The Thai Journal of Veterinary Medicine

Volume 51
Issue 3 *September, 2021*

Article 17

9-1-2021

Association of clinical parameters and a formula for predicting the total antioxidant power in cats

Kanissarinn Sakundech

Worapol Aengwanich

Original Article

Association of clinical parameters and a formula for predicting the total antioxidant power in cats

Kanissarinn Sakundech[1] Worapol Aengwanich[1*]

Abstract

The objective of this study was to investigate external appearances, vital signs, hematological parameters, malondialdehyde and total antioxidant power to find correlations and to predict total antioxidant power in cats. Body condition scores, mucous membrane color scores, heart rate, respiratory rate, body temperature, hematological parameters, malondialdehyde and ferric reducing antioxidant capacity of 27 cats were measured. Correlation and multiple regression models were analyzed. The results revealed the following information: respiratory rate vs. heart rate, packed cell volume vs. hemoglobin, total red blood cell vs. packed cell volume, total red blood cell vs. hemoglobin, mean corpuscular hemoglobin vs. mean corpuscular volume, total white blood cell vs. neutrophil, mean corpuscular hemoglobin concentration vs. mean corpuscular hemoglobin, mean corpuscular volume vs. lymphocyte and total white blood cell vs. monocyte were significant positive correlation between pairs of these parameters (P<0.05), while mucous membrane color scores vs. mean corpuscular volume, mean corpuscular hemoglobin concentration vs. lymphocyte, neutrophil vs. lymphocyte, platelet vs. mean corpuscular volume, platelet vs. mean corpuscular hemoglobin demonstrated were significant negative relation between pairs of these parameters (P<0.05), respectively. A formula for predicting the total antioxidant power was obtained based on the measured levels of FRAP. Measured levels and values calculated using the formula were not significantly different (P>0.05). These results indicated that there was a correlation between physical examination and hematological parameters. Lastly, total antioxidant power in cats could be predicted by using some hematological values.

Keywords: Total antioxidant power, malondialdehyde, physical examination, hematology

[1]*Stress and Oxidative Stress Research Unit, Faculty of Veterinary Sciences, Mahasarakham University, Mahasarakham 44000, Thailand*

****Correspondence**: worapol.a@msu.ac.th* (W. Aengwanich)

Received February 11, 2021.
Accepted May 14, 2021.
doi: https://doi.org/10.14456/tjvm.2021.67

Introduction

Oxidative stress results from an oxidant/antioxidant imbalance (Kayar *et al*., 2015). Intracellular and extracellular macromolecules (i.e., proteins, lipids, and nucleic acids) can suffer oxidative damage (Lim and Lim, 2013). Lipid peroxidation is one of the most-commonly reported indices of oxidative stress (Moselhy *et al*., 2013). Malondialdehyde is a lipid peroxidation product and is a reliable marker that signals oxidative stress in clinical situations (Ayala *et al*., 2014). Generally, free radicals are removed or inactivated *in vivo* by a coordinated sequence of antioxidants. The body's antioxidant defense system is comprised of antioxidant enzymes, transition metal binding proteins, and hormones. Other antioxidants of dietary origin include vitamins, polyphenols, and carotenoids. Blood contains many antioxidants that inhibit or limit oxidative damage. The most common laboratory test of "total antioxidant power" is ferric reducing antioxidant power (FRAP) (Lim and Lim, 2013). This method has been used to measure the ability of plasma to withstand the oxidative effects of reactive species (Benzie and Strain, 1996). The FRAP method, however, has its own limitations, especially for measurements under non-physiological pH values. In addition, this method is unable to detect slowly-reacting polyphenolic compounds and thiols (Moniruzzaman *et al*., 2012).

