

# Investigation of changes in serum thiols and neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and mean platelet volume/platelet count ratio indices in cats undergoing ovariohysterectomy

Investigación de los cambios en los tioles séricos y la proporción de neutrófilos a linfocitos, la proporción de monocitos a linfocitos, la proporción de plaquetas a linfocitos y los índices promedio de volumen de plaquetas/recuento de plaquetas en gatas sometidas a ovariohisterectomía

Tuğra Akkuş<sup>1\*</sup> , Mehmet Ekici<sup>2</sup> 

<sup>1</sup>Harran University, Faculty of Veterinary Medicine, Department of Veterinary Obstetrics and Gynaecology, Sanliurfa, Turkey.

<sup>2</sup>Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Veterinary Physiology, Sivas, Turkey.

\*Corresponding author: [tugraakkus08@hotmail.com](mailto:tugraakkus08@hotmail.com)

## ABSTRACT

The aim of this study was to investigate complete blood indices [neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR) and mean platelet volume/platelet count ratio (MPV/PLT)] and thiols (native and thiol) in cats undergoing ovariohysterectomy (OHE). The study sample comprised a total of 10 female cats of mixed breeds. Ovariohysterectomy operation was performed under Ketamine/Xylazine anesthesia with the appropriate technique. Blood samples were collected from the cephalic vein pre-OHE and post-OHE at 2 h, 24 h, and 7 d. Total and native thiol levels in the serum were analyzed using a colorimetric technique, and whole blood analysis was carried out using anticoagulant tubes. White Blood Cell (WBC) and neutrophil increased significantly at 2 h post-OHE, peaked at 24 h post-OHE, then returned to baseline levels by 7 d ( $P<0.001$ ). The lymphocyte and thiols decreased at 2 h, 24 h, and 7 d post-OHE, and the lowest lymphocyte count was observed at 24 h ( $P<0.001$ ). The lowest monocyte count was observed on d 7 post-OHE ( $P<0.001$ ). NLR increased significantly 2 h after OHE, peaked at 24 h after OHE, and remained high on d 7 ( $P<0.001$ ). MLR increased at 2 h, peaked at 24 h, then returned to baseline levels on d 7 ( $P<0.001$ ). PLR increased gradually and peaked at 24 h and on d 7 ( $P<0.001$ ). MPV/PLT after OHE was statistically lowest on d 7 ( $P<0.05$ ). A negative correlation was determined between NLR, MLR, PLR, and thiols ( $P<0.001$ ). In conclusion, thiols and complete blood indices (NLR, MLR, and PLR) may be important in the assessment of inflammation and stress responses after OHE in cats.

**Key words:** Monocyte-to-lymphocyte ratio; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; ovariohysterectomy

## RESUMEN

El objetivo de este estudio fue investigar los índices sanguíneos completos [proporción de neutrófilos a linfocitos (NLR), proporción de monocitos a linfocitos (MLR), proporción de plaquetas a linfocitos (PLR) y proporción media de volumen de plaquetas/recuento de plaquetas (MPV/PLT)] y tioles (nativos y tiol) en gatas sometidas a ovariohisterectomía (OHE). La muestra del estudio estuvo compuesta por un total de 10 gatas de razas mixtas. La operación de ovariohisterectomía se realizó bajo anestesia con ketamina/xilazina con la técnica adecuada. Se recogieron muestras de sangre de la vena cefálica antes de la OHE y después de la OHE a las 2 h, 24 h y 7 d. Los niveles de tiol total y nativo en el suero se analizaron mediante una técnica colorimétrica, y el análisis de sangre total se realizó utilizando tubos con anticoagulante. Los glóbulos blancos (WBC) y los neutrófilos aumentaron significativamente a las 2 h posteriores a la OHE, alcanzaron su punto máximo a las 24 h posteriores a la OHE y luego regresaron a los niveles iniciales a los 7 d ( $P<0,001$ ). Los linfocitos y tioles disminuyeron a las 2 h, 24 h y 7 d después de la OHE, y el recuento de linfocitos más bajo se observó a las 24 h ( $P<0,001$ ). El recuento de monocitos más bajo se observó el d 7 después de la OHE ( $P<0,001$ ). NLR aumentó significativamente 2 h después de OHE, alcanzó su punto máximo a las 24 h después de OHE y permaneció alto el d 7 ( $P<0,001$ ). MLR aumentó a las 2 h, alcanzó su punto máximo a las 24 h y luego volvió a los niveles de referencia el d 7 ( $P<0,001$ ). PLR aumentó gradualmente y alcanzó su punto máximo a las 24 h y el d 7 ( $P<0,001$ ). MPV/PLT después de OHE fue estadísticamente más bajo en el d 7 ( $P<0,05$ ). Se determinó una correlación negativa entre NLR, MLR, PLR y tioles ( $P<0,001$ ). En conclusión, los tioles y los índices de sangre completa (NLR, MLR y PLR) pueden ser importantes en la evaluación de las respuestas de inflamación y estrés después de la OHE en las gatas.

