Determination of blood heavy metal concentrations and oxidant-antioxidant capacities in Angora cats at different age and gender

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Abstract

This study was conducted to investigate heavy metal concentrations and oxidative status of plasma and erythrocytes in Angora cats at different ages and gender. Sixteen young (less than 1 year old) and 14 adult (1–6 years old) cats were also grouped according to gender as male (n = 17) and female (n = 13). The separated plasma samples from cat's blood were analyzed for selected heavy metals and total oxidant and antioxidant capacities (TOC and TAC) and calculated for oxidative stress index (OSI). The erythrocyte hemolysates were also evaluated for malondialdehyde (MDA), and super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Results of the study showed that most of the measured metals were not varied statistically according to age or gender. However, adult cats had significantly ($P<0.01$) higher Cu and lower Fe levels compared to young cats. Plasma levels of TOC, TAC and OSI, and erythrocyte MDA concentrations in young cats were significantly ($P<0.05$) higher than that of adults. While the SOD activity was decreased by the age, GPX activity was increased ($P<0.05$). However, the activity of CAT was changed by only gender, which was higher in males ($P<0.01$). In conclusion, metals, especially trace elements, are required for many kinds of physiological processes and the synthesis of antioxidant enzymes. Therefore, it can be suggested that the periodic measurement of metals and the addition of common antioxidant supplements to the diet of adult Angora cats will support weakening antioxidant mechanisms by age.

Introduction

The elements that had a density greater than 5 g/cm^3 water are called heavy metals. They have generally toxic effects even at low concentrations. While some of them are nonessential for the body such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), some others are essential, such as aluminum (Al), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), tin (Sn), and zinc (Zn). The latter is also defined as trace elements, which involve many kinds of metabolic events in humans and animals. However, both of them (essential and non-essential metals) may be toxic unless taken/given appropriate concentrations (20, 25).

Heavy metals are the most widespread chemical groups among the potential environmental contaminants because of an exponential increase in their usage in industry, agriculture, and technology. They are transported to the body through digestion, respiration and other routes as they are persistent in the environment for a long time, and threaten man and animals' health (20, 24). Pet animals may also be exposed to these chemicals continuously that resulting in bioaccumulation in many tissues in relation to the long lifespan of pets (30).

Moreover, canine and feline may have similar metabolic and clinical responses to toxic substances as humans (24). Exposure to high levels of heavy metals has toxic, teratogenic, and mutagenic effects and leads to the production of free radicals as a result of oxidative degradation of lipids (13, 20).

Although reactive oxygen species (ROS) are generated as a consequence of cellular metabolism in many physiological processes, the harmful effects of them are inhibited by endogenous antioxidant mechanisms, which are classified as nonenzymatic and enzymatic antioxidants (31). However, they can be potentially toxic and cause oxidative stress if the oxidant-antioxidant balance is disturbed. For instance, heavy metals may cause to the oxidative damage (13). Moreover, McMichael (23) reported an age-related increase in ROS formation in mitochondria during aging.

Angora cats that have gold/blue colored eyes and generally white hairs are one of the kind cat breeds lived in the Central Anatolia Region of Türkiye. Many studies have been conducted to get more information about them from all perspectives to save them as they are endangered (4, 14, 21, 26). In this study, it was aimed to investigate blood heavy metal concentrations and oxidant-antioxidant status in Angora cats, which drink tap water in Kırıkkale province at different age and gender.

Materials and Method

Animals: Thirty Angora cats were grouped according to age as young (less than 1 year old, $n = 16$) and adult (1–6 years old, $n = 14$) or gender as male ($n = 17$) and female ($n = 13$). The healthy and vaccinated cats housed in Kırıkkale University, Faculty of Veterinary Medicine at the same conditions were fed ad libitum with commercial dry cat food (Fit32, Royal Canin, France) and tap water during the investigation. All the procedures of this research were conducted with the approval of the Local Ethical Committee of Kırıkkale University (document number: 2019/110).