Body condition scores are used for many purposes such as to indicate evaluated nutritional status (Sapowicz *et al*., 2016), survival and lifespan (Teng *et al*., 2018[a]), and are used for diagnosis of some abnormalities or diseases (Teng *et al*., 2018[b]). Normal mucous membrane color should be pale pink to pink but abnormal mucous membrane color such as pale, muddy, and white mucous membranes are commonly caused by poor peripheral perfusion, including vasoconstriction from shock or anemia (Norkus, 2018). Vital signs, ie respiration and heart rates are the most critical objective parameters of the physical examination. A significant increase or decrease in the respiration or heart rate of the cat may be a sign of a major illness. Besides, heart rate is also commonly used as an emotion-related physiological indicator for assessing the mental state of cats (Wang *et al*., 2020). Furthermore, body temperature is frequently measured to assess the health status of cats (Levy *et al*., 2015). Hematological parameters are considered to reflect the general health status of animals, including physiological condition and the function of important organs and are commonly applied to evaluate the trend of physiological status (Hwang *et al*., 2015).

In this study, we hypothesize that physical examination, hematological parameters, malondialdehyde and total antioxidant power are correlated and could be used for predicting the total antioxidant power in cats. Therefore, the objective of this study was to examine physical examination, hematological parameters, malondialdehyde and total antioxidant power for analyzing correlation and formula for predicting total antioxidant power. Formula from this study might be used for prediction of total antioxidant status of cats in cases of working in the field in situations without access to a laboratory.

Materials and Methods

This study was approved by the Institution's Ethics Committee on Animal Experimentation of Mahasarakham University (license number: IACUC-MSU-004/2020). All procedures were performed with the owner's consent.

Animals and FIV/FeLV testing: The test group for this study consisted of sixty-two domestic short hair cats, 1 to 3 years old. Cats were obtained from households and shelters in Maha Sarakham province, Thailand. The nature of measured laboratory data of cats is shown in Table 1. All cats were fed with commercial foods. Cats were evaluated for FIV/FeLV infection at the Veterinary Teaching Hospital, Faculty of Veterinary Sciences, Mahasarakahm University, Thailand between May – June 2020 by an immunochromatographic assay (Anigen Rapid FIV Ab/FeLV Ag Test Kit, Korea). Cats that tested FIV/FeLV-positive were eliminated from the study. After testing for FIV/FeLV infection, it was found that twenty-seven cats (3.93±1.03kg) tested negative. All cats underwent a body condition score and mucous membrane color score evaluation, vital signs investigation, and then blood was collected for laboratory analysis.

Table 1 Collection of laboratory data of cats.

Parameters	Cats (n=27)		Total
	Male (n=13)	Female (n=14)	
1. Reproductive status	8*/13	4**/14	12***/27
2. Pregnant/ lactating	-	none	none
3. External/ internal parasite	none	none	none
4. Drug administration	none	none	none
5. Vaccination	none	none	none

* Castration; ** Spay; *** Neuter

Physical examination: Body condition score and mucous membrane color score evaluation and vital signs examination were performed in a laboratory.

Body condition score: The body condition score of the cats was determined using a 9-point scale with body composition as reported by Bjornvad *et al*. (2011).

Mucous membrane color score: Mucous membrane color score was determined with a method adapted from the explanation of Englar (2019). Scores were recorded as follows: White mucous membrane = 0, pale pink mucous membrane = 1 and pink mucous membrane = 2.

Body temperature: Each cat was acclimatized to the temperature in the room for 30 mins before measurements were taken. Auricular infrared thermometers (Polygreen KI-8170, Germany) were positioned in the ear canal descending to the eardrum, then activated to provide readings within seconds (Humm and Kellett-Gregory, 2016).

Respiratory rate: The rate of breathing was observed when the cat was resting. One breath was counted every time the

chest rose and fell (up and down equals one breath) (Duguma, 2016). The number of breaths in one minute was counted.

Heart rate: Heart rate was determined by placing a stethoscope (3M™ Littmann® Cardiology III™ Stethoscope) to the left side of the chest and counting how many pulses in 60 seconds to obtain the number of beats per minute (Taylor, 2020).