**Palabras clave:** Proporción de monocitos a linfocitos; proporción de neutrófilos a linfocitos; proporción de plaquetas a linfocitos; ovariohisterectomía

## INTRODUCTION

Surgical sterilization (ovariohysterectomy, OHE) is the most widely practiced and chosen procedure for controlling the pet population [1]. Ovariohysterectomy in small animal veterinary surgery includes the removal of sex hormone-producing organs and the uterus, and the most important reasons include preventing unwanted pregnancies, reducing the risk of developing mammary tumors, pyometra, and eliminating estrus and related problems [2]. Surgical stress results from psychological stress, tissue damage and circulatory changes, anesthetic agents, and complications before, during, and after surgery, including sepsis. Surgery-related stress responses stimulate the sympathoadrenal medullary and hypothalamic-pituitary-adrenal axis, which results in post-traumatic endocrine and immunomodulatory alterations [3, 4]. The stress response is a physiological response to trauma or surgery and is generally considered to be proportional to the degree of surgical trauma [5].

The evaluation of hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in animals. Analysis of blood indices has proven to be a valuable approach to analyze the health status of animals and these indices provide reliable information about health and stress status [6]. Hematological biomarkers such as neutrophil, lymphocyte and neutrophil/lymphocyte percentage in humans and animals are widely used for the evaluation of inflammation and stress response [7, 8].

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of reactive oxygen species (ROS), and trauma from a surgical procedure can contribute to oxidative stress [9]. Under increased oxidative stress, thiol levels decrease to neutralize ROS, and in this condition, the sulfhydryl groups of thiols play an important role [10]. During oxidative stress, reversible formation of disulfide bonds between protein thiols and low molecular weight thiols is observed. These bonds can be reduced back to thiols to maintain thiol/disulfide homeostasis [11]. It is well known that the extracellular space has a relatively more oxidized redox state than the interior of the cell under physiological conditions. The extracellular supply of thiols is critical for maintaining the redox state of the extracellular space [12]. Protein-SH groups are important circulating antioxidant defences [13]. During surgery, the extracellular redox state may change, and the sulfhydryl group of thiols may undergo oxidation reactions. From a review of literature, no study could be found that has investigated the effect of OHE on plasma thiol/disulfide levels in domestic cats (*Felis catus*). The aim of this study was to determine the ratio of serum thiol levels and complete blood indices neutrophil/lymphocyte ratio (NLR), monocyte/lymphocyte ratio (MLR), platelet/lymphocyte ratio (PLR) and mean platelet volume/platelet count (MPV/PLT) in domestic cats undergoing OHE and to reveal the correlation between them.

## MATERIALS AND METHODS

This study was conducted with the permission of Harran University Animal Experiments Local Ethics Committee (HRU-HADYEK)(decision no: 2022/006, dated 07/09/2022).

### Selection of animals

The cats comprising the study material were taken to Harran University Veterinary Faculty Animal Hospital with a request for sterilization by the owner. The study group was formed of a total of 10

domestic cats of various breeds, aged 1-3 years, each weighing 2.75-3.34 (2.99±0.19) kg, which had never given birth, were healthy with no signs of disease, had routine care and feeding procedures, complete vaccinations and parasitic practices, and were housed in similar conditions. General clinical examinations of the cats were performed and recorded. The study exclusion criteria were defined as aggression, cardiac arrhythmia on auscultation, pregnancy, breastfeeding, obesity (body condition score >7 on a scale of 1 to 9) [14], anemia (hematocrit <30%), or any symptoms of clinical disease. Access to food and water was restricted 8-12 h before the operation. Operations of 10 cats were performed on different d. The average operation time was 15-20 min.

### Anesthesia and ovariohysterectomy operation

All cats undergoing OHE were injected intramuscularly (IM) at a dose of 1 mg·kg<sup>-1</sup> with Xylazine hydrochloride (Rompun 2%®, Bayer) for sedation. Approximately 10 min later, Ketamine (Ketasol 10%®, Richter Pharma) was administered IM at a dose of 15 mg·kg<sup>-1</sup>. Premedication with atropine administration before anesthesia was not performed due to early awakening and lack of complete induction. The OHE operation was performed on all cats by the same experienced veterinarian using the ventral midline approach technique with a 1 cm incision line [15]. No undesirable complications were encountered in all of the cats during the operation. Vital parameters (respiration rate, body temperature and pulse rate) were monitored in all the cats before, during and after the operation (0, 2, 24 h and 7 d).

