

Journal of the Hellenic Veterinary Medical Society

Vol 73, No 1 (2022)



Age-and sex-related changes in selected hematological parameters, lipid peroxidation and erythrocytes osmotic fragility of Turkish Angora cats

Ruhi Kabakci

doi: [10.12681/jhvms.25515](https://doi.org/10.12681/jhvms.25515)

Copyright © 2022, Ruhi Kabakci



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

To cite this article:

Kabakci, R. (2022). Age-and sex-related changes in selected hematological parameters, lipid peroxidation and erythrocytes osmotic fragility of Turkish Angora cats. *Journal of the Hellenic Veterinary Medical Society*, 73(1), 3715–3722. <https://doi.org/10.12681/jhvms.25515>

Age-and sex-related changes in selected hematological parameters, lipid peroxidation and erythrocytes osmotic fragility of Turkish Angora cats

R. Kabakci^{1*}, A.A. Yigit^{1#}, E. Kara², M. Cinar³, H. Ekici⁴, Y. Aluc⁵

¹Department of Physiology, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey

² Department of Internal Medicine, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey

³Department of Biochemistry, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey

⁴Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey

⁵Scientific and Technological Research Application and Research Center, Kirikkale University, Kirikkale, Turkey

#Current affiliation of Dr. Yigit is "Department of Physiology, Faculty of Medicine, Baskent University, 06790, Ankara, Turkey"

ABSTRACT: This study was conducted to investigate the changes in selected hematological parameters, lipid peroxidation and osmotic fragility of erythrocytes in Angora cats depending on age and gender. For this purpose, the blood samples were collected from *vena saphena medialis* of 9 young and 14 adult cats which were also classified as male (n=12) and female (n=11). Following hematological analysis, samples were washed with PBS by centrifugation and 10% hematocrit suspension was prepared from the erythrocytes pellet for the osmotic fragility test. The concentration of malondialdehyde (MDA) was also measured from lysed erythrocytes to determine lipid peroxidation level. Red blood cells (RBCs), hemoglobin, and hematocrit were significantly ($P<0.001$) high in adults while the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly ($P<0.05$) high in young cats. Erythrocyte MDA level was also higher statistically ($P<0.05$) in adult cats than in young cats. There was no significance ($P>0.05$) in these parameters between male and female cats. Findings of fragility tests showed that erythrocytes of young and male cats were statistically more susceptible to hypotonic NaCl solutions than those of adult and female cats, respectively ($P<0.01$, $P<0.05$). It was concluded that erythrocytes related parameters in Angora cats changed depending on age rather than gender except for stability of RBCs.

Keywords: Angora cat, age, erythrocyte membrane stability, gender, osmotic fragility, oxidative stress

Corresponding Author:

Ruhi Kabakci Address: Department of Physiology, Faculty of Veterinary Medicine, Kirikkale University, 71450, Yahsihan, Kirikkale, Turkey
E-mail address: ruhikabakci@kku.edu.tr

Date of initial submission: 09-12-2020
Date of revised submission: 27-05-2021
Date of acceptance: 04-10-2021

INTRODUCTION

Angora cat is one of the kind cat breeds mainly originated from Ankara, Turkey. They have gold, blue or gold-blue colored eyes and generally white hairs. Researching Angora cats is vital as they are now an endangered species. Analysis of hematological parameters is the most common procedure in human and animal clinics for the diagnosis of many diseases. However, species-specific reference blood values can be affected by different factors such as age, breed, sex, environment and toxicants, and should be available to interpret the findings accurately (Spada et al., 2015; Şimşek et al., 2015; Turgut, 2000; Yiğit and Kabakçı, 2018). Therefore, it is very critical to investigate normal blood parameters of Angora cats from many perspectives.

The erythrocyte osmotic fragility (OF) is a commonly used test to measure the membrane resistance of RBCs for the assessment of blood-related disorders such as anemia. Although the OF test is not routinely used as a diagnostic hematological parameter by clinicians, it is known that erythrocyte is affected in some disease by increasing or decreasing fragility (Slappendel, 1998). Erythrocyte OF also provides information about the surface/volume ratio of RBCs, which may change in many pathological conditions (Beutler, 1990). This technique is based on the colorimetric measurement of the hemoglobin (Hb) content released from the RBCs, exposed to the progressively hypotonic NaCl solution. The level of hemolysis is expressed as a percentage by comparing RBCs completely lysed in distilled water (Perk et al., 1964). It was previously well reviewed that OF could be affected by some extrinsic (temperature, pH, oxygenation, and drugs) and intrinsic (age, gender, breed, species, genotype and phenotype) factors (Igbokwe, 2018). It was shown that OF decreased aging in sheep (Asri et al., 2006) and cattle (Basarab et al., 1980). It was determined that the osmotic fragility of aged erythrocytes in circulation decreased in elderly humans and bovine (Mosior and Gomulkiwicz, 1988). On the contrary, Rifkind et al. (1983) reported that OF increased in aged-erythrocytes of human. It was also found that osmotic fragility of erythrocytes was higher in male than female in cattle (Olayemi, 2007), fowls (Durotoye and Oyewale, 1988) and sheep (Durotoye, 1987), while it was lower in male than female in dogs (Ogunyemi and Olayemi, 2016), goats (Habibu et al., 2014), turkeys (Azeez et al., 2011) and humans (Olorunshola et al., 2012).

