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Effects of Age and Gender on The Concentrations of Plasma Homocysteine, Vitamin B12 and Folic Acid in Angora Cats

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Abstract

Homocysteine (Hcy) is an amino acid containing sulfur group in methionine metabolism regulating by vitamin B_{12} and folic acid. In recent years, it has been used as a biomarker for the diagnosis of several diseases. Therefore, in this study, it was aimed to investigate normal values of plasma Hcy, vitamin B_{12} and folic acid in Angora cats considering the age and gender. Thirty-two blood samples were collected from healthy Angora cats. They were classified as young (5.8±2.6 months, n=16) and adults (76.6±52.6 months, n=16) according to age, and as male (n=16) and female (n=16) according to gender as well. The findings of this study showed that plasma Hcy, vitamin B_{12} and folate concentrations of all cats were totally 29.94±3.20 nmol/mL, 1179.25±156.96 pg/mL, 28.10±1.50 ng/mL, respectively. These parameters were not differed statistically according to gender ($P>0.05$). On the other hand, it was observed that plasma concentrations of Hcy, and vitamin B_{12} were significantly ($P<0.05$) increased by ageing, except folate levels. In conclusion, this is the first study represents the normal values of plasma Hcy, vitamin B_{12} and folic acid in Angora cats considering age and gender It is supposed that findings of this study may offer scientific data for researchers, veterinarians, or further comprehensive studies.

Keywords: Angora cats, folic acid, homocysteine, vitamin B_{12}.

INTRODUCTION

Homocysteine (Hcy) is a nonessential amino acid containing thiol group (-SH) and an intermediate metabolite produced by the metabolism of methionine which is an essential amino acid and the only source of Hcy in the body as it could not be taken directly by diet (Finkelstein and Martin 2000; Nekrassova et al. 2003). During the metabolism of methionine and generation of Hcy, folate and B vitamins play roles as co-factors, which indicate the close relationship between them (Aksoy et al. 2006; Nursalim et al. 2013). The level of Hcy is under the effect of its remethylation to methionine regulated by folic acid and vitamin B_{12} or its renal transsulfuration to cystathionine regulated by vitamin B_6. Therefore, the deficiencies of these vitamins lead to increased Hcy levels (Aksoy et al. 2000; Lippi and Plebani 2012).

The increased plasma level of Hcy has been associated with early period of several diseases in man and animals, particularly renal, cardiovascular, and reproductive diseases (Robbe et al. 2003; Trisolini et al. 2008; Rizzo and Sciorsci 2019). Homocysteine, known for a long time, has been evaluated in many kinds of studies in human medicine. However, it is not sufficiently detailed in veterinary medicine (McMichael et al. 2000). Unlike most other species, cats have more requirements to methionine and vitamin B_6 because they are obligate carnivore (Baker and Czarnecki-Maulden 1991). Due to insufficient intake or excessive loses of these vitamins in cats may be faced to absolute or relative deficiencies of them which resulted in cardiovascular and renal disorders in relation to hyperhomocysteinaemia (McMichael et al. 2000; Yu 2007). However, the diagnosis of hyperhomocysteinemia necessitates the establishment of reference ranges for Hcy which is affected by various factors such as age and gender (Refsum et al. 2004). Although, it is well known that determining the normal values of Hcy in cats and the variables affecting them, are of clinical importance for the evaluation of Hcy status in diseases states (Drut et al. 2020), species-specific plasma Hcy levels are still not fully established, particularly in terms of breeds.

Angora cats are one of the significant and special cat breeds mainly originating from the vicinity of Ankara, Turkey. They have generally gold-blue colored eyes and white hairs (Erat and Arikan 2012). Until now, Turkish Angora cats as an important and endangered breed are researched in many kinds of studies in terms of hematological, biochemical, or characteristic genetic parameters in Turkey (Macun et al. 2010; Erat and Arikan 2012; Atmaca et al. 2014; Simsek et al. 2015; Şimşek et al. 2015), however, no studies focused on levels of plasma Hcy and related vitamins were found during the literature review. Therefore, the main object of this study was to investigate the effects of age and gender on the concentrations of plasma Hcy, vitamin B_{12} and folic acid in Angora cats.

MATERIALS AND METHODS

Animals

The experimental material of this study obtained from 16 male and 16 female Angora cats at varying ages were grouped as young (5.8±2.6 months, n=16) and adults (76.6±52.6 months, n=16). The healthy and vaccinated

cats were living at the same conditions during the investigation fed with standard commercial dry cat food (Fit 32, Royal Canin, France). The experimental design of the present study was approved by the Local Ethical Committee of Kirikkale University (2021/02-06).

Sampling and biochemical analysis

Blood samples were collected from the cephalic ven of all cats into the heparinized test tubes for biochemical analysis at 09:00 – 12:00 am. The samples were centrifuged at 1000 g for 10 min at 4 °C to obtain plasma which were kept at -20 °C until further analysis.

