminobenzoic acid
tHcy	total homocysteine
tT4	total thyroxine

In canine medicine research results on homocysteine are disputable due to conflicting data, and publications concerning this amino acid as a marker for diseases and prognosis are scarce.[2,3,7] There is a statistically significant increase in homocysteine levels in dogs with heart disease and kidney disease as compared to healthy dogs, and the use of homocysteine as a biomarker of heart and kidney diseases is suggested.[7] Elevated homocysteine and decreased folic acid concentrations are observed in human patients with hypothyroidism, and this disease is one of the most common endocrinopathies in dogs, although the relationship between these variables has yet to be studied in this species.[8,9]

The aim of the this study was to assess the concentration of total homocysteine (tHcy) in the serum and compare it to the concentration of folic acid and thyroid hormones (tT4 and fT4) in hypothyroid dogs, as compared to a control group of healthy dogs.

Materials and Methods

The study was conducted between January 2016 and June 2016 at the Endocrine Office of the Faculty of Veterinary Medicine of University of Life Sciences in Lublin, Poland. The study involved a total of 29 client-owned mongrel dogs of different sexes, aged between 4 and 12 years (average age: 7.6 years). Due to potential influence of sex hormones on homocysteine levels,[10] only sterilized animals were used in the study. Control group (C) comprised 10

clinically healthy mongrels—5 castrated males and 5 spayed females, aged between 5 and 10 years (average age: 7.3 years)—was routinely physically examined, with total thyroxine (TT4) of a reference range (25–50 nmol/L). Experimental group (E) comprised 19 mongrels (n = 19)—8 castrated males and 11 spayed females, aged between 4 and 12 years (average age: 7.7 years), with clinical signs of hypothyroidism (e.g, excess weight, obesity, apathy, exercise intolerance, bradycardia, myxedema, hair loss, symmetrical alopecia) confirmed by additional tests—with concentration of total thyroxine (tT4) in serum below 20 nmol/L, and free thyroxine (fT4) in blood serum below 9 pmol/L. Exclusion criteria applied to control group C included the occurrence of any clinical signs and abnormalities in laboratory tests. In group E, animals exhibiting signs of organ failure, that is liver or kidneys and those showing signs of gastrointestinal tract diseases, were excluded as well. All animals were privately owned and were fed balanced commercial feed without any dietary supplements, especially containing methionine, cobalamin, and folic acid. All study protocols were approved by the Local Ethics Committee of University of Life Sciences in Lublin.

Animals fasted overnight, and blood samples were collected from cephalic vein in K_2EDTA tubes to perform hematology tests and in tubes for coagulation to obtain serum. Serum was obtained by centrifuging at 1,500 × *g* for 15 minutes, and stored at −70°C for a maximum of 6 months before determination—homocysteine is stable at −20°C for 5 years[11]; similarly, folic acid remains stable at −70°C for 1 year.[12] Routine hematology tests and biochemical serum tests were performed to exclude other diseases. tT4 and fT4 concentrations in serum were determined by immunofluorescent method with the use of AIA 360 analyzer (Tosoh) in accordance with our reference range for TT4 25–50 nmol/L, inconclusive range for TT4 20–25 nmol/L, low range for TT4 < 20 nmol/L, and reference range for fT4 9–39 pmol/L. Total homocysteine (tHcy) concentrations in serum were determined by a chemiluminescent immunoassay using a homocysteine reagent and ADVIA Centaur (ADVIA Centaur XP Immunoassay System) with our reference range 8.89–17.2 μmol/L, which has been proven to be reliable to be used in dogs.[2] Folic acid (FA) concentrations in serum were determined by a chemiluminescent immunoassay using a folic acid reagent and ADVIA Centaur with our reference range 11.3–39.6 nmol/L.

The median and range were calculated for all variables. Statistical analysis was conducted with the use of Mann–Whitney *U*-test at *P*-values of $P \leq 0.05$ (Statistica 10.0 software). For each parameter, statistically significant differences were calculated between the control and experimental groups. Correlations were calculated with the Spearman rank method.

Results

The total homocysteine concentration in the serum of hypothyroid dogs was statistically significantly higher than in healthy animals ($P < 0.001$). The difference between groups was 10.68 μmol/L (77%). The group of hypothyroid dogs had statistically significantly lower concentrations of folic acid ($P = 0.0023$), total thyroxine ($P < 0.001$), and free thyroxine ($P < 0.001$) compared to healthy animals (Table 1). The homocysteine concentration in 18 sick dogs (95%) was above the reference range, while 1 sick dog (5%) had folic acid concentration below the reference range. In the group of dogs with hypothyroidism, a negative correlation was observed between total homocysteine and the concentrations of folic acid ($\rho = -0.47$, $P < 0.001$), total thyroxine ($\rho = -0.69$, $P = 0.0092$), and free thyroxine ($\rho = -0.56$, $P = 0.0302$).

Table 1. Results of the concentration of determined total homocysteine (tHcy), folic acid (FA), total thyroxine (tT4), and free thyroxine (fT4).

Group	tHcy (μmol/L)	FA (nmol/L)	tT4 (nmol/L)	fT4 (pmol/L)
C				
n = 10				
Median	11.52	30.68	28.96	11.97
Range	10.00–16.65	22.84–38.52	25.88–33.46	10.81–21.88
E				
n = 19				
Median	22.20*	20.62*	15.44*	5.40*
Range	16.50–37.75	10.54–26.35	9.00–19.30	3.35–7.46
P value	<0.001	0.0023	<0.001	<0.001

C, control group; E, experimental group.
*Statistically significant differences compared to control.

Discussion

Measurement of total homocysteine concentration in dogs with kidney diseases or heart diseases (cardiomyopathy, mitral insufficiency, pulmonic stenosis, congenital arrhythmia, and patent ductus arteriosus) is useful in assessing the severity of the diseases. In the case of inflammation, gastroenterological disturbances, cancers, and injuries, the results were not conclusive and did not differ from the results obtained in healthy animals. The results obtained in this study suggest that elevated serum total homocysteine concentration in dogs might be related to hypothyroidism. The evidence for this is the negative correlation between total homocysteine concentration and thyroid hormone concentration, with the concentration of this amino acid being 77% higher in hypothyroid dogs than in healthy animals. This finding has been supported by the results of the latest research in human patients,[8] but not exclusively.[13] The presence of hyperhomocysteinemia might be caused by a decrease in glomerular filtration rate (GFR), which is common in dogs with hypothyroidism, and a decrease in homocysteine clearance, which leads to blocking of the process of amino acid elimination.[8,14,15] Hyperhomocysteinemia is also caused by aberrations in homocysteine metabolism in the liver. A decrease in thyroid hormone concentration results in a decrease in the activity of methylenetetrahydrofolate reductase (MTHFR), which participates in the process of remethylating homocysteine to methionine.[8,16]

Mild folic acid decrease in hypothyroid dogs can also be responsible for the increase in homocysteine concentration in serum. We assumed that all animals were on folic acid-oriented, balanced diets, and thus, its decrease, as compared to the control group, could stem from its abnormal absorption or metabolic processes resulting in homocysteine removal. The effectiveness of supplementation of folic acid has been confirmed in human patients.[4]

In conclusion, the hypothyroid dogs were diagnosed with hyperhomocysteinemia and mild folic acid decrease in comparison with the control group. However, studies

should be repeated on a larger group of animals in consideration of different breeds of dogs.

Acknowledgments

The authors thank the owners of the dogs for their participation in this study.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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