The membranes of RBCs consist of polyunsatu-

rated fatty acids, phospholipids and cholesterol which relate to membrane stability and functions (Sako et al., 1989). Also, erythrocytes are constantly exposed to oxidative stress by free radicals produced by hemoglobin oxidation (Akila et al., 2007). Disruption of the redox activities of erythrocytes may result in membrane damage (Ojo et al., 2006). Aging might be a potential process for irreversible alterations related to the bioaccumulation of such oxidative disturbance in the cell (Akila et al., 2007). Lipid peroxidation is a series reaction producing free radicals in cell membranes and serves as an indicator of oxidative stress. It could be easily determined by the measurement of malondialdehyde (MDA), a product of lipid peroxidation, for determining oxidative disturbance (Halliwell, 1994; van Ginkel and Sevanian, 1994).

In Turkey, there are only few cat houses where Angora cats are saved and maintenance, except individual Angora cats keeper. It is aimed to create reference information about these cats while ensuring the protection and reproduction of these endangered animals. There are several pieces of research (Atmaca et al., 2014; Erat and Arıkan, 2012; Simsek et al., 2015a; Şimşek et al., 2015) about Angora cats related to their physiological values. However, to the best of our knowledge, variations of the erythrocyte membrane resistance in Angora cats have not been defined depending on the age and gender previously. Therefore, the purpose of the present study was to determine the age-and sex-related changes in selected hematological parameters, lipid peroxidation and erythrocytes osmotic fragility of Turkish Angora cats.

MATERIAL AND METHODS

This study was carried out with nine young (less than 1 year old, 6.3 ± 1.9 months) and fourteen adult (1-6 year-old, 44.6 ± 18.6 months) Turkish Angora cats all healthy, vaccinated, fed with standard commercial dry cat food, and housed in Kirikkale University in the same conditions throughout the investigation. We only used the cats in our center for this study in case of changes in blood parameters depending on housing and feeding conditions. They were also grouped as male ($n=12$) and female ($n=11$) to investigate the effects of gender. The study design was approved by the Local Ethical Committee of Kirikkale University (2019/3-21).

Blood was collected into the heparinized test tubes by puncturing of *vena saphena medialis* for hematological analysis and obtaining hemolysate. The blood samples were immediately transported to the labora-

tory in the next building and analyzed for red blood cells (RBC), hemoglobin (Hb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) by using an automatic blood analyzer (Abacus Junior Vet 5, Austria).

The rest of the blood samples were centrifuged at 1000 g for 10 min at 4 °C in the laboratory. The plasma and buffy coat were removed, and erythrocytes were washed 3 times with phosphate buffered sodium chloride solution (PBS, 154 mM NaCl, 10 mM sodium phosphate, pH 7.4). After the last centrifugation, a little part of the erythrocyte pellet was resuspended in PBS-NaCl to prepare 10% hematocrit to use the osmotic fragility test. The rest of erythrocyte pellet was hemolyzed with cold-deionized distilled water and stored at -80 °C for further MDA analysis. The MDA levels of erythrocytes were analyzed as a classical method previously described by Buege and Aust (1978). The hemoglobin content of erythrocytes was determined by the cyanmethemoglobin method (Drabkin and Austin, 1932).

The osmotic fragility of erythrocytes was evaluated in 2.5 mL of PBS containing gradually increased concentrations of NaCl from 0 to 0.85 in 13 tubes as previously defined by Tritschler et al. (2016) with some modification. Ten microliters of 10% hematocrit suspension were mixed with 2.5 mL of each PBS-NaCl dilutions and distilled water in different glass tubes and incubated for 30 min at laboratory temperature. Afterwards, all samples were centrifuged at 1000 g for 10 min, and the optical density of the supernatants was read with a spectrophotometer (Multiskan Go, Thermo Scientific, Finland) at 540 nm. The percentage of hemolysis in each tube containing different concentrations of NaCl was calculated by comparison with that of distilled water which was assumed 100 % hemolysis by using formula; % hemolysis = (absorbance of test tubes/absorbance of 0% NaCl dilution tube) x 100. The results were expressed as % hemolysis.

The threshold of P-value is considered 0.3 instead of 0.05 to obtain more accurate results of sample distribution tests for determining of reference interval (RI) in veterinary species that have a smaller population, according to guideline developed by the American Society of Veterinary Clinical Pathology (ASVCP) (Friedrichs et al., 2012). The Gaussian distribution of samples was determined by the Shapiro-Wilks test and the RI calculation of Gaussian and non-Gaussian samples were performed with parametric and robust methods us-

ing MedCalc Software Version 20 (Ostend, Belgium), respectively. Further statistical analysis of the data obtained from two groups (young and adult or male and female) was performed with SPSS 18.0 package program by using Student's T-test and Mann Whitney U test for parametric and non-parametric data, respectively. $P < 0.05$ was considered statistically significant. It is also recommended when the number of between 20 and 40 reference samples are available, a table of ascending values along with a histogram and mean or median values should be reported (Friedrichs et al., 2012).