### Collection of blood and plasma samples

A pediatric intravenous cannula (24G, yellow) was placed in the cephalic vein in the sedated cats to collect the blood samples. Catheter patency was provided with heparinized saline (20 UI·mL<sup>-1</sup>) and the catheter was fixed to the skin with an elastic bandage. The blood samples from the cats were taken into vacuum tubes containing Ethylenediaminetetraacetic acid (K<sub>3</sub>-EDTA), approximately 0.75-1 mL, pre-OHE and post-OHE at 2 h, 24 h, and 7 d. Lactated Ringer's solution was administered equivalent to the amount of blood taken. The catheter was removed after blood collection at the postoperative 24th h. First, hematological analyses of the blood samples were performed with an automatic hemogram device (Mindray BC-30Vet, Shenzhen, China). Then, the blood in the vacuum tubes containing K<sub>3</sub>-EDTA was centrifuged (NÜVE NF 200, Ankara, Turkey) at 3000 G for 10 min. The extracted plasma samples were stored at -20°C (Ugur, UED 5175 DTK, Aydin, Turkey) for later analysis. Plasma total thiol (Cat. No. MT2101N, Rel Assay, Gaziantep, Turkey) and native thiol (Cat. No. MT2101T, Rel Assay, Gaziantep, Turkey) levels were determined spectrophotometrically (Molecular Device SpectraMax M5 Plate Reader, Pleasanton, CA, USA) using a commercial kit (FIG. 1).

### Statistical analysis

Data analyses were performed using GraphPad Prism 8.00 software (GraphPad Software, San Diego, CA, USA). The normal distribution of the data was confirmed with the Shapiro-Wilk test. The repeated measures One-way ANOVA test followed by Tukey's multiple comparisons test was used to evaluate differences in analytes between different sampling time-points in all the cats that underwent OHE. The results were expressed as mean ± standard deviation (SD) values. Correlations of NLR, MLR, and PLR with Total thiol and native thiol were examined using Spearman correlation analysis. A value of  $P < 0.05$  was considered statistically significant.

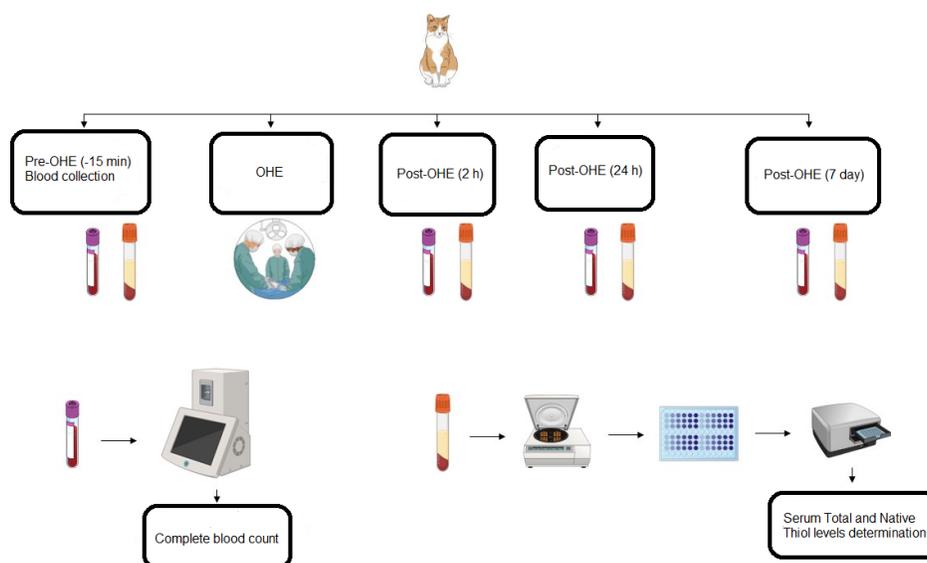


FIGURE 1. Study design

## RESULTS AND DISCUSSION

Heart rate, respiration, and temperature were significantly reduced at 2 h following OHE in all the cats ( $P < 0.05$ ). At 24 h and 7 d after OHE, these physiological parameters reached their normal values and were statistically significantly higher than at 2 h ( $P < 0.05$ ) (FIG. 2). No statistically significant difference was determined before and after OHE in the RBC, Hb, Hct, MCV, MCHC, PLT, and MPV values ( $P > 0.05$ )

(FIG. 3). The WBC and neutrophil counts increased significantly at 2 h and peaked at 24 h after OHE ( $P < 0.001$ ), with no significant difference seen between 0 hr and 7 d ( $P > 0.05$ ). The lymphocyte count decreased significantly at 2 h, 24 h, and 7 d after OHE, and the lowest lymphocyte value was observed at 24 h ( $P < 0.001$ ). Statistically, the lowest monocyte count was observed on d 7 after OHE ( $P < 0.001$ ). The eosinophil count gradually increased after OHE and the highest eosinophil level was observed on d 7 (FIG. 4).