Sample collection and heavy metal analysis: Blood samples taken from *vena saphena medialis* into the heparinized test tubes were centrifuged at 1000 g for 10 min at 4 °C to obtain plasma and erythrocytes. Plasma samples were analyzed using inductively coupled plasma optical emission spectrometer (ICP-OES, Spectroblue, Germany) for Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, vanadium (V), Zn. Then the measured values were quantified by using calibration curves plotted from analytical standards (Merck, Germany) as previously described by Aluc and Ekici (3). The rest of the plasma and hemolysates prepared re-suspending of erythrocytes in an equal volume of phosphate-buffered solution (PBS, pH 7.4), were stored at −80° C for further analysis of the oxidant and antioxidants.

Evaluation of oxidative status of plasma: Plasma TOC levels of cats were measured via commercial Total Oxidant Status kits (Rel Assay Diagnostic, Türkiye) as previously described by Erel (16), and the results were expressed as µmol H_2O_2 equivalent/L. Plasma TAC level was also measured using commercial Total Antioxidant Status kits (Rel Assay Diagnostic, Türkiye) according to the novel automated method described by Erel (15), in which the results were expressed as mmol Trolox equivalent/L. The TOC/TAC ratio is expressed as oxidative stress index (OSI) (32). It was calculated according to the following formula: OSI (arbitrary unit, AU) = TOC (µmol H_2O_2 equivalent/L) / TAC (µmol Trolox equivalent/L).

Assessment of oxidative status of erythrocytes: The erythrocyte hemolysate was used to determine the level of cellular oxidant and antioxidants. The MDA concentrations, an indicator of lipid peroxidation, were measured as previously described by Buege and Aust (10). The SOD, CAT, and GPX activities of erythrocytes were analyzed with the same named commercial assay kits (Rel Assay Diagnostics, Türkiye) according to the manufacturer's instructions.

Data analysis: Descriptive analysis of all data was performed using SPSS 18.0 package program for Windows. After the distribution of normality of data checked by Shapiro-Wilk test, the effects of age and gender on the measured data were investigated using two-way ANOVA for statistical significance. The non-normally distributed data were analyzed using Mann–Whitney U test as a non-parametric test for young and adult, or male and female groups. $P<0.05$ was considered statistically significant.

Results

Plasma heavy metal accumulation in cats: Plasma heavy metal levels in cats at different ages and sex are shown in Table 1. Arsenic, Co, Cd, and Hg have not included in the table because they were under the detection limit of the used technique in all plasma samples. The limits of detection values of these metals were 1.60, 1.38, 0.79, and 1.60 ppb for As, Co, Cd, and Hg, respectively. It was observed that most of the measured elements were not varied statistically according to age or gender. However, the plasma levels of Cu in adults were significantly higher than the young cats ($P<0.01$). The concentrations of Fe in the plasma of young cats were higher compared to adults ($P<0.01$). Additionally, plasma Se levels in females ($P<0.01$), and V levels ($P<0.001$) in male Angora cats were significantly higher than those of the other gender, respectively.

Table 1. Variations of plasma heavy metal concentrations according to age and gender in Angora cats.