RESULTS

The histograms in Figure 1 show the population distributions of selected blood parameters while the histograms in Figure 2 represent the population distributions of erythrocyte MDA levels and hemolysis degrees of RBCs in different % NaCl concentrations.

Selected hematological parameters of young, adult, male, and female Angora cats, and reference ranges of them for domestic cats were shown in Table 1. There were significant differences between erythrocyte related values of young and adult cats while there were no significant differences between the same parameters of male and female cats. According to these findings, RBC, Hb, and PCV were higher in adults, while MCV, MCH, and MCHC were lower than in young cats.

Table 2 represents the effects of age and gender on the MDA level of cats' erythrocytes. Malondialdehyde levels of adult cats were significantly ($P < 0.05$) higher than that of young cats. However, the erythrocyte MDA levels of males or females were not different statistically ($P > 0.05$).

Table 2 also shows the % NaCl concentrations of 10% and 90% hemolysis which are accepted initial and complete hemolysis, of erythrocytes in young, adult, male, and female cats. The erythrocytes OF curve of young and adult cats, and male and female cats represented in Figure 3 and Figure 4, respectively, show % hemolysis of RBCs in different dilutions of NaCl. Paired comparisons were made only at the NaCl concentrations range from initial hemolysis to complete hemolysis of RBCs. While erythrocytes of young cats were significantly more fragile than the adults at 0.50, 0.55, and 0.60 % of NaCl concentrations ($P < 0.01$, $P < 0.001$, $P < 0.001$ respectively), according to Figure 1, the erythrocytes fragility of male cats was significantly higher than that of females at 0.55 % ($P < 0.01$) and 0.60 % ($P < 0.05$) of NaCl concentrations (Figure 2).

Table 1. Changes in erythrocytes related values of Angora Cats depending on the age and gender

Parameters	Reference ranges*	Groups	n	Mean	SD	Median	Min	Max	Reference Interval	CI 90% of LRL	CI 90% of URL	P value
RBC ($\times 10^6/\mu\text{L}$)	5 - 10	Young	9	6.69	1.24	6.56	5.18	8.95	4.65-8.73	3.55-5.75	7.63-9.83	<0.001
		Adult	14	9.72	1.29	9.62	8.36	12.97	7.10-11.87	5.91-8.45	10.56-13.18	
		Male	12	8.62	1.73	8.78	5.18	10.45	5.83-12.29	3.97-7.51	10.80-13.42	NS
		Female	11	7.89	2.24	7.17	5.52	12.97	3.03-11.92	1.12-5.05	9.10-14.10	
Hb (g/dL)	8 - 15	Young	9	11.30	0.92	11.40	10.10	12.80	9.78-12.81	8.97-10.60	11.99-13.62	<0.001
		Adult	14	13.01	0.69	13.01	11.30	13.90	11.88-14.42	11.21-12.51	1.68-14.92	
		Male	12	12.33	1.23	12.85	10.10	13.70	10.39-15.32	9.04-11.69	14.00-15.88	NS
		Female	11	12.05	1.16	12.10	10.60	13.90	10.14-13.94	9.12-11.17	12.92-14.97	
PCV (%)	24 - 45	Young	9	30.54	4.02	29.21	24.67	37.10	23.93-37.14	20.36-27.44	33.58-40.71	<0.001
		Adult	14	42.21	3.82	43.06	34.21	48.76	35.92-48.49	32.68-39.16	45.26-51.73	
		Male	12	38.35	7.17	41.04	24.67	45.45	25.93-54.66	19.52-34.53	48.17-60.63	NS
		Female	11	34.75	6.81	34.21	27.55	48.76	21.27-47.37	13.28-26.25	39.15-53.57	
MCV (fL)	39 - 45	Young	9	46.00	3.22	46.00	41.00	51.00	40.69-51.30	37.83-43.55	48.44-54.16	<0.05
		Adult	14	42.58	4.12	42.50	36.00	50.00	35.80-49.36	32.31-39.29	45.87-52.85	
		Male	12	44.50	2.88	44.50	41.00	50.00	38.93-49.83	35.44-40.62	46.88-51.91	NS
		Female	11	43.91	5.15	45.00	36.00	51.00	35.44-52.37	30.87-40.00	47.80-56.94	
MCH (pg)	12.5 - 17.5	Young	9	17.19	1.99	16.88	14.30	20.65	13.92-20.46	12.16-15.68	18.70-22.22	<0.001
		Adult	14	13.55	1.30	13.51	10.70	15.38	11.40-15.69	10.30-12.50	14.59-16.80	
		Male	12	14.66	2.11	14.00	12.46	19.50	11.20-18.17	9.41-12.98	16.34-19.91	NS
		Female	11	15.98	2.75	16.46	10.70	20.65	11.45-20.50	9.01-13.89	18.06-22.04	
MCHC (g/dL)	30 - 36	Young	9	37.24	2.28	36.97	34.40	40.94	33.48-40.99	31.45-35.50	38.98-43.02	<0.001
		Adult	14	30.99	2.71	30.07	28.40	37.10	24.66-35.03	23.04-28.20	31.60-38.12	
		Male	12	32.79	4.05	30.47	28.56	40.94	22.08-39.62	20.14-27.08	34.90-43.38	NS
		Female	11	35.27	3.75	35.86	28.40	40.76	29.10-41.44	25.78-32.43	38.12-44.78	

SD: Standard deviation, CI: Confidence interval, LRL: Lower reference limits, URL: Upper reference limits, NS: Non-significant.