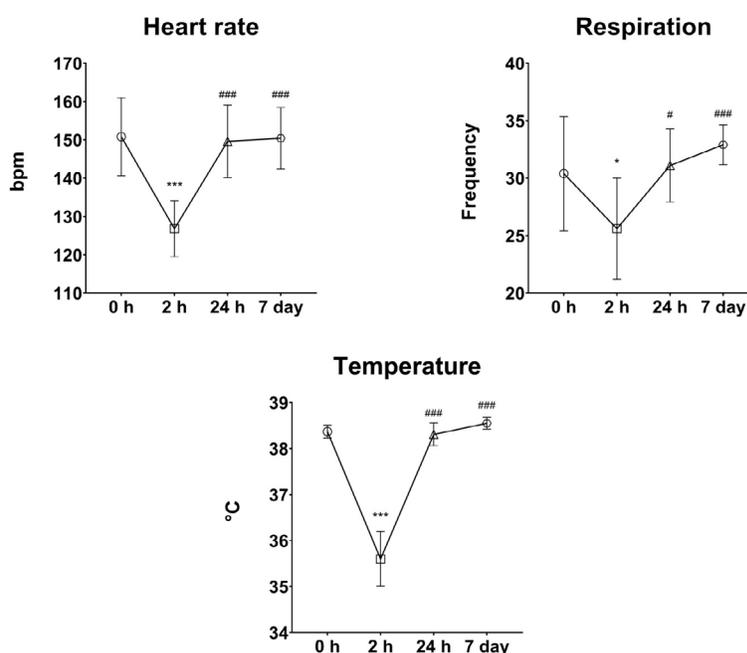


FIGURE 2. Heart rate, respiration, and temperature changes in cats undergoing OHE. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  indicates significance between 0 h and other time-points. #  $P < 0.05$  and ### $P < 0.001$  indicates significance between 2 h and other time-points (24 h)

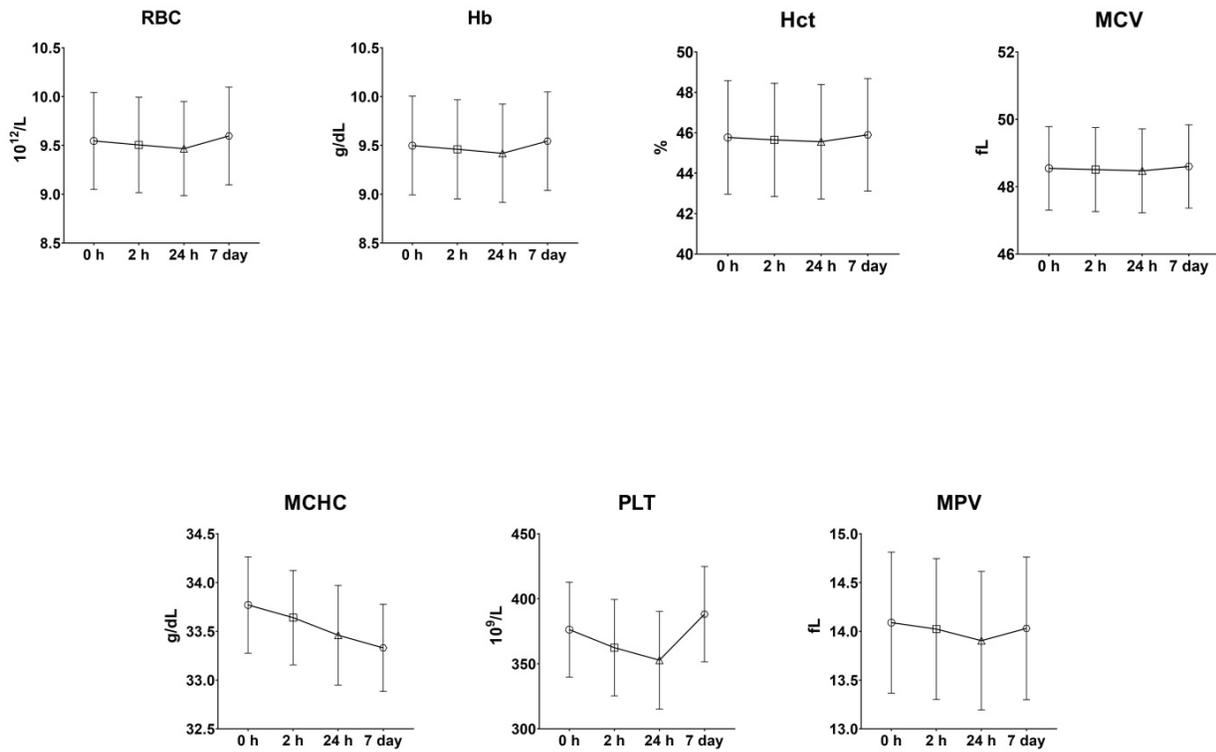


FIGURE 3. RBC, Hb, Hct, MCV, MCHC, PLT, and MPV concentration changes in cats undergoing OHE

