

Original Article

## Serum homocysteine, vitamin B<sub>12</sub> and folate levels in healthy Turkish Van Cats

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### ABSTRACT

**Objective:** The present study was conducted for the purpose of setting forth the normal serum Hcy, vitamin B<sub>12</sub> and folate levels in Van cats of varying ages and genders, and the age-dependent variations of these parameters.

**Materials and methods:** The material of the study consisted of a total of 60 healthy Van cats including 30 female and 30 male cats. Cats from both genders were separated into 3 groups on the basis of their ages. While the cats of 6 - 12 months of age were included in the first group, cats of 12-24 months of age were included in the second and those of more than 24 months of age were included in the third group.

**Results:** From the blood samples collected; serum normal homocysteine, vitamin B<sub>12</sub> and folate levels were determined as  $7.1 \pm 2.2$  nmol/mL,  $850.7 \pm 231.8$  pg/mL and  $16.7 \pm 0.8$  ng/mL, respectively. In the statistical comparison of the determined serum homocysteine, vitamin B<sub>12</sub> and folate levels; some variations among different groups of age and genders were determined. However, none of these differences were determined to be statistically significant.

**Conclusion:** The normal levels of serum Hcy, vitamin B<sub>12</sub> and folate of healthy Van cats were set forth for the first time by the present study. It is believed that the normal values of these parameters in Van cats can be used in the diagnosis and prognosis of various diseases and particularly cardiovascular diseases, that they will be helpful for researchers and will serve as a guideline to the studies to be conducted in the future.

### CORRESPONDENCE

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### KEYWORDS

Homocysteine, Folate, Vitamin B<sub>12</sub>, Turkish Van Cat

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## INTRODUCTION

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Turkish Van cat is a significant endemic cat species living in the area of Van province of Turkey and attracting great attention all around the world. With qualities of having odd colored eyes, being affectionate, the white and silky fur and being quite fond of water, Van cats have built a well-earned reputation worldwide. Due to their diminishing numbers in the recent years, they have been taken into protection and subjected to many scientific studies ([Sonmez and Agaoglu, 2010](#); [Ozkan et al., 2013](#); [Kayar et al., 2014](#)).

Homocysteine (Hcy) is a sulfuric amino acid generated in consequence of the metabolism of the essential amino acid methionine and is not taken in the primary structure of proteins. It is known that there are close relations between Hcy and folic acid, B<sub>6</sub> and B<sub>12</sub> vitamins. In the body it is metabolized through remethylation where folic acid and vitamin B<sub>12</sub> serves as cofactors and transsulfuration where vitamin B<sub>6</sub> is the cofactor ([Aksoy et al., 2006](#); [Nursalim et al., 2013](#); [Ganguly and Alam, 2015](#)). Generated through transsulfuration, Hcy is disintegrated and excreted via urine and during these reactions the cofactor vitamin B<sub>6</sub> is needed. Therefore, it is reported that Hcy levels increase in cases of folic acid, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> deficiencies, and that these vitamins are essential for Hcy metabolism ([Lippi and Plebani, 2012](#); [Nursalim et al., 2013](#); [Geesaman et al., 2016](#)).

It is reported in recent studies that Hcy levels may increase in the early periods of several diseases and particularly cardiovascular diseases ([Uren et al., 2009](#); [Lippi and Plebani, 2012](#); [Amin et al., 2016](#); [Smith and Refsum, 2016](#)). Due to this reason blood Hcy levels has become a significant parameter in the early diagnosis of many diseases. Although there are many studies conducted on Hcy levels within the scope of human medicine, the same topic is not studied in details in veterinary medicine ([McMichael et al., 2000](#); [Ozden et al., 2005](#); [Geesaman et al., 2016](#)).

Until now Van cats as an endemic species gaining more importance and fame everyday were subjected to many studies in Turkey ([Yuksekk et al., 2005](#); [Macun et al., 2010](#); [Ozkan et al., 2013](#); [Kayar et al., 2014](#)), however none of these studies examined the serum Hcy, vitamin B<sub>12</sub> and folate levels of Van cats. The present study was conducted for the purpose of setting forth the normal values of serum Hcy, vitamin B<sub>12</sub> and folate levels in Van cats of varying ages and genders, and the age-dependent variations of these parameters.