* Reference ranges of selected blood parameters for domestic cats (Blood and Studdert, 1988; Schalm et al., 1975).

Table 2. Changes in lipid peroxidation level and hemolytic range of erythrocytes of Angora cats depending on the age and gender

Parameters	Groups	n	Mean	SD	Median	Min	Max	Reference Interval	CI 90% of LRL	CI 90% of URL	P value
MDA nmol/mL	Young	9	29.31	9.69	32.69	16.67	43.59	11.51-50.63	4.72-21.73	37.42-56.09	<0.05
	Adult	14	38.97	10.69	39.10	21.79	57.69	21.39-56.55	13.34-29.44	48.50-64.60	
	Male	12	34.01	8.28	34.61	19.23	53.20	20.39-47.63	14.36-26.42	41.60-53.66	NS
	Female	11	36.28	15.17	38.46	16.67	57.69	6.48-66.32	5.80-21.90	52.64-72.74	
Initial Hemolysis (%NaCl)	Young	9	0.65	0.05	0.65	0.60	0.75	0.55-0.73	0.51-0.57	0.70-0.78	NS
	Adult	14	0.65	0.05	0.65	0.60	0.75	0.56-0.76	0.54-0.59	0.73-0.79	
	Male	12	0.65	0.04	0.65	0.60	0.75	0.57-0.74	0.55-0.60	0.71-0.77	NS
	Female	11	0.65	0.05	0.60	0.60	0.75	0.55-0.73	0.50-0.60	0.67-0.78	
Complete Hemolysis (%NaCl)	Young	9	0.50	0.06	0.50	0.40	0.55	0.35-0.57	0.31-0.40	0.53-0.61	NS
	Adult	14	0.45	0.04	0.40	0.40	0.50	0.38-0.49	0.34-0.40	0.46-0.52	
	Male	12	0.45	0.05	0.40	0.40	0.55	0.35-0.53	0.31-0.39	0.49-0.57	NS
	Female	11	0.45	0.04	0.45	0.40	0.50	0.39-0.50	0.36-0.43	0.47-0.54	

SD: Standard deviation, CI: Confidence interval, LRL: Lower reference limits, URL: Upper reference limits, NS: Non-significant.

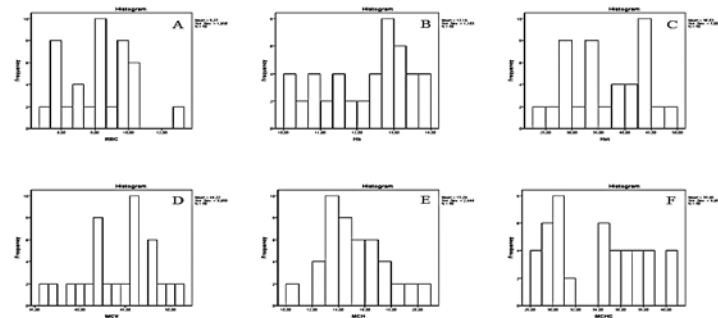


Figure 1. Histograms showing the 6 tested analyte population distributions: (A) RBC: Red blood cells, (B) Hb: Hemoglobin, (C) PCV: Package cell volume, (D) MCV: Mean corpuscular volume, (E) MCH: Mean corpuscular hemoglobin, (F) MCHC: Mean corpuscular hemoglobin concentration

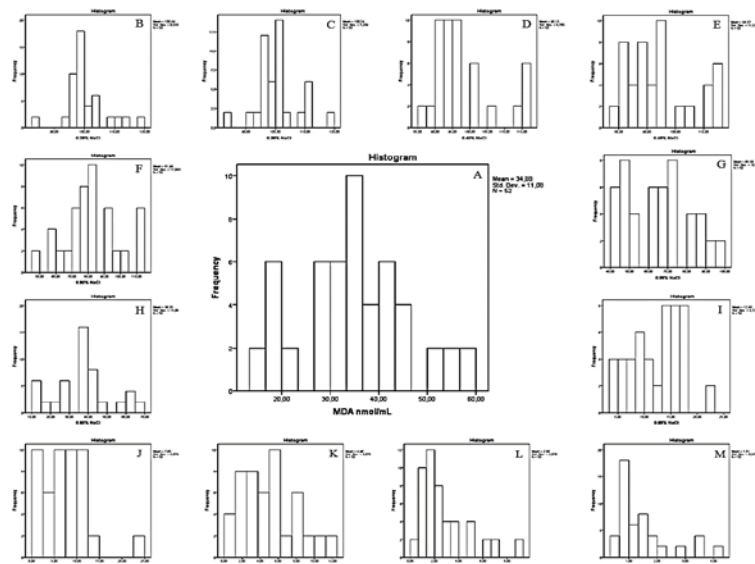


Figure 2. Histograms showing the 13 tested analyte population distributions: (A) MDA levels of erythrocytes, (B-M), hemolysis degrees of erythrocytes in different NaCl dilutions