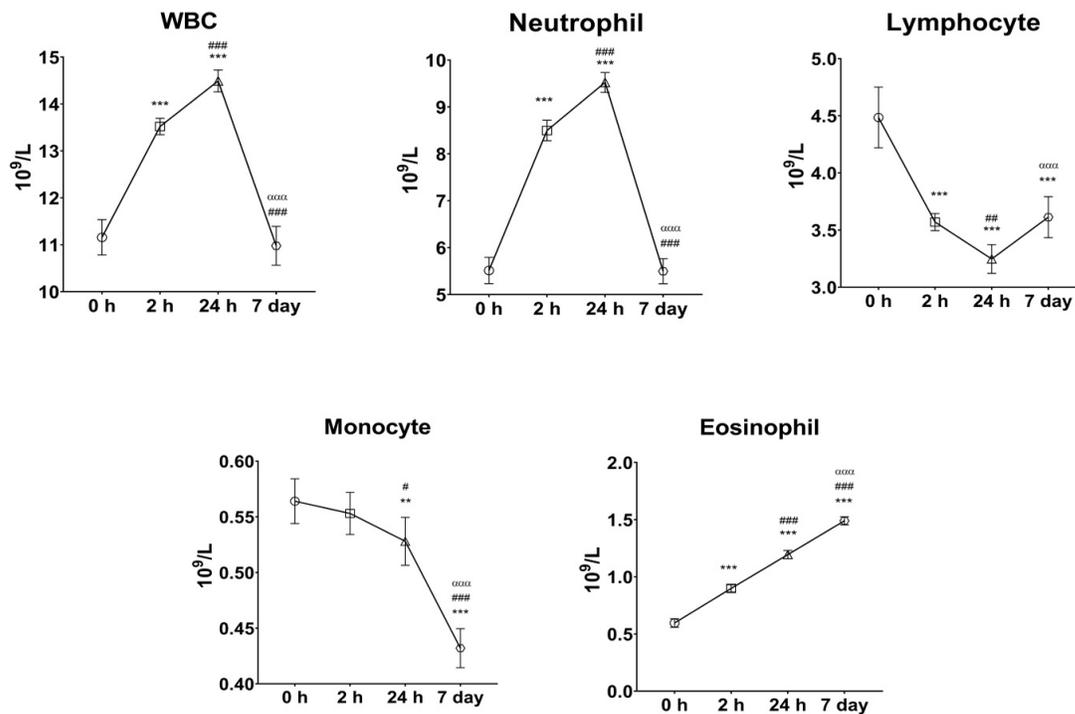


FIGURE 4. WBC, neutrophil, lymphocyte, monocyte, eosinophil concentration changes in cats undergoing OHE. \*\* $P < 0.01$  and \*\*\* $P < 0.001$  indicates significance between 0 hr and other time-points. # $P < 0.05$ , ## $P < 0.01$  and ### $P < 0.001$  indicates significance between 2 h and other time-points (24 h).  $\alpha\alpha\alpha P < 0.001$  indicates significance between 24 h and 7 d

The hematological indices (NLR, MLR, PLR, and MPV/PLT) are shown in FIG. 4. NLR increased significantly at 2 hrs, peaked at 24 h after OHE ( $P<0.001$ ) and remained significantly higher on d 7 after OHE ( $P<0.001$ ). After OHE, MLR increased significantly at 2 h, peaked at 24 h ( $P<0.001$ ) and returned to the pre-OHE value on the 7th d ( $P>0.05$ ). The PLR increased significantly at each time-point ( $P<0.01$ ), showing a significant peak at 24 h and 7 d after OHE ( $P<0.001$ ). The MPV/PLT value after OHE was statistically lowest on the 7th d ( $P<0.05$ ) (FIG. 5).

Total and native thiol counts were significantly reduced at 2 h, 24 h, and 7 d after OHE ( $P<0.001$ ). The lowest serum total and native thiol levels were determined at 24 h after OHE ( $P<0.001$ ) (FIG. 6).

The correlations between the hematological indices (NLR, MLR, PLR, and MPV/PLT) and thiols (total and native thiol) are shown in FIG. 7. A very strong positive correlation was determined between

native and total thiol ( $r=0.978$ ,  $P<0.001$ ). A very strong negative correlation was determined between NLR and total ( $r=-0.913$ ,  $P<0.001$ ) and native ( $r=-0.915$ ,  $P<0.001$ ) thiol. A strong negative correlation was determined between MLR and total ( $r=-0.719$ ,  $P<0.001$ ) and native ( $r=-0.737$ ,  $P<0.001$ ) thiol. A moderate negative correlation was determined between PLR and total ( $r=-0.639$ ,  $P<0.001$ ) and native ( $r=-0.671$ ,  $P<0.001$ ) thiol. No correlation was determined between MPV/PLT and total and native thiol ( $P>0.05$ ).