Blood sample collection, hematological and biochemical analysis

Blood sample collection: 4 mL of blood samples of experimental groups were taken from a cephalic vein using a butterfly needle into vacuum ethylenediaminetetraacetic acid (EDTA) (for hematological analysis) and vacuum heparin tubes. The vacuum heparin tubes were centrifuged using a refrigerated centrifuge (Hettich Rotina 380R, Andreas Hettich GmbH & Co. KG, Germany) at 2500 rpm for 5 mins. The obtained heparinized plasma was frozen in cryotubes and stored in a freezer at −20 °C before biochemical (malondialdehyde and FRAP) analysis.

Hematological analysis: Blood samples with added EDTA as an anticoagulant were analyzed by a IDEXX ProCyte Dx Hematology Autoanalyzer (IDEXX Laboratories, Inc., USA) for RBC count, white blood cell (WBC) count, platelets, hematocrit and hemoglobin. Blood films were prepared, fixed with methanol and stained with Giemsa-Wright solution, and then used for a white blood cell differential count.

Biochemical analysis

Plasma malondialdehyde: Malondialdehyde in plasma was investigated using the following procedure. A 0.01 mL sample was assayed by the addition of 3 mL (0.05 mol/L) of HCl (QRëC™) and 1 mL (0.67%) of thiobarbituric acid (Fluka). The mixtures were heated for 30 mins at 100 °C, cooled with running tap water and then 4mL of n-butyl alcohol (QRëC™) was added. The mixture was shaken in a vortex mixer and centrifuged using a refrigerated centrifuge (Hettich Rotina 380R, Andreas Hettich GmbH & Co. KG, Germany) at 3,000 rpm (1008Xg) for 10 mins. The absorbance at 532 nm was measured by a Tecan Infinite® 200 Microplate Reader (Tecan Trading AG, Männedorf, Switzerland) and compared with that of a 1,1,3,3 tetramethoxypropane (Aldrich) standard.

Plasma total antioxidant power: Plasma total antioxidant power was evaluated using the ferric reducing ability of plasma (FRAP) assay. The procedure was as follows; 300 mmol/L of acetate (Ajax Finechem Pty. Ltd.) buffer (pH 3.6), 10 mmol/L of 2,4,6-tri-pyridyl-s-triazine (Aldrich) in 40 mmol/L of HCl (QRëC™); and 20 mmol/L of FeCl3.6H2O (*PanReac AppliChem*) were prepared. 20 mL of acetate (Ajax Finechem Pty. Ltd.) buffer, 2.5 mL of 2,4,6-tri-pyridyl-s-triazine (Aldrich) and 2.5 mL of FeCl3.6H2O (Ajax Finechem Pty. Ltd.) yielded the working FRAP reagent and then 0.5 mL of plasma, 0.5 mL of deionized distilled water and working FRAP reagent were mixed. After exactly 6 minutes at room temperature, the absorbance at 593 nm was measured by Tecan Infinite® 200 Microplate Reader (Tecan Trading AG, Männedorf, Switzerland). FeSO4.7H2O (Ajax Finechem Pty. Ltd.) at 100-1000 µmol/L was used as the standard.

Statistical analysis: Data was prepared with Excel software. The correlation analyses for estimating the level of association between quantitative variables were performed using Pearson's correlation test. A *p*-value <0.05 was accepted as significant. The multiple regression model was analyzed by using the stepwise method for selection of significant independent variables ($P < 0.05$).

Results

Body condition scores, mucous membrane color scores, vital signs, hematological parameters, malondialdehyde and ferric reducing antioxidant power in cats were investigated. Then the correlation and multiple regression model were analyzed. The results revealed the following information: Respiratory rate vs. heart rate, packed cell volume vs. hemoglobin, total red blood cell vs. packed cell volume, total red blood cell vs. hemoglobin, mean corpuscular hemoglobin vs. mean corpuscular volume, total white blood cell vs. neutrophil, mean corpuscular hemoglobin concentration vs. mean corpuscular hemoglobin, mean corpuscular volume vs. lymphocyte and total white blood cell vs. monocyte were significant positive correlation between a pair of parameters ($P<0.05$), while mucous membrane color scores vs. mean corpuscular volume, mean corpuscular hemoglobin concentration vs. lymphocyte, neutrophil vs. lymphocyte, platelet vs. mean corpuscular volume, platelet vs. mean corpuscular hemoglobin demonstrated significant negative relation between pairs of parameters ($P<0.05$), respectively (Table 2). The formula for predicting total antioxidant capacity in cats showed in Table 3.