Plasma levels of folate and vitamin B12 were measured using electrochemiluminescence immunoassay kits (Elecsys Vitamin B_{12} II and Elecsys Folate III, Roche Diagnostic, USA) on the Roche Cobas E801 analyzer according to the recommendations of manufacturer. The concentrations of plasma Hcy were determined using high-performance liquid chromatography (HPLC) system with fluorescence detection (ImmuChrom GmbH, Germany) of chromatograms revealed by oxidation-reduction processes. Quantifications were conducted with the kit-calibrator.

Data analysis

Descriptive and further statistical analysis of the data were performed with SPSS 18.0 package program. Parametric and non-parametric data of female and male animals were analyzed with Mann Whitney U and Student's T tests, respectively, after the normality was checked with Shapiro-Wilk test. The data obtained from all cats at different ages were analyzed by using One-way ANOVA, then Tukey test for post-hoc multiple comparison between groups. $P<0.05$ was considered as statistically significant.

RESULTS

Plasma Hcy, vitamin B_{12} and folic acid concentrations of Angora cats were totally 29.94 ± 3.20 nmol/mL, 1179.25 ± 156.96 pg/mL, 28.10 ± 1.50 ng/mL, respectively, which were represented in Table 1. In addition, according to this table, female Angora cats have slightly higher levels of all parameters compare to males. However, these differences seen between both genders were not statistically significant ($P>0.05$).

Table 1. Plasma concentrations of homocysteine, vitamin B_{12} and folate in male and female Angora cats.

	Male (n=16)	Female (n=16)	Total (n=32)	P value
Parameters	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Homocysteine (nmol/mL)	28.74 ± 3.90	31.14 ± 5.18	29.94 ± 3.20	P>0.05
Vitamin B_{12} (pg/mL)	895.59 ± 123.24	1462.91 ± 266.07	1179.25 ± 156.96	P>0.05
Folate (ng/mL)	26.75 ± 1.50	29.44 ± 2.61	28.10 ± 1.50	P>0.05

SEM: Standard error mean.

Age-related changes in plasma levels of Hcy, vitamin B_{12} and folate were shown in Table 2 and Figure 1. It was observed that adult male and female cats have significantly higher concentrations of Hcy compare to young male and female cats ($P<0.05$). In addition, plasma levels of vitamin B_{12} in adult female cats were significantly higher than the other groups ($P<0.05$). As seen in the same table, the highest level of folate (32.07 ± 4.71) was in adult females while the lowest level of folate (22.31 ± 1.04) was in adult males. However, there were not statistically differences between each group ($P>0.05$).

Table 2. Plasma concentrations of homocysteine, vitamin B_{12} and folate in Angora cats at different ages.

	Young Male (n=8)	Young Female (n=8)	Adult Male (n=8)	Adult Female (n=8)
Parameters	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Homocysteine (nmol/mL)	18.77 ± 2.19 ^{b}	13.58 ± 1.38 ^{b}	38.70 ± 5.66 ^{a}	48.70 ± 4.99 ^{a}
Vitamin B_{12} (pg/mL)	922.00 ± 161.71^{b}	679.88 ± 100.02 ^{b}	869.19 ± 196.84^{b}	2245.94 ± 343.8 ^{a}
Folate (ng/mL)	31.94 ± 1.30 ^{a}	26.56 ± 2.37 ^{a}	22.31 ± 1.04 ^{a}	32.07 ± 4.71^{a}

Different letters in the same row represent statistically significance which is accepted $P<0.05$. SEM: Standard error mean.

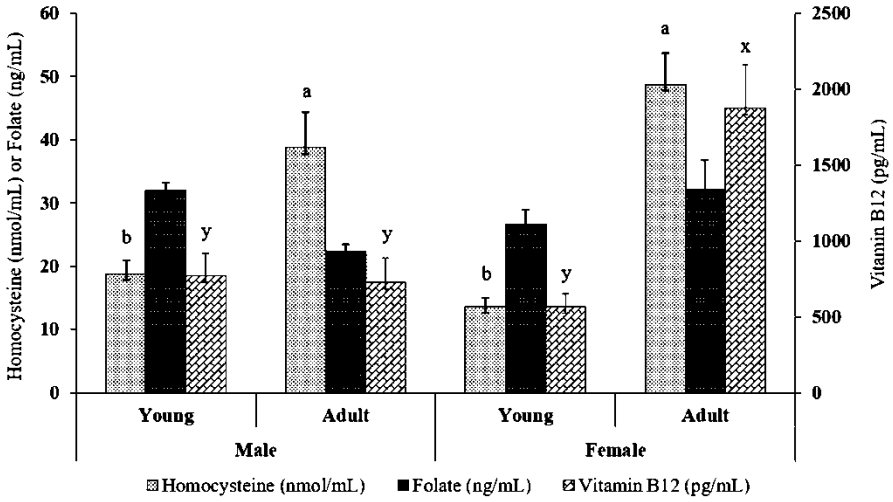

Figure 1. Plasma concentrations of homocysteine, vitamin B_{12} and folate in Angora cats at different ages. The letters a and b represent the differences in homocysteine levels between each group, while x and y represent the differences in vitamin B_{12}. $P<0.05$ was accepted as statistically significant.