## MATERIALS AND METHODS

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**Animals:** The animal material of the present study consisted of a total of 60 (30 female and 30 male) clinically healthy Van cats of varying ages living in the Van Cat House operated by the Van Cat Research Center of the Yuzuncu Yil University. Cats from both genders were separated into 3 groups on the basis of their ages. While the cats of 6-12 months of age were included in the first group, cats of 12-24 months of age were included in the second and those of more than 24 months of age were included in the third group. This research was approved (26.11.2015 and 2015/13) by the Animal Research Ethics Committee of Yuzuncu Yil University in Van, Turkey.

**Blood sampling and biochemical analyses:** For biochemical analysis, blood samples were collected from vena radialis (V. cephalica) to vials without anticoagulant. Blood samples collected in vials without anticoagulant were centrifuged for 10 min at 3000 rev/min (Rotofix 32®-Hettich) and serums were prepared. Until the time of measurements, obtained serums were kept at -20 °C.

Serum vitamin B<sub>12</sub> and folate levels were examined by means of Cobas-6000 e601 (Roche-Hitachi Japan) hormone analyzer. Homocysteine levels, on the other hand, were determined by means of an ELISA equipment (ELISA reader®-DAS) and through the use of a commercial homocysteine test kit (Human Homocysteine ELISA Kit-CUSABIO, Catalog Number; CSB-E13814h), at 450 nm wavelength as advised in the user manual of the test kit.

**Statistical analysis:** Kalmagrov Simirnov was performed on the data of the study as a normality test. Parametric methods were used for statistical analyses because the data showed normal distribution. Definitive statistics were performed for analysing different gender and age groups. While independent *t*-test was used for comparing the data of female and male animals, One WAY ANOVA test was used for comparing data of different ages belonging to female and male animals. Tukey test was used as multiple comparison test between groups. For this purpose SPSS 20.0 software was used. For making the calculations, *P*<0.05 values were accepted as statistically significant.

## RESULTS

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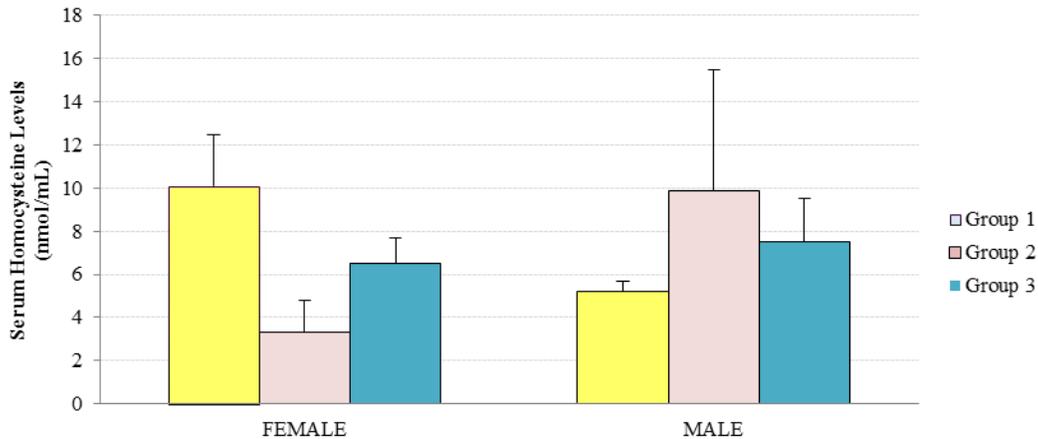
While the arithmetic means and standard deviations of the serum Hcy, vitamin B<sub>12</sub> and folate levels of different genders of Van cats and the results of the statistical