Elements	Groups	n	Mean (mg/L)	SEM	Min.	Max.
Lead (Pb)	Young	16	0.206	0.028	0.149	0.616
	Adult	14	0.174	0.007	0.148	0.232
	Male	17	0.198	0.023	0.149	0.616
	Female	13	0.177	0.010	0.148	0.232
Aluminum (Al)	Young	16	3.380	0.159	2.159	4.578
	Adult	14	3.363	0.092	2.964	4.288
	Male	17	3.449	0.137	2.159	4.578
	Female	13	3.217	0.029	3.092	3.331
Chromium (Cr)	Young	16	0.316	0.049	0.217	0.977
	Adult	14	0.240	0.009	0.210	0.305
	Male	17	0.302	0.040	0.214	0.977
	Female	13	0.237	0.008	0.210	0.282
Copper (Cu)	Young	16	0.495	0.024	0.254	0.638
	Adult	14	0.687**	0.066	0.390	1.462
	Male	17	0.565	0.028	0.254	0.773
	Female	13	0.625	0.100	0.390	1.462
Iron (Fe)	Young	16	2.902**	0.354	1.517	6.804
	Adult	14	1.952	0.305	1.058	5.636
	Male	17	2.452	0.271	1.129	5.636
	Female	13	2.472	0.535	1.058	6.804
Manganese (Mn)	Young	16	0.035	0.004	0.022	0.086
	Adult	14	0.031	0.004	0.021	0.069
	Male	17	0.034	0.004	0.022	0.086
	Female	13	0.033	0.004	0.021	0.056
Nickel (Ni)	Young	16	0.129	0.019	0.092	0.409
	Adult	14	0.099	0.006	0.080	0.145
	Male	17	0.120	0.016	0.084	0.409
	Female	13	0.105	0.007	0.080	0.145
Selenium (Se)	Young	16	0.147	0.090	0.000	1.275
	Adult	14	0.277	0.188	0.000	1.979
	Male	17	0.000	0.000	0.000	0.000
	Female	13	0.622**	0.256	0.000	1.979
Tin (Sn)	Young	17	35.550	1.297	30.133	48.014
	Adult	13	34.425	0.945	31.306	43.232
	Male	16	35.304	1.152	30.133	48.014
	Female	14	34.467	0.859	31.306	38.138
Vanadium (V)	Young	17	0.009	0.002	0.000	0.022
	Adult	13	0.005	0.001	0.000	0.015
	Male	16	0.010***	0.002	0.000	0.022
	Female	14	0.001	0.001	0.000	0.004
Zinc (Zn)	Young	17	0.825	0.128	0.503	2.573
	Adult	13	0.651	0.023	0.546	0.868
	Male	16	0.777	0.104	0.503	2.573
	Female	14	0.676	0.031	0.558	0.868

The detection limits of the used technique for the metals were just like the following; Al: 0.47, As: 1.60, Cd: 0.79, Co: 1.38, Cr: 8.50, Cu: 0.09, Fe: 0.19, Hg: 1.60, Mn: 4.26, Ni: 0.29, Pb: 1.68, Se: 1.87, Sn: 0.13, V: 4.72, Zn: 0.31 ppb. The As, Cd, Co, and Hg were not included the table because they were under the limit of detection. SEM: Standard error mean, Asterisk represents the statistical significance, **: P<0.01, ***: P<0.001.

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Plasma oxidant and antioxidant status of cats: The TOC levels of plasma in young (P<0.001) and male cats (P<0.05) were significantly higher than that of the adult and female cats, respectively (Table 2). The plasma levels of TAC in young cats were significantly (P<0.001) higher than the adults. It was also higher in male cats but not statistically significant (P>0.05) according to females. The OSI of plasma in young cats was remarkably higher than that of adults (P<0.01). It was insignificantly different in males from females (P>0.05).

Erythrocyte oxidant and antioxidant status of cats: As shown in Table 3, the concentrations of MDA in erythrocytes of young cats were significantly higher than in adults (P<0.05). The increased level of MDA in male cats compared to females was not significant (P>0.05). While SOD activity of erythrocytes was higher in youngers than adults (P<0.001), GPX activity was higher in adults than in young cats (P<0.01). Additionally, CAT activity was higher in males than the female (P<0.001). Although GPX activity of female cats was higher than the male, it was not seen statistically significant because of the wide interval of the values.

Table 2. Plasma oxidative stress status of Angora cats according to age and gender.

	Groups	n	Mean	SEM	Min.	Max.
TOC (µmol/L)	Young	16	1.70***	0.13	0.89	2.60
	Adult	14	0.89	0.08	0.49	1.52
	Male	17	1.53*	0.15	0.74	2.60
	Female	13	1.05	0.11	0.49	1.71
TAC (mmol/L)	Young	16	1.61***	0.08	1.00	1.98
	Adult	14	1.30	0.10	0.86	1.84
	Male	17	1.53	0.07	1.00	1.95
	Female	13	1.38	0.13	0.86	1.98
OSI (arbitrary unite)	Young	16	0.11**	0.01	0.05	0.21
	Adult	14	0.07	0.00	0.05	0.10
	Male	17	0.11	0.01	0.05	0.21
	Female	13	0.08	0.00	0.05	0.10

SEM: Standard error mean, Asterisk represents the statistical significance, *: P<0.05, **: P<0.01, ***: P<0.001.