To the best of the knowledge, this is the first study to have thoroughly investigated the responses of thiols (total and native) and hematological indices (NLR, MLR, PLR, and MPV/PLT) in cats undergoing OHE. After OHE, The measurement of thiols is a novel method that could help to quantify oxidative stress in post-surgical patients, the leukogram in the hemogram was the most affected hematological parameter, but no change was observed in the

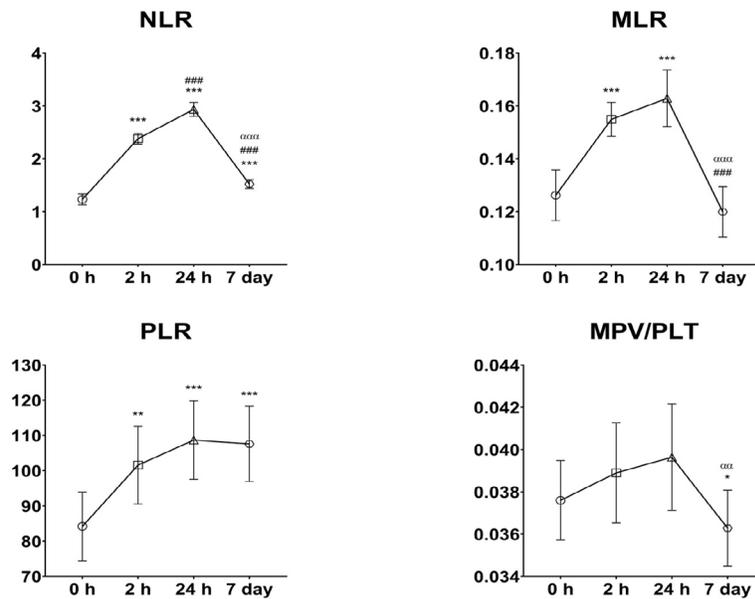


FIGURE 5. The hematological indices (NLR, MLR, PLR, and MPV/PLT) changes in cats undergoing OHE. \* $P<0.05$ , \*\* $P<0.01$  and \*\*\* $P<0.001$  indicates significance between 0 hr and other time-points. ### $P<0.001$  indicates significance between 2 h and other time-points (24 h). α $P<0.01$  and αα $P<0.001$  indicates significance between 24 h and 7 d

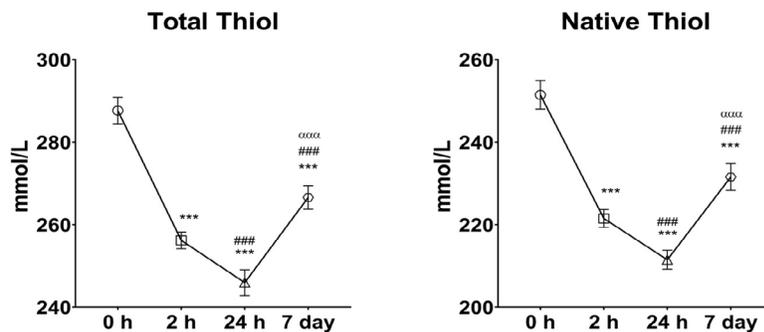
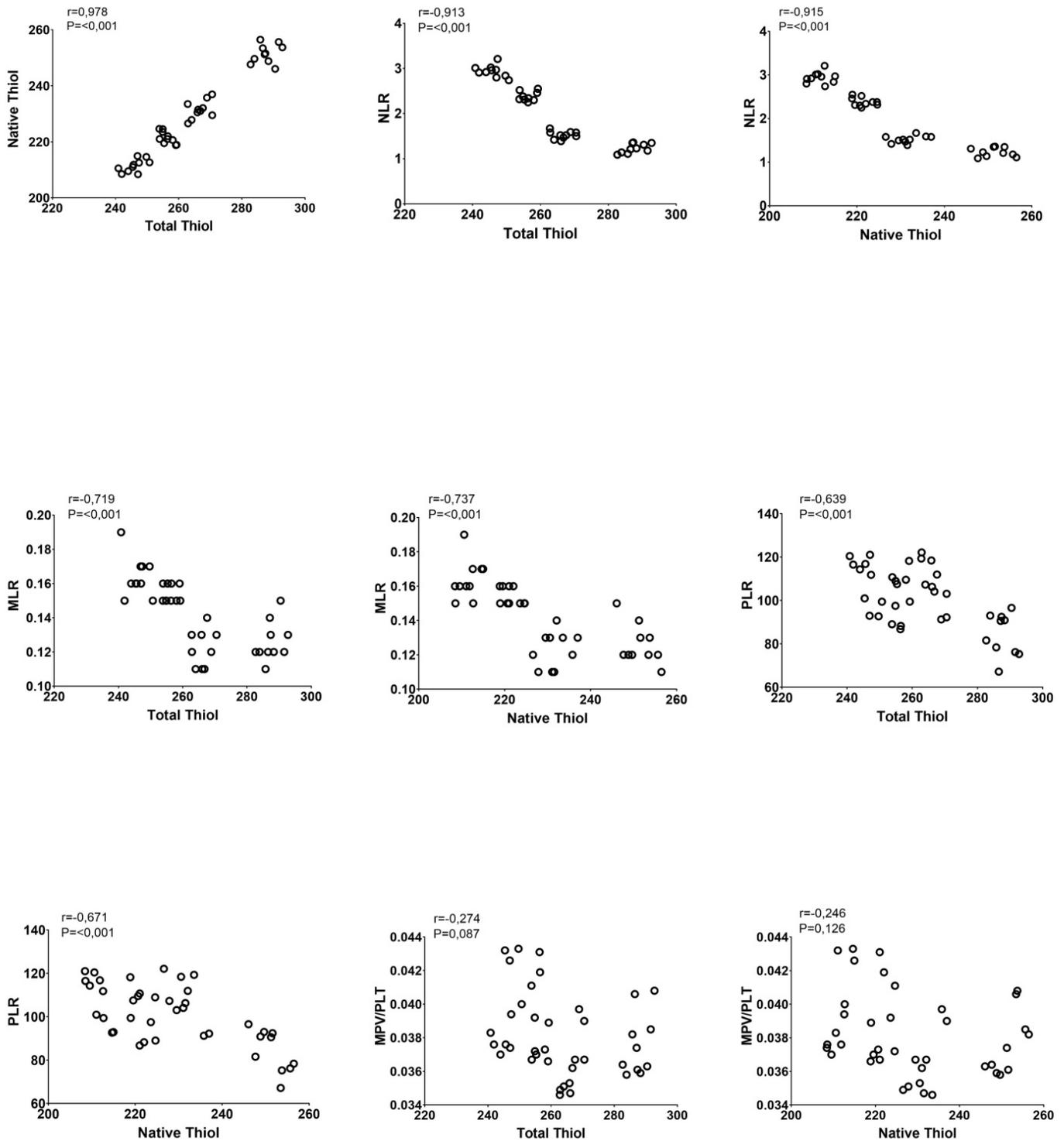