**Table 1.** Serum Hcy, vitamin B<sub>12</sub> and folate levels of healthy male and female Van Cats.

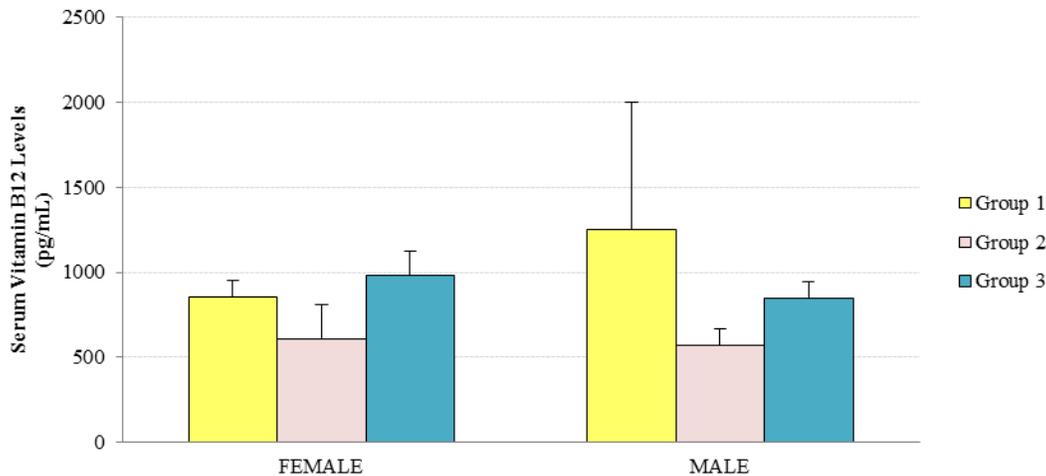
Parameters	Female (n: 30)	Male (n: 30)	Total (n: 60)	P-value
	x±Sx	x±Sx	x±Sx	
Homocysteine (nmol/mL)	6.7±1.7	7.5±2.6	7.1±2.2	>0.05
Vitamin B <sub>12</sub> (pg/mL)	813.6±146.6	887.8±317.0	850.7±231.8	>0.05
Folate (ng/mL)	17.0±0.8	16.4±0.8	16.7±0.8	>0.05

**Table 2.** Serum Hcy, vitamin B<sub>12</sub> and folate levels of male and female Van cats of different groups of age.

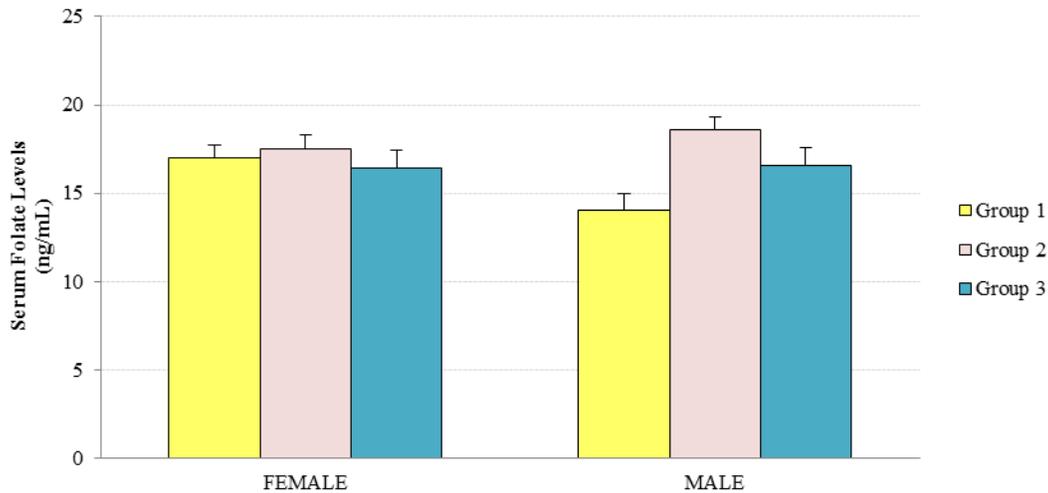
Parameters	FEMALE			MALE			P-value
	Group 1 (n: 12)	Group 2 (n: 8)	Group 3 (n: 10)	Group 1 (n: 7)	Group 2 (n: 10)	Group 3 (n: 13)	
	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	
Homocysteine (nmol/mL)	10.1±2.4	3.3±1.5	6.5±1.2	5.2±0.5	9.9±5.6	7.5±2.0	>0.05
Vitamin B <sub>12</sub> (pg/mL)	856.6±96.7	605.9±200.4	978.3±142.8	1249.0±751.0	569.9±99.4	844.6±100.7	>0.05
Folate (ng/mL)	17.0±0.7	17.5±0.8	16.4±0.9	14.0±1.0	18.6±0.7	16.6±0.8	>0.05



**Figure 1.** Serum homocysteine levels of male and female Van cats of different groups of age.



**Figure 2.** Serum vitamin B<sub>12</sub> levels of male and female Van cats of different groups of age.



**Figure 3.** Serum folate levels of male and female Van cats of different groups of age.

comparisons between the groups are presented in [Table 1](#). The arithmetic means, standard deviations of the same values for age-based groups and the statistical comparisons between these groups are presented in [Table 2](#), and [Figure 1](#), [2](#) and [3](#).