Table 3. Erythrocyte oxidative stress status of Angora cats according to age and gender.

	Groups	n	Mean	SEM	Min.	Max.
MDA (nmol/gHb)	Young	16	288.70*	45.43	114.02	489.34
	Adult	14	199.18	15.62	104.52	318.77
	Male	17	258.57	34.62	114.02	489.34
	Female	13	202.62	15.70	104.52	265.31
SOD (U/ gHb)	Young	16	1898.65***	43.51	1701.65	2064.36
	Adult	14	1639.72	36.18	1449.64	1911.93
	Male	17	1749.93	51.30	1449.64	2064.36
	Female	13	1720.72	50.23	1578.74	1911.93
CAT (U/ gHb)	Young	16	0.59	0.09	0.27	1.01
	Adult	14	0.53	0.08	0.13	0.89
	Male	17	0.68***	0.06	0.27	1.01
	Female	13	0.26	0.05	0.13	0.51
GPX (U/ gHb)	Young	16	3.02	0.45	0.63	5.13
	Adult	14	5.14**	0.37	2.85	7.76
	Male	17	4.01	0.43	0.63	7.76
	Female	13	5.00	0.58	2.85	6.61

SEM: Standard error mean, Asterisk represents the statistical significance, *: P<0.05, **: P<0.01, ***: P<0.001.

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Discussion and Conclusion

Angora cat is one of the special cat breeds grown and lived in Ankara and its surrounding provinces such as Kırıkkale and Çankırı of Türkiye. This study is conducted in Kırıkkale, which is a small industrial region containing arms factories, ammunition supply industry, and oil refineries. It is well known that industrial wastes contaminate environmental sources such as air, soil, and water with many kinds of pollutants including heavy metals. Kızılırmak, the longest river in Türkiye passes through Kırıkkale, and heavy metal contamination in the Kızılırmak river has been shown in several previous studies (7, 11). It has also been reported that some heavy metals were detected in soil, water and feedstuff samples collected from close to industrial areas in Kırıkkale (9). Therefore, in the present study, it was investigated plasma heavy metal concentrations and oxidant-antioxidant status of Angora cats, which drink tap water in Kırıkkale at different age and gender.

According to findings of this study, nonessential metals except Pb were not detected in plasma samples of cats. The levels of lead and most of the trace elements were varied, but not significant in cats' plasma at different age and gender as seen in Table 1. However, Cu concentration increased while Fe decreased by aging. Simsek et al. (27) has previously reported that serum Al, V, Mn, Ni, As and Sn levels were significantly higher in adult Angora goats that bred in Çankırı province compared to young goats while Cr, Fe, Co, Cu, Zn, Se, Cd, and Pb were similar levels in both. However, Esposito et al. (17) showed that Pb concentrations in the liver and kidney of stray cats in Naples, Italy were decreased by the age while Cd concentrations were increased. In another study, it was reported that while most of the metal levels accumulated in various organs and tissues were significantly higher in the adults than in the chicks, Ni and Pb in the brain, skeletal muscles from pectoral and femoral regions, liver, kidney and skin, and Cu in liver were seen lower concentrations in the adults compared to White Egret chicks (19). The only metal accumulated in the plasma by the age was Cu in our study. This situation was compatible with the findings of Doong et al. (12) who have reported that plasma Cu level in adult cats was higher than that of the kitten.