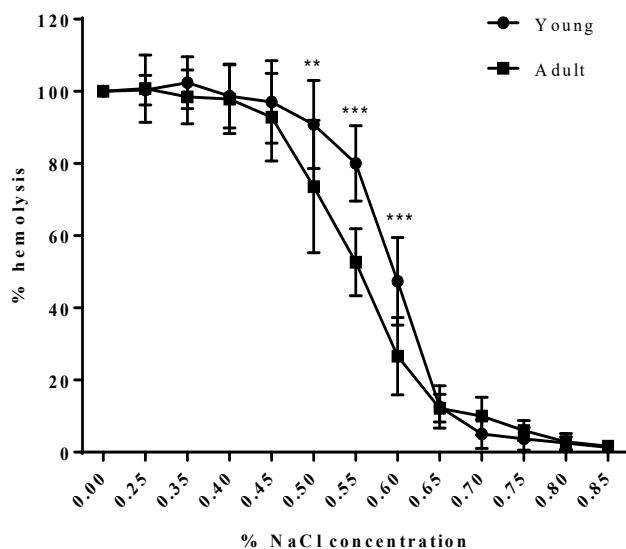


Figure 3. Erythrocyte osmotic fragility curve of young and adult Angora cats. The data expressed as mean plus standard error mean. Asterisk represents the statistically significance, **:P<0.01, ***:P<0.001

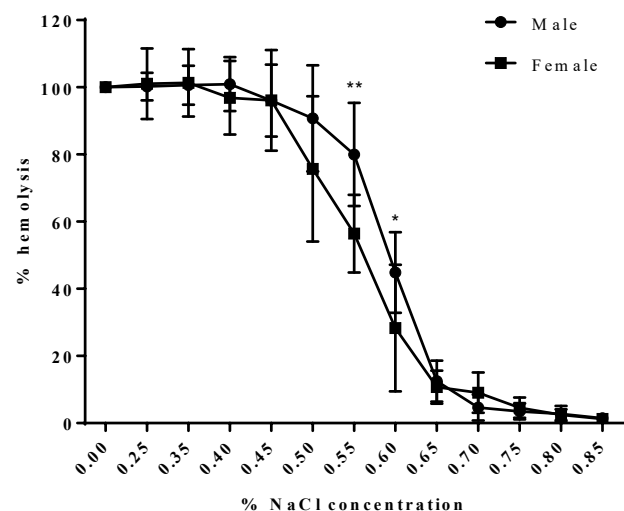


Figure 4. Erythrocyte osmotic fragility curve of male and female Angora cats. The data expressed as mean plus standard error mean. Asterisk represents the statistically significance, *:P<0.05, **:P<0.01

DISCUSSION

The blood parameters are determined not only for the diagnosis of diseases but also for evaluating the health status of living organisms since they are supplied much specific knowledge on the metabolic events in the body (Theml et al., 2004). In this study, we investigated some hematological parameters, lipid peroxidation level, and osmotic resistance of RBCs in healthy Angora cats in terms of age and gender. This study could be criticized for using a small sample size. However, we should note that Angora cat is an important and special cat breed belonging to Turkey, and well-known throughout the world. Unfortunately, it is an endangered breed (Erat and Arikan, 2012). Although, in guidelines the Clinical and Laboratory Standards Institute (CLSI), it is recommended minimum 120 samples for the preparation of reference limits (CLSI, 2010), it is difficult to find enough number of cats that are available, because there are only two centers which tries to protect and maintenance of Angora cats in our country. One of these centers is in our Faculty at Kırıkkale University where 25 cats are housed. The other Angora Cat House belongs to Ankara Pursaklar Municipality, where 40 cats have been saved and maintain here since 2017 (İnal, 2019). We did not want to include them, because their housing, feeding, and drinking conditions were different. In addition, two cats in our facility were excluded from the study because they are too older (13- and 14-years-old). Therefore, the present study was carried out total of 23 cats, and both classification and grouping of them were performed as previously described by Olayemi et al. (2009).

The results of the hematologic parameters were within the reference ranges and highly similar to suggested standard values for cats (Blood and Studdert, 1988; Schalm et al., 1975). While erythrocyte related values were significantly different between young and adult cats, they did not differ between male and female cats. According to our study, RBC, Hb, and PCV values were higher in adults than in young cats whereas MCV, MCH, and MCHC were higher in young than in adults. Similarly, Simsek et al. (2015a) reported that RBC, PCV, and Hb values in adult Angora cats were higher than in 1.5-3 month-old kittens. Authors also showed that MCHC was higher in adults than in 1.5-3 month-old kittens, while MCV and MCH values were statistically the same in both groups. These findings were also in agreement with the reports on dogs (Olayemi et al., 2009) and goats (Elitok, 2012), in which RBC, Hb and PCV were significantly higher in

adults than in the young. Whereas Yiğit et al. (2002) showed the same parameters were higher in young sheep compared to adults. Elitok (2012) showed that high level of MCV, MCH, MCHC in young goats significantly decreased with age which was parallel with our results. We suggest the differences between young and adult cats could be related to the life span or higher oxygen transport capacity of RBCs.