A reverse test was used to check the reliability of the formula by using packed cell volume and mean corpuscular hemoglobin concentration from laboratory measurement for calculation of the FRAP level of each cat. A test was performed to check the normal distribution of FRAP data from laboratory investigation and values calculated using the formula. Data were analyzed using a *t*-test. The level of significance was determined at $P<0.05$ and it was found that mean levels of FRAP from laboratory measurement and values obtained by calculation using formula were not significantly different ($P>0.05$) (Table 3).

Table 2 Correlation between pair of some external appearance, vital signs and hematological parameters in cats.

Parameters	*P*-value	Pearson's correlation coefficient between parameters (r)
1. Heart rate vs. Respiratory rate	0.0417	0.3946
2. Packed cell volume vs. Hemoglobin	<0.001	0.9974
3. Total red blood cell vs. Packed cell volume	<0.001	0.9107
4. Total red blood cell vs. Hemoglobin	<0.001	0.9070
5. Mucous membrane color scores vs. Mean corpuscular volume	0.0323	-0.4130
6. Mean corpuscular hemoglobin vs. Mean corpuscular volume	<0.001	0.7407
7. Total white blood cell vs. Neutrophil	<0.001	0.9607
8. Mean corpuscular hemoglobin concentration vs. Mean corpuscular hemoglobin	0.0021	0.5650
9. Mean corpuscular volume vs. Lymphocyte	0.0230	0.4360
10. Mean corpuscular hemoglobin concentration vs. Lymphocyte	0.0334	-0.4105
11. Neutrophil vs. Lymphocyte	0.0191	-0.4653
12. Total white blood cell vs. Monocyte	0.0256	0.4289
13. Platelet vs. Mean corpuscular volume	0.0110	-0.5996
14. Platelet vs. Mean corpuscular hemoglobin	0.0321	-0.5206

Table 3 Comparison of FRAP from laboratory measurement with value calculated from packed cell volume and mean corpuscular hemoglobin concentration of cats using the formula.

Parameter	Laboratory measurement (n=27)	Calculation using formula (FRAP (mmol/l) = 1.97390 -0.00706*PCV (%)-0.03179*MCHC(g/dl)) (MSE=0.00835; r^2=0.32), packed cell volume and mean corpuscular hemoglobin concentration data of each cats (n=27)	*P*-value
FRAP*(mmol/l)	0.7109±0.0237	0.6795 ± 0.0154	0.2732

FRAP*=mean±SE
FRAP= ferric reducing antioxidant power; SE=standard error; PCV= packed cell volume; MCHC= mean corpuscular hemoglobin concentration

Discussion

Generally, the cardiac and respiratory systems are important for gas exchange. These two systems involve the autonomous nervous system and act via a variety of mechanical and neuronal regulatory mechanisms. This modulation is known as respiratory sinus arrhythmia, which manifests itself through the number of heart beats per breath changing according to the respiratory cycle, with the heart rate increasing during inspiration and decreasing during expiration (Perry *et al*., 2019). The relationship between heart rate and respiratory rate in our study were positively correlated and it was in accordance with the report of Perry *et al*. (2019), who explained that the peripheral nervous regulation is considered to be the predominant factor. Determining blood parameters was helpful in assessing the health status of animals. Many reports have found that these parameters had correlation such as Turkson and Ganyo (2015) and Velguth *et al*. (2010) reported that packed cell volume correlated with hemoglobin. McManus *et al*. (2009) also found that packed cell volume correlated with hemoglobin, and that total red blood cell, total white blood cell, mean corpuscular volume, and mean corpuscular hemoglobin concentration; total red blood cell correlated with hemoglobin, total white blood cell, mean corpuscular volume and mean corpuscular hemoglobin concentration; hemoglobin correlated with total white blood cell, mean corpuscular volume and mean corpuscular hemoglobin concentration; total white blood cell correlated with mean corpuscular volume and mean corpuscular hemoglobin concentration; mean corpuscular volume correlated with mean corpuscular hemoglobin concentration, respectively. In addition, Tiwari *et al*. (2013) reported that hemoglobin was correlated with mean corpuscular volume.