Examining [Table 1](#) shows that while the serum Hcy and vitamin B<sub>12</sub> levels of male cats are higher than those of female cats, folate levels are higher in female cats. However, the differences in these parameters were not found out to be statistically significant in terms of the cats' genders. While the average Hcy level was determined to be  $7.1 \pm 2.2$  nmol/mL, the average levels of vitamin B<sub>12</sub> and folate were determined to be  $850.7 \pm 231.8$  pg/mL and  $16.7 \pm 0.8$  ng/mL respectively.

Although statistically insignificant, these parameters were determined to be different among the groups of different ages, and the values are presented in [Table 2](#). While the highest Hcy levels were observed in the 1<sup>st</sup> female group, the highest levels of vitamin B<sub>12</sub> were found out to be in the 1<sup>st</sup> male group, and the highest folate levels were determined to belong the 2<sup>nd</sup> male group ([Figure 1](#), [2](#) and [3](#)).

## DISCUSSION

While there has been many studies conducted on several haematological and biochemical parameter values of Van cats ([Eksen et al., 1992](#); [Yukseket al., 2005](#); [Macun et al., 2010](#); [Sonmez and Agaoglu, 2010](#)), their serum Hcy, vitamin B<sub>12</sub> and folate levels were never taken as the topic of a study. Due to this reason, in the present study it was aimed to determine the normal values serum Hcy, vitamin B<sub>12</sub> and folate levels of healthy Van cats of

varying ages and different genders, and to set forth the variations in these values based on cats' age and gender.

Homocysteine is a sulfuric amino acid generated in consequence of the metabolism of methionine and is not taken in the primary structure of proteins ([Gauthier et al., 2003](#); [Lippi and Plebani, 2012](#); [Nursalim et al., 2013](#); [Çayır and Kozat, 2016](#)). Several studies conducted in the recent years report that the increases in homocysteine levels cause several negative effects in the organism ([Bostom and Lathrop, 1997](#); [Lippi and Plebani, 2012](#); [Geesaman et al., 2016](#)).

Increased homocysteine level in the body causes endothelium damage by acting as free radicals. Consequently, the increase in platelet activation results in thrombosis formation and the peroxydation of membrane lipids in connection with the increase of coagulation factors. Thereby, particularly the risk of atherosclerosis formation increases ([Uren et al., 2009](#); [Nursalim et al., 2013](#)). High total homocysteine (tHcy) concentrations constitute a high risk factor in terms of cardiovascular, cerebrovascular and peripheral vascular diseases. It is reported that increases of the homocysteine levels can be observed in the early periods of several and particularly cardiovascular diseases, and that therefore blood homocysteine levels can be used in the diagnosis of several diseases ([Aksoy et al., 2000](#); [Ozden et al., 2005](#)).

Conducted studies report that there are close relations between Hcy and the vitamins that are essential for Hcy metabolism, namely folic acid, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> and that in the case of the deficiency of these, Hcy levels may increase ([Klee, 2000](#); [Collin et al., 2010](#); [Nursalim et al., 2013](#); [Geesaman et al., 2016](#)). In addition Hcy levels increase also as a consequence of insufficient

consumption of vitamin B containing foods, ageing, kidney dysfunctions and the deficiency of the enzyme activities effective on homocysteine metabolism ([Bostom and Lathrop, 1997](#); [Gauthier et al., 2003](#)).

As a matter of fact in the study conducted by [Moreiras et al., \(2009\)](#), it was reported that the decreases in serum folate levels cause increases in Hcy levels, and in the study conducted by [Klee \(2000\)](#) it was stated that decreases in folate and vitamin B<sub>12</sub> levels would cause increase in Hcy levels. It is further reported that the insufficient intake of folate and B<sub>12</sub> vitamins, or their decrease due to any other reason, causes Hcy levels to increase, and that increasing the intake of folate and vitamin B<sub>12</sub> would result in a decrease in Hcy levels ([Aksoy et al., 2006](#)).