Our findings on RBC, PCV, Hb, MCV, MCH, and MCHC in female cats shown in Table 1 were fairly compatible with a previous study in which reported erythrocytes-related hematological parameters in 1-3-year-old female Angora cats (Şimşek et al., 2015). Olayemi et al. (2009) also did not find significant differences in RBC, PCV, Hb, MCV, MCH and MCHC in male and female Nigerian indigenous dogs. Furthermore, in a study carried out on Van cats, the PCV and Hb values did not differ according to age and gender (Sönmez and Ağaoğlu, 2010). However, Özkan et al. (2016) reported that PCV and Hb values of Van cats were significantly increased with the age, and in male cats. These different reports may be explained by the effects of other factors such as season or breed on blood parameters.

In this present study, lipid peroxidation level in erythrocytes was measured by MDA concentration and was significantly increased by age but there was no difference between male and female cats. Similarly, Simsek et al. (2015b) showed that healthy adult Angora goats had higher MDA levels in erythrocytes than that of young goats. Our results also support the findings of previous studies carried out on dogs (Gaál et al., 1996), mares (Aydilek and Şimşek, 2006), sheep (Salar-Amoli and Baghbanzadeh, 2010) and humans (Akila et al., 2007) which reported MDA concentration was affected by aging, but not gender. Denaturation of lipids, proteins and nucleic acids, and production of free radicals are normal process of cellular metabolism and are balanced by antioxidant mechanisms. Inadequate neutralization of free radicals emerging with ageing, leads to oxidation of cellular lipids, proteins, and carbohydrates (Matsubara and Machado, 1991). High MDA levels in adult cats may be associated with cellular membrane damage because of aging.

According to the findings of the present study, the concentration of NaCl lead to initial (10%) hemolysis was 0.65 % in all groups, and NaCl dilution lead to complete (90%) hemolysis was 0.50 % and 0.45 % in young cats and the others, respectively. These results

were in accordance with standard values of domestic cats as described by Schalm et al. (1975), in which minimum hemolysis was shown at 75% NaCl solution while maximum hemolysis was shown at lower 50% NaCl solution. Kohn et al. (2000) also reported that the initial and complete hemolysis of erythrocytes in non-anemic healthy (Abyssinian and Somali) cats were seen at 0.55 % and 0.45 % NaCl dilutions, respectively. Erythrocytes of young and male cats in the current study were more susceptible to hypotonic NaCl solutions than those of adult and female cats. In some previous reports, the erythrocytes OF in younger sheep (Asri et al., 2006), cattle (Basarab et al., 1980), and turkey (Azeez et al., 2011) were higher than that of older animals, which were compatible with the findings of the present study. This may be related to the short life span of RBCs in young as well as high MCV since it is considered to increase macrocytes (immature erythrocytes) count in blood. Macrocytes may have been easily broken in capillaries during circulation, which resulted in increased fragility when comparing in young and adult animals. Moreover, lower erythrocyte OF in adult cats may be associated with increased stability of cell membrane by aging as previously described by Grinna (1977). According to his report, cholesterol, phospholipids, and saturated fatty acid concentrations of erythrocyte's membrane increased during aging. However, this situation may lead to increased oxidative stress markers such as MDA, GSH, CAT in elderly/geriatric people (Akila et al., 2007). This agrees with the findings of the current study on MDA level (lipid peroxidation) in adult cats which was a higher concentration than that of young cats. It is well known that erythrocytes are more vulnerable to rupture because of the increased oxidative stress by the age, especially in old animals (Tyan, 1982). Contrary to expectations, higher MDA levels in adults did not cause lower OF in erythrocytes compared to young cats. Therefore, it is suggested that increased MDA level in adult Angora cats is not yet enough to increase OF in erythrocytes because they were not senior cats.

Erythrocytes OF in male Angora cats were higher than those in females especially at 0.55 and 0.60 % concentrations of NaCl solution. There are many incompatible reports on variations of erythrocyte OF according to gender in the literature. As compatible

with the current study, RBCs of male cattle (Olayemi, 2007), sheep (Durotoye, 1987), and fowls (Durotoye and Oyewale, 1988) were more fragile to hypotonic NaCl solution compared to females. The authors have suggested that this may be related to the stabilizing effect of estrogen. However, erythrocytes OF in female Nigerian Indigenous dogs were higher than that of males (Olayemi et al., 2009) while there were no significant differences in erythrocytes OF between male and female horses (Andriichuk et al., 2014) and German Shepherd dogs (Ogunyemi and Olayemi, 2016). Unfortunately, the effects of gender on erythrocytes OF are still unclear.

CONCLUSION

In conclusion, the results of this study showed that erythrocytes related values of Angora cats may have variations depending on age and gender. Total RBCs, Hb, and PCV values were high in adults while MCV, MCH, and MCHC were high in young cats. These parameters were not altered according to gender. Malondialdehyde level, an indicator of lipid peroxidation was also higher in adults compared to young cats, but statistically the same in males and females. Osmotic fragility of erythrocytes decreased by age and male Angora cats have higher OF than females. It is suggested that the life span of RBCs may play a role in these age-related differences. However, no difference between genders in these values may be due to low levels of sex steroids in females during long anestrus periods. It was concluded that not only the age and gender difference affect the RBC related values, but also breed, season, and hormonal fluctuation might be responsible for these variations. In addition, although this is a small-scale qualitative study related to some blood parameters of Angora cats, these data may be useful for veterinarians, and seen as an opportunity to generate further hypotheses for researchers.