FIGURE 6. The serum total and native thiol concentration changes in cats undergoing OHE. \*\*\* $P<0.001$  indicates significance between 0 hr and other time-points. ### $P<0.001$  indicates significance between 2 h and other time-points (24 h and 7 d). αα $P<0.001$  indicates significance between 24 h and 7 d



**FIGURE 7.** The correlation between hematological indices (NLR, MLR, PLR, and MPV/PLT) and thiols (native and total) in cats undergoing OHE

erythrogram. While NLR, MLR, and PLR were the most affected in hematological indices, negligible changes were seen in MPV/PLT. After OHE, total and native thiol plasma concentrations decreased

dramatically in the first 24 h, and showed lower levels than before OHE even at one week after OHE.

The results of this study showed that the vital parameters of heart rate, respiration, and temperature were depressed at 2 h post-OHE and then returned to normal after the recovery phase. In the study, anesthesia was provided with Ketamine, a centrally effective N-methyl-D-aspartate (NMDA) antagonist, and xylazine, an alpha 2 adrenergic receptor agonist. The suppression of all vital parameters at 2 h post-OHE shows that the depth of pharmacological action of the anesthetics continued. With the return of vital values to normal levels in the following h the effect of the anesthetics was seen to have disappeared. Numerous studies that have used various surgical procedures and anesthesia regimens to assess the alterations in hematological parameters in OHE bitches have reported varying results [16]. Intraoperative blood loss of less than 15% of blood volume is not anticipated to result in clinical symptoms in non-anemic human patients, although the loss of less than 30% of intraoperative blood volume has been proposed as a red cell transfusion trigger in otherwise healthy people [17].

Dogs (*Canis lupus familiaris*) and cats have been reported to be more likely to develop hospital-acquired anemia when cumulative phlebotomy volumes exceed 3% of their total blood volume and when undergoing surgery [18]. No difference in RBC, Hb, Hct, MCV, MCHC, PLT and MPV values was determined in the current study, which was consistent with the findings of a previous study of OHE in cats [19]. This can be attributed to the fact that the OHE surgery performed was minimally invasive with minimal bleeding.

OHE is an intrusive operation with postoperative pain ranging from mild to severe [5, 20]. Surgical trauma induces a stress reaction and increases muscle activity because of injury to superficial nerve terminals, which stimulates the corticotrophic releasing hormone (CRH). Following hypothalamic activation, CRH promotes adrenocorticotropin hormone (ACTH) and causes the adrenal gland to produce cortisol [21]. As a result of tissue damage, organ manipulation, and inflammation, OHE is well known to produce pain and stress. In a previous study, 2 h after OHE, dogs had considerably greater peak plasma cortisol levels [22]. Neutrophils migrate from the neutrophil pool to the circulating neutrophil pool in response to cortisol, although this can also be augmented by neutrophil release from the bone marrow storage pool and decreased migration of neutrophils to tissues [23]. It has been reported that neutrophilia and lymphocytopenia are seen in dogs undergoing OHE [24]. Another study of dogs showed that OHE led to stress leukogram such as leukocytosis, neutrophilia, lymphocytopenia, and eosinopenia [25]. However, in a recent study of cats, neutrophilia and lymphocytopenia were observed on the 2nd d after OHE, and eosinophilia on the 10th d [19]. In the current study, leukocytosis with neutrophilia started to appear at 2 h after OHE and peaked at 24 h, but then returned to the baseline level. However, lymphocytopenia, monocytopenia, and interestingly eosinophilia was noted in the cats after OHE in the current study. This may have been due to circulating stress hormones originating from OHE.