In the study conducted on humans by [Collin et al., \(2010\)](#), the folate and vitamin B<sub>12</sub> levels of control group test subjects were determined to be 15.8 nmol/L and 299 pmol/L respectively, with a tHcy level of 11.1 µmol/L. [Aksoy et al., \(2000\)](#) reported the average Hcy levels of a group of people suffering from coronary artery disease 12.6 µmol/L, while reporting the same value as 11.1 µmol/L for the control group. [Kural et al. \(2003\)](#), on the other hand, determined the serum tHcy, vitamin B<sub>12</sub> and folate levels of the control group of their study as 11.36 µmol/L, 252 pmol/L and 15.9 nmol/L, respectively.

In a study conducted on cats with chronic kidney failure ([Uren et al., 2009](#)), the Hcy levels of ill cats were determined to be (41.68±9.97-13.03±2.81 µmol/L) higher than that of healthy cats. [McMichael et al. \(2000\)](#), on the other hand determined Hcy levels to be 7.6±4.1 µmol/L in healthy cats, 10.1±10.6 µmol/L in cats with cardiomyopathy and 8.0±4.1 µmol/L in cats with thromboembolism. According to these data, the authors determined that the Hcy levels of cats with cardiomyopathy are statistically higher than that of healthy cats. In this study the researchers found the vitamin B<sub>12</sub> levels to be 1650±700 pg/mL in healthy cats, 939±389 pg/mL in cats with cardiomyopathy and 866±367 pg/mL in cats with thromboembolism. With this, the authors concluded that vitamin B<sub>12</sub> levels of healthy cats are significantly higher than those of cats with cardiomyopathy and thromboembolism ([McMichael et al., 2000](#)).

The serum tHcy (7.1±2.2 nmol/mL), vitamin B<sub>12</sub> (850.7±231.8 pg/mL) and folate (16.7±0.8 ng/mL) levels determined in healthy Van cats within the scope of our study are considered to be close to the normal values reported for cats and humans in various studies ([Aksoy et](#)

[al., 2000](#); [McMichael et al., 2000](#); [Aksoy et al., 2006](#); [Uren et al., 2009](#); [Geesaman et al., 2016](#)).

In another study ([Kocabalkan et al., 2000](#)), tHcy levels were reported to be 6.7±1.7 nmol/mL in female and 7.5±2.6 nmol/mL in male test subjects, and that serum Hcy levels may be about 10% higher in males when compared to females. In a study conducted by [Aksoy et al. \(2006\)](#), levels of Hcy, vitamin B<sub>12</sub> and folate were reported to be 10.3 µmol/L, 357±163 pmol/L and 8.8±4.1 ng/mL respectively. They reported plasma Hcy levels of males (11.8 µmol/L) to be statistically higher than those of females (10.6 µmol/L).

Also in our present study serum Hcy levels of male animals (7.5±2.6 nmol/mL) were determined to be higher than those of female animals (6.7±1.7 nmol/mL), yet the difference was not found out to be statistically significant. This finding is in line with several studies reporting that the Hcy levels of males are higher than the Hcy levels of females ([Kocabalkan et al., 2000](#); [Aksoy et al., 2006](#)). In addition, it was reported in studies conducted on humans that serum or plasma Hcy levels may increase in line with the increasing age ([Kocabalkan et al., 2000](#); [Aksoy et al., 2006](#)). Examining the Hcy levels determined in our study also revealed that there are some differences among the Hcy levels of different groups of age, while none of these differences were determined to be statistically significant ([Figure 1, 2, 3](#)).

## CONCLUSION

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In conclusion, the normal levels of serum Hcy, vitamin B<sub>12</sub> and folate of healthy Van cats were set forth for the first time by the present study. It is believed that the normal values of these parameters in Van cats can be used in the diagnosis and prognosis of various diseases and particularly cardiovascular diseases, that they will be helpful for researchers and will serve as a guideline to the studies to be conducted in the future.

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## CONFLICT OF INTEREST

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The authors declare that they have no conflict of interest.

## AUTHORS' CONTRIBUTION

CÖ, SK, AK and YA designed the study and completed most of the works. CÖ and AK analyzed the test results and collected materials. CÖ, SK, AK and YA gave experiment instruction. CÖ wrote of the manuscript. All authors read and approved the final manuscript.

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