ACKNOWLEDGMENTS

This research was funded by the Kirikkale University Scientific Research Projects Coordination Unit with the project number of 2019/110.

CONFLICT OF INTEREST

It is declared that there is no conflict of interest between the authors.

REFERENCES

- Akila VP, Harishchandra H, D'souza V, and D'souza B (2007) Age related changes in lipid peroxidation and antioxidants in elderly people. *Indian J Clin Biochem* 22: 131-134.
- Andriichuk A, Tkachenko H, and Kurhaluk N (2014) Gender differences of oxidative stress biomarkers and erythrocyte damage in well-trained horses during exercise. *J Equine Vet Sci* 34: 978-985.
- Asri RS, Ramin A, and Koukalanifar M (2006) Evaluation of correlation between serum vitamin E status and the erythrocyte osmotic fragility in sheep. *J Vet Sci* 61: 155-159.
- Atmaca N, Simsek O, Arıkan S, and Kabakci R (2014) Evaluation of some electrocardiographic parameters of kitten and adult angora cats. *Res J Vet Pract* 2: 113-116.
- Aydilek N, and Şimşek H (2006) Yaşlanmanın kısırlıklarda plazma oksidan ve antioksidan parametreleri üzerine etkisi. *J Fac Vet Med Univ Erciyes* 3: 73-77.
- Azeez O, Olayemi F, and Olanrewaju J (2011) Age and sex influences on the haematology and erythrocyte osmotic fragility of the nigerian turkey. *Res J Vet Sci* 4: 43-49.
- Basarab J, Berg R, and Thompson J (1980) Erythrocyte fragility in "double-muscling" cattle. *Can J Anim Sci* 60: 869-876.
- Beutler E (1990) Osmotic fragility. in Williams JW, Beutler E and Erslev AJ (eds.) *Hematology*, 4th ed, McGraw Hill Publishing Co, New York: pp 1726-1728
- Blood DC, and Studdert VP (1988) Baillière's comprehensive veterinary dictionary. Baillière Tindall: London, UK.
- Buege JA, and Aust SD (1978) Microsomal lipid peroxidation. in Abelson J, Simon M, Verdine GL and Pyle AM (eds.) *Methods enzymol*, 1st ed, Academic Press, USA: pp 302-310
- CLSI (2010) Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guidelines. Clinical and Laboratory Standards Institute. 3rd ed. EP28-A3C. Wayne, PA, USA
- Drabkin DL, and Austin JH (1932) Spectrophotometric studies I. Spectrophotometric constants for common hemoglobin derivatives in human, dog, and rabbit blood. *J Biol Chem* 98: 719-733.
- Durotoye L (1987) Effect of sex, pregnancy and lactation on the osmotic fragility of the west african dwarf sheep. *Bull Anim Health Prod Afr* 35: 29-33.
- Durotoye L, and Oyewale J (1988) A comparative study of erythrocyte osmotic fragility between the guinea fowl (*Numida meleagris* galeata) and the nigerian domestic fowl (*Gallus domesticus*). *Bull Anim Health Prod Afr* 36: 73-76.
- Elitok B (2012) Reference values for hematological and biochemical parameters in saanen goats breeding in afyonkarahisar province. *Kocatepe Vet J* 5: 2012517117-2012517121.
- Erat S, and Arıkan S (2012) The hair characteristics of turkish angora and van cats. *Turk J Vet Anim Sci* 36: 215-221.
- Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, and Blanco-Chavez J (2012) Asvcp reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol* 41: 441-453.
- Gaál T, Speake B, Mezes M, Noble R, Surai P, and Vajdovich P (1996) Antioxidant parameters and ageing in some animal species. *Comp Haematol Int* 6: 208-213.
- Grinna LS (1977) Changes in cell membranes during aging. *Gerontology* 23: 452-464.
- Habibu B, Kawu M, Makun H, Aluwong T, Yaqub L, Ahmad M, Tauheed M, and Buhari H (2014) Influence of sex, reproductive status and foetal number on erythrocyte osmotic fragility, haematological and physiologic parameters in goats during the hot-dry season. *Vet Med (Praha)* 59: 479-490.
- Halliwell B (1994) Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? *Lancet (British edition)* 344: 721-724.
- Igbokwe N (2018) A review of the factors that influence erythrocyte osmotic fragility. *Sokoto J Vet Sci* 16: 1-23.
- İnal A (2019) Ankara kedisi 'sevgiyle' hayata tutunuyor <https://www.aa.com.tr/tr/yasam/ankara-kedisi-sevgiyle-hayata-tutunuyor/1353391> [accessed 24 May 2021].
- Kohn B, Goldschmidt MH, Hohenhaus AE, and Giger U (2000) Anemia, splenomegaly, and increased osmotic fragility of erythrocytes in abyssinian and somali cats. *J Am Vet Med Assoc* 217: 1483-1491.