In the present study, we found that pairs of hematological parameters in cats which had correlations, i.e., packed cell volume vs. hemoglobin, total red blood cell vs. packed cell volume and total red blood cell vs. hemoglobin were similar to reports of Turkson and Ganyo (2015), McManus *et al*. (2009), Tiwari *et al*. (2013) and Velguth *et al*. (2010). Whereas, the correlation between other parameters, (i.e., mucous membrane color scores vs. mean corpuscular volume, mean corpuscular hemoglobin vs. mean corpuscular volume, total white blood cell vs. neutrophil, mean corpuscular hemoglobin concentration vs. mean corpuscular hemoglobin, mean corpuscular volume vs. lymphocyte, mean corpuscular hemoglobin concentration vs. lymphocyte, neutrophil vs. lymphocyte, total white blood cell vs. monocyte, platelet vs. mean corpuscular volume and platelet vs. mean corpuscular hemoglobin of cats) found in this study has not been previously reported. Malondialdehyde and total antioxidant power of cats in this study were not correlated with other parameters.

After analyzing body condition scores, mucous membrane color scores, vital signs, hematological values, malondialdehyde and total antioxidant power by using multivariate regression, we found a simple formula that

only used packed cell volume and mean corpuscular hemoglobin concentration for calculation of total antioxidant power. However, the r^2 value of this formula may not be very high. This may be explained because the FRAP value is difficult to measure and has low correlation with other observations. In addition, the cat population may influence the r^2 value as an increased population sampled might increase the r^2 value. When comparing FRAP levels, measured in the laboratory and that calculation using a formula, it was found that the FRAP levels were not different. Readers might consider using this formula carefully when a laboratory is not available, or when working in the field. In addition, this formula was obtained from only healthy cats, but other conditions such as stress, oxidative stress, some abnormalities and diseases should be further studied.

In conclusion, Body condition scores, mucous membrane color scores, vital signs, hematological values, malondialdehyde and total antioxidant power of cats were determined. Correlations were then analyzed and multiple regression models were evaluated and found that had a correlation occurred between heart rate vs. respiratory rate; mucous membrane color scores vs. mean corpuscular volume; some red blood cell parameters; some white blood cell parameters; some red blood cell parameters and white blood cell parameters; and platelet with some red blood cell parameters, respectively. Furthermore, a formula for predicting the total antioxidant power of cats was obtained.

Acknowledgements

This research was supported by Faculty of Veterinary Sciences, Mahasarakham University, Thailand (grant numbers: 006/2563). We would like to thank Dr. Wootichai Kenchaiwong and Assoc. Prof. Dr. Bungon Kumphon for statistical support.

References

Ayala A, Muñoz MF and Argüelles S 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev. Volume 2014, Article ID 360438. https:// doi.org/10.1155/2014/360438

Benzie IFF and Strain JJ 1996. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay. Anal Biochem. 239: 70–76.

Bjornvad CR, Nielsen DH, Armstrong PJ, McEvoy F, Hoelmkjaer KM, Jensen KS, Pedersen GF and Kristensen AT 2011. Evaluation of a nine-point body condition scoring system in physically inactive pet cats. Am J Vet Res. 72: 433–437.