DISCUSSION AND CONCLUSION

During the last few decades, plasma Hcy levels have been researched as a diagnostic marker in terms of cardiovascular diseases, renal disorders, tumors, neurological behaviors, oxidative stress etc. in a lot of studies. Therefore, reference values of plasma Hcy concentrations in humans have been well defined. However, veterinary medicine is still required research for determining the normal values of plasma Hcy levels in animals, particularly in cats (Baker and Czarnecki-Maulden 1991; McMichael et al. 2000; Drut et al. 2020). In this study, therefore, it was investigated age and sex related changes in plasma homocysteine, vitamin B_{12} and folate in Angora cats. According to our findings, all these parameters were significantly affected by the age but not by gender.

Homocysteine is an amino acid containing sulfur group (-SH) generated from metabolic pathways of methionine dependent on the vitamin B_{12} and folic acid. Biological determinants such as age, gender, breed, diet, exercise etc. could affect the plasma concentrations of Hcy (Refsum et al. 2004; Kakimoto et al. 2014; Cotul et al. 2020). In our study, the mean plasma concentration of Hcy for all cats was 29.94 ± 3.20 nmol/mL. This is in compliance with the report of Drut et al. (2020) in which plasma Hcy levels of healthy cats were range from 6.2 to 52.3 nmol/mL. In contrast, Hcy levels in healthy cats have been reported as 3.0 µg/mL by Fulmer et al. (2008); 7.1 ± 2.2 nmol/mL by Özkan et al. (2017); 7.6 ± 4.1 by McMichael et al. (2000); and 13.0 ± 2.8 by Üren et al. (2009) which were higher than that of the results of the present study. The main responsible for this difference may be breed. This situation is not well defined in cats because of the limited studies in terms of comparing breeds. In dogs, however, Kakimoto et al. (2014) have reported that plasma Hcy levels of Labrador Retrievers were significantly higher than that of Miniature Dachshunds, Chihuahuas, and Papillons. Authors also suggested that these differences may be the result of genetic factors related to Hcy metabolism. Therefore, this high Hcy level in Angora cats may be species-specific concentrations.

In some previous studies conducted in humans, serum Hcy levels in males have been found to be higher than in females (Kocabalkan et al. 2000; Aksoy et al. 2006). However, the findings of some other studies on cats have shown that there were no statistical differences on plasma levels of Hcy, vitamin B_{12} and folate in both sexes (McMichael et al. 2000; Özkan et al. 2017). Similarly, it was also found in the present study that these parameters did not change according to gender. Therefore, it is well understood that multiple further studies on cats are required in order to explain association with gender, reproduction, estrous cycle, or pregnancy and Hcy metabolism.

The mean plasma concentrations of vitamin B_{12} and folate were 1179.25 ± 156.96 pg/mL and 28.10 ± 1.50 ng/mL, respectively. These results are in line with the report of McMichael et al. (2000) on vitamin B_{12} levels and Fulmer et al. (2008) on folate concentrations in cats while incompatible with the findings of Özkan et al. (2017) on both parameters. This situation may be resulted from the differences of cat foods because vitamin B_{12}, and folate need to be taken by diet.

The findings of this study showed that Hcy, and vitamin B_{12} were changed according to age, except folate levels. Plasma Hcy levels of Angora cats significantly increased by aging in both genders. Similarly, Brattström et al. (1994) showed that total plasma Hcy in men and women increased markedly with age. It was also higher in elderly people compare to youngs (Kocabalkan et al. 2000; Aksoy et al. 2006). In addition, McMichael et al. (2000) have found a positive correlation between age and plasma level of vitamin B_{12} in cats. This vitamin is also increased in our study, particularly in adult female cats. However, in a study performed with Turkish Van cats it was shown that no statistical differences on Hcy, vitamin B_{12} and folate levels in any age groups. Homocysteine is metabolized by folic acid and vitamin B_{12}, so deficiencies of these vitamins may cause elevation of Hcy. It may also increase because of aging, kidney or cardiovascular diseases, and abnormal enzyme activity of Hcy metabolism (Bostom and Lathrop 1997; Gauthier et al. 2003). Therefore, the high level of both Hcy and vitamin B_{12} in adult cats may

be associated with the weakness in cellular uptake of vitamin B_{12} or dysfunction of vitamin B_{12}-dependent enzyme (methionine synthase) activity (Dhobale et al. 2012).

In conclusion, this study presented normal values of plasma Hcy, vitamin B_{12}, and folate levels in Angora cats considering the age and gender. Although these parameters did not change according to gender, they were significantly increased by age, expect folate. Because Hcy is used to predict a risk factor of several diseases in cardiovascular, renal, or reproductive systems, it is supposed that findings of this study may offer scientific data for researchers, veterinarians, or further comprehensive studies.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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