NLR, PLR, MLR, and MPV/PLT are biomarkers of systemic inflammatory response, which are potentially diagnostic [26, 27]. There is a growing amount of evidence indicating the potential utility of these indexes in veterinary care. Previous research in veterinary medicine has associated increased NLR with canine sepsis [26], dogs with chronic enteropathy [28], feline hypertrophic cardiomyopathy [29], and tumor size in cats [30]. Recently, findings have been reported proving that NLR can be an effective stress and inflammation marker in cats undergoing OHE [19]. In the current study, NLR increased in

the first two h following OHE, peaked in the next 24 h, and then started to decline on the 7<sup>th</sup> d to the pre-OHE level.

Previous studies support that this may be related to OHE-induced stress and inflammatory status. Related to systemic inflammatory reactive diseases and PLT function and activity, the rise in PLR has been reported to be caused by an increase in platelet count and a reduction in lymphocyte count in peripheral blood [31]. Increased thrombopoietin and cytokine levels during inflammation result in increased megakaryocytes, which stimulate PLT production and decrease their size [32]. PLT are important in initial hemostasis because they adhere to the damaged vascular bed induced by subendothelial collagen exposure [33]. MPV/PLT and PLR indices have been previously shown to be ineffective as systemic biomarkers for disease activity in dogs, such as periodontitis and oropharyngeal cancers [27]. In a previous study, increases in the mean PLT values were observed in cats after OHE [34], whereas another cat study showed that PLT, MPV and PLR were not significantly changed post-OHE [19]. PLR increased following OHE in the current study, and the MPV/PLT ratio decreased on the 7<sup>th</sup> d post-OHE. In general, stress-induced hypercortisolemia, followed by platelet release into blood cells and transient lymphopenia, influences the degree of PLR elevation in a variety of proinflammatory and prothrombotic states [31]. MLR is a simple biomarker of the host immune system. Research has linked a high monocyte count to immune gene upregulation and the generation of monocyte/macrophage-related cytokines [35]. Although a previous study showed that monocyte levels did not change, lymphocytes decreased after OHE in cats [19]. Another study of domestic dogs in Nigeria reported a decrease in monocyte levels 2 h after OHE, returning to pre-OHE levels, while decreased lymphocyte levels were reported [3]. MLR and NLR formed similar curves in the current study, increasing at 2 h post-OHE, peaking at 24 h, and then reverting to normal on the 7<sup>th</sup> d. OHE-induced stress and inflammation may have contributed to this response.

Native thiols (-SH) and total thiols (-SH+ -SS) are critical for the detoxification of extracellular and intracellular reactive nitrogen and oxygen species, which is a crucial component of cellular antioxidant defences. Importantly, this oversees preservation of the redox state of protein thiols necessary for deoxyribonucleic acid (DNA) synthesis and repair [13]. Protein structural and functional alterations are commonly caused by the loss of thiol groups, which is the principal molecular process. It has been reported that surgery and trauma decrease protein synthesis and increase protein catabolism and oxidation, and these changes are related to the level and duration of trauma [36]. In a previous study, total thiol was reported to be a therapeutically relevant indicator of oxidative stress in cats with pyometra that had undergone elective OHE [37]. In the current study, it was hypothesized that the body's efforts to detoxify oxidative stress and the oxygen free radicals generated by surgical trauma would be the main cause of the greatly reduced thiol levels post-OHE. The study results showed that natural and total thiol have a very high negative correlation with NLR, a strong negative correlation with MLR, and a moderate negative correlation with PLR. However, MPV/PLT and native and total thiol did not correlate. Although there are many human studies in the literature to explain the relationship between thiols and NLR, PLR, and MLR, animal research is limited. A negative correlation has been previously reported between NLR and native and total thiol after gunshot injury in humans [38]. Total thiol and native thiol levels have also been shown to be positively correlated with lymphocyte levels and negatively correlated with NLR

and PLR in COVID-19 patients [39]. In a clinical study of patients who presented at the Emergency Department with ischemic stroke, NLR values were found to be significantly higher, and total and native thiols were significantly lower [38]. Thiol homeostasis could potentially be beneficial in the inflammatory response and negatively correlated with the degree of inflammation.

## CONCLUSIONS

The results of this study demonstrated that the whole blood indices of NLR, MLR, and PLR increased and thiols (native and total) decreased in cats after OHE, which was related to the inflammatory and stress response. This information indicates that changes in serum concentrations of thiols as well as NLR, MLR, and PLR are important in the assessment of inflammation and stress response after OHE.

## Conflict of interests

The authors have read and approved the article, and have no conflict of interests to declare.

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