Heavy metal deposits can also differ according to gender. In this study, it was observed that plasma Se level in female cats were significantly higher than in males and, V levels in males were significantly higher than that of female cats. Altunok et al. (2) have shown that Ba, Al, Cu, Mn, and Sr were detected in high levels in female Van cats than in males, and none of the metals were differed statistically by the age as in Fe which was high level in young. Alternatively, Al-Kalidi et al. (1) demonstrated that the plasma Cu levels of female home cats was significantly higher than that of males while Fe levels did not differ according to gender. In accordance with our results, high Se levels in hair samples have been detected in female healthy cats compared to males (6). Altunok et al. (2) have also showed that serum Se level was higher in female Van cats compared to males, but was statistically insignificant. In another study, V level in the hair samples of male cats was higher compared to females (29), which is in agreement with our findings in terms of plasma V levels.

Exposure to inappropriate concentrations of heavy metals may have adverse effects even if they are required for several physiological processes (20). Although the detected concentrations of these metals in Angora cats are not high enough to cause acute or serious toxic effects, possible chronic toxicity cannot be ignored, since these chemicals can accumulate in the body over time. On the other hand, the measured levels of metal may be related to metal contamination of tap water, which can be affected by location. The study conducted by Behrooz and Poma (8) also supports this sight, in which different heavy metal levels were detected in plasma samples of wild cats taken from different regions.

Heavy metals may also lead to oxidative damages in the body (13). Although heavy metal levels did not appear high in young cats, the results of TOS, MDA and OSI show that the young cats have taken tap water had higher oxidative stress than their older ones in this study. It was seen that the TAC and SOD levels were also increasing against increased oxidative stress in young cats. A similar study conducted by Simsek et al. (26) showed that the concentration of MDA was higher in adult Angora goats compared to young goats, and SOD activity of goat erythrocytes was decreased by the age. Mitochondrial SOD plays a major role in defense mechanisms against oxidants. However, it is well known that the concentrations of mitochondrial antioxidants decrease by age (23). This situation may be the responsible lower level of SOD activity in adult cats.

Destructive effects of oxidative stress on biological macromolecules are inhibited by endogenous antioxidants such as GPX and CAT. Catalase is a heme protein located in peroxisomes and converts H_2O_2, generated in the cytosol or peroxisomes, to water and oxygen while GPX is protective against lipid peroxides (23). There are different reports of variations of enzymatic antioxidant activity according to age. For example, Tekeli et al. (31) have showed that serum MDA levels, and GSH and GPX activity of erythrocytes in Saanen goats were increased while the CAT activity was decreased by the age. In another study, it was found an age-related decrease in SOD activity while GPX increase in humans (22). Aydilek and Şimşek (5) have also reported increased GPX activity in mares and Gaál et al. (18) have revealed increased SOD

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and GPX activity in dogs, while the latter researchers have showed decreased GPX and CAT activity in cows during aging as well. However, in the present study, adult cats had similar CAT and higher GPX activity compared to young cats in compliance with some previous studies. It is thought that oxidative stress during aging may increase the levels of GHS and enzymatic activities of GPX (28). Despite all the efforts, we have not found any study examining the heavy metal-oxidant and antioxidant relationship in cats or dogs depending on age or gender, and we think that our findings may be useful for future studies in this regard.

Finally, this study specified the age- and sex-related variations of some heavy metals and oxidant-antioxidant capacities of Angora cats given tap water and lived in Kırıkkale. It was revealed that toxic heavy metals such as As, Cd, Hg, and Co were not detected in plasma samples, and the levels of some others (Cu, Fe, Se, and V) changed according to age or gender. Additionally, young cats had higher levels of TOS, OSI, MDA, and SOD, whereas lower GPX activity compared to adults. Only CAT activity was differed by gender. In conclusion, metals, especially trace elements, are required for many kinds of physiological processes and the synthesis of antioxidant enzymes. Therefore, it can be suggested that the periodic measurement of metals and the addition of common antioxidant supplements to the diet of adult Angora cats will support weakening antioxidant mechanisms by the age.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

RK, AAY and HE conceived and planned the experiments. RK, YA and EK collected blood and plasma samples and carried out the experiments. YA and HE measured heavy matal concentratios of samples. RK and AAY analyzed the samples for oxidative stress parametes. RK, AAY and HE contributed to the interpretation of the results. RK and AAY took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Local Ethical Committee of Kırıkkale University with the document number of 2019/3-21.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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