Duguma A 2016. Practical manual on veterinary clinical diagnostic approach. J Vet Sci Technol. 7: 337. https://doi.10.4172/2157-7579.1000337

Englar RE 2019. Common Clinical Presentations in Dogs and Cats. New Jersey: John Wiley & Sons, Inc. 1040 pp.

Humm K and Kellett-Gregory L 2016. Monitoring small animal patients in the intensive care unit. In Pract. 38 (Suppl 4):12–17.

Kayar A, Dokuzeylul B, Kandemir FM, Kirbas A, Bayrakal A and Or ME 2015. Total oxidant and antioxidant capacities, nitric oxide and malondialdehyde levels in cats seropositive for the feline coronavirus. Vet Med-Czech. 60: 274–281.

Levy JK, Nutt KR and Tucker SJ 2015. Reference interval for rectal temperature in healthy confined adult cats. J Feline Med Surg. 17: 950–952.

Lim CSH and Lim SL 2013. Ferric reducing capacity versus ferric reducing antioxidant power for measuring total antioxidant capacity. Lab Med. 44: 51–55.

McManus C, Paludo GR, Louvandini H, Gugel R, Sasaki LCB and Paiva SR 2009. Heat tolerance in Brazilian sheep: Physiological and blood parameters. Trop Anim Health Prod. 41: 95–101.

Moniruzzaman M, Khalil MI, Sulaiman SA and Gan SH 2012. Advances in the analytical methods for determining the antioxidant properties of honey: A review. Afr J Tradit Complement Altern Med. 9: 36–42.

Moselhy HF, Reid RG, Yousef S, and Boyle SP 2013. A specific, accurate, and sensitive measure of total plasma malondialdehyde by HPLC. J Lipid Res. 54: 852–858.

Norkus CL 2018. Veterinary Technician's Manual for Small Animal Emergency and Critical Care. 2nd ed. Pennsylvania: Wiley Blackwell. 640 pp.

Perry S, Khovanova NA and Khovanov IA 2019. Control of heart rate through guided high-rate breathing. Sci Rep. 9, 1545 (2019). https://doi.org/10.1038/s41598-018-38058-5

Sapowicz SA, Linder DE and Freeman LM 2016. Body condition scores and evaluation of feeding habits of dogs and cats at a low cost veterinary clinic and a general practice. Sci World J. Article ID 1901679. https://doi.org/10.1155/2016/1901679

Taylor SM 2020. Small Animal Clinical Techniques. Missouri: Elsevier. 320 pp.

Teng KT, McGreevy PD, Toribio JAML, Raubenheimer D, Kendall K, Dhand NK 2018[a]. Strong associations of nine-point body condition scoring with survival and lifespan in cats. J Feline Med Surg. 20: 1110–1118.

Teng KT, Toribio JALML, Raubenheimer D, Kendall K and Dhand NK 2018[b]. Associations of body condition score with health conditions related to overweight and obesity in cats. J *Small* Anim Pract. 59: 603–615.

Tiwari LM, Kotwal CJ, Kotwal A, Mishra MP, Dutta BV and Chopra BS 2013. Correlation of haemoglobin and red cell indices with serum ferritin in Indian women in second and third trimester of pregnancy. Med J *Armed Forces India*. 69: 31–36.

Turkson PK and Ganyo EY 2015. Relationship between haemoglobin concentration and packed cell volume in cattle blood samples. *Onderstepoort* J Vet Res. 82(1), Art. #863, 5 pages. http:// dx.doi.org/10.4102/ojvr. v82i1.863

Velguth KE, Payton ME and Hoover JP 2010. Relationship of hemoglobin concentration to packed cell volume in avian blood samples. J Avian Med Surg. 24:115–121.

Wang P, Ma Y, Liang F, Zhang Y, Yu X, Li Z, An Q, Lv H and Wang J 2020. Non-contact vital signs monitoring of dog and cat using a UWB radar. Animals. 10(2): 205, https://doi.10.3390/ ani10020205