- Matsubara LS, and Machado P (1991) Age-related changes of glutathione content, glutathione reductase and glutathione peroxidase activity of human erythrocytes. *Braz J Med Biol Res* 24: 449.
- Mosior M, and Gomulkiewicz J (1988) Osmotic properties of bovine erythrocytes aged in vivo. *Gen Physiol Biophys* 7: 73-79.
- Ogunyemi AD, and Olayemi FO (2016) Comparative assessment of erythrocyte osmotic fragility in two breeds of dog: Nigerian indigenous breed and the german shepherd. *Comp Clin Path* 25: 79-83.
- Ojo O, Kabutu F, Bello M, and Babayo U (2006) Inhibition of paracetamol-induced oxidative stress in rats by extracts of lemongrass (*Cymbopogon citratus*) and green tea (*Camellia sinensis*) in rats. *Afr J Biotechnol* 5: 1227-1232.
- Olayemi F (2007) The effect of sex on the erythrocyte osmotic fragility of the nigerian white fulani and ndama breeds of cattle. *Top Vet* 25: 106-111.
- Olayemi F, Azeez I, Ogunyemi A, and Ighagbon F (2009) Study on erythrocyte values of the nigerian indigenous dog. *Folia Vet* 53: 65-67.
- Olorunshola K, Eze K, and Achie L (2012) Effect of road transport stress on erythrocyte osmotic fragility (eof) of healthy young adult nigerians during the harmattan season. *Niger J Physiol Sci* 27: 157-164.
- Özkan C, Kozat S, Kaya A, and Akgül Y (2016) Some hematological and biochemical parameter levels in healthy van cats at different age and gender. *Eurasian J. Vet. Sci* 32: 214-219.
- Perk K, Hort I, and Perri H (1964) The degrees of swelling and osmotic resistance in hypotonic solutions of erythrocyte from various domestic animals. *Refuah vet* 20: 122-128.
- Rifkind JM, Araki K, and Hadley EC (1983) The relationship between the osmotic fragility of human erythrocytes and cell age. *Arch Biochem Biophys* 222: 582-589.
- Sako T, Takeda S, Shibuya M, Koyama H, Uchino T, and Motoyoshi S (1989) Seasonal changes in the phospholipids and fatty acid composition of the bovine erythrocyte membrane. *The Japanese journal of veterinary science* 51: 1243-1245.
- Salar-Amoli J, and Baghbanzadeh A (2010) Oxidative stress in shaal sheep in different age groups. *Turk J Vet Anim Sci* 34: 379-383.
- Schalm OW, Jain NC, and Carroll EJ (1975) *Veterinary hematology*. Lea & Febiger: Philadelphia, USA.
- Simsek O, Cinar M, and Arıkan S (2015a) Changes in selected hematology and serum biochemistry in turkish angora cats (*Felis catus*) during growth period. *J adv vet* 2: 34-39.
- Simsek O, Ekici H, Cinar M, Atmaca N, Arıkan S, and Guner B (2015b) Heavy metal accumulation and oxidative-antioxidative status in angora goats depending on age. *Fresenius Environ Bull* 24: 2659-2663.
- Slappendel R (1998) Abnormal osmotic fragility of erythrocytes in dogs and cats. *Vet Q* 20: S38-S39.
- Sönmez N, and Ağaoglu ZT (2010) Van kedilerinde kardiyak troponin seviyelerinin araştırılması. *Van Vet J* 21: 21-25.
- Spada E, Antognoni MT, Proverbio D, Ferro E, Mangili V, and Miglio A (2015) Haematological and biochemical reference intervals in adult maine coon cat blood donors. *J Feline Med Surg* 17: 1020-1027.
- Şimşek Ö, Arıkan Ş, and Çınar M (2015) Reference values for selected hematological and biochemical blood parameters from pre-pregnancy to advanced gestation in angora cats. *Turk J Vet Anim Sci* 39: 29-33.
- Thieml H, Diem H, and Haeflacher T (2004) *Color atlas of hematology: Practical microscopic and clinical diagnosis*. Thieme: New York.
- Tritschler C, Mizukami K, Raj K, and Giger U (2016) Increased erythrocyte osmotic fragility in anemic domestic shorthair and purebred cats. *J Feline Med Surg* 18: 462-470.
- Turgut K (2000) Veteriner klinik laboratuvar teshis. *Bacıvanlar Basım Sanayi AŞ: Konya*.
- Tyan ML (1982) Age-related increase in erythrocyte oxidant sensitivity. *Mech Ageing Dev* 20: 25-32.
- van Ginkel G, and Sevanian A (1994) Lipid peroxidation-induced membrane structural alterations. *Methods Enzymol* 233: 273-288.
- Yiğit AA, and Kabakçı R (2018) Çevre kirlenmelerden ağır metallerin hayvanlarda hematopoetik sistem üzerine etkileri. *Türkiye Klinikleri J Vet Sci Pharmacol Toxicol-Special Topics* 4: 9-15.
- Yiğit AA, Kısa Ü, Arıkan Ş, Akçapınar H, and Taşdemir U (2002) Sakız x karayaka melezi gı koyunlarının kan parametreleri üzerine cinsiyet ve yaşın etkisi. *Ankara Univ Hekim Fak Derg* 49: 101-106.