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Dynamic changes in thiol–disulfide homeostasis and oxidative stress during vincristine treatment in Kangal bitches with transmissible venereal tumor

Cambios dinámicos en la homeostasis tiol–disulfuro y el estrés oxidativo durante el tratamiento con vincristina en perras Kangal con tumor venéreo transmisible

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ABSTRACT

The aim of this study was to evaluate time-dependent changes in oxidative stress biomarkers and thiol-disulfide homeostasis in dogs diagnosed with canine transmissible venereal tumor and treated with vincristine sulfate. Eighteen female Kangal dogs were included in the study, nine with canine transmissible venereal tumor and nine clinically healthy (control group). Dogs in the canine transmissible venereal tumor group received vincristine sulfate once a week until complete tumor regression was achieved. Analyses were performed at pre-treatment, week 1 (T1), week 3 (T3), and end-treatment. Only one sample was taken from the control group dogs. Serum levels of malondialdehyde, glutathione, total thiol, native thiol, disulfide, and thiol-disulfide ratios were determined. In dogs with canine transmissible venereal tumor, malondialdehyde levels were significantly higher (P = 0.001) during the pre-treatment period compared to the healthy control group, while glutathione (P = 0.002), total thiol (P = 0.001), and native thiol (P = 0.001) levels were significantly lower. During the same period, disulfide levels (P = 0.001), disulfide/ total thiol × 100 (P = 0.001) and disulfide/ native thiol × 100 (P = 0.001) ratios increased significantly, while the native thiol/total thiol × 100 ratio (P = 0.001) decreased significantly. Although a gradual improvement in oxidative stress parameters and thiol-disulfide homeostasis was observed during vincristine treatment, malondialdehyde levels remained significantly higher than the control group at the end of treatment (P = 0.001), and thiol-disulfide homeostasis did not fully return to normal. In conclusion, Kangal female dogs with canine transmissible venereal tumor exhibit a marked state of oxidative stress along with impaired thiol-disulfide homeostasis. Vincristine treatment partially improves this redox imbalance but does not provide complete normalization. Thiol-disulfide homeostasis parameters can reflect changes in redox status and may be useful indicators in evaluating treatment-related biochemical changes in canine transmissible venereal tumor.

Key words: Venereal tumors; glutathione; malondialdehyde; oxidative stress; vincristine.

RESUMEN

El objetivo de este estudio fue evaluar los cambios dependientes del tiempo en los biomarcadores de estrés oxidativo y la homeostasis tiol-disulfuro en perras diagnosticados con tumor venéreo transmisible canino y tratados con sulfato de vincristina. Dieciocho perras Kangal fueron incluidas en el estudio, nueve con tumor venéreo transmisible canino y nueve clínicamente sanas (grupo control). Las perras en el grupo tumor venéreo transmisible canino recibieron sulfato de vincristina una vez por semana hasta que se logró la regresión completa del tumor. Los análisis se realizaron en el pretratamiento, semana 1 (T1), semana 3 (T3) y final del tratamiento. Solo se tomó una muestra de las perras del grupo control. Se determinaron los niveles séricos de malondialdehído, glutatión, tiol total, tiol nativo, disulfuro y las relaciones tiol-disulfuro. En perros con tumor venéreo transmisible canino, los niveles de malondialdehído fueron significativamente más altos (P = 0,001) durante el período pretratamiento en comparación con el grupo control sano, mientras que los niveles de glutatión (P = 0,002), tiol total (P = 0,001) y tiol nativo (P = 0,001) fueron significativamente más bajos. Durante el mismo período, los niveles de disulfuro (P = 0,001), disulfuro/tiol total × 100 (P = 0,001) y las razones disulfuro/tiol nativo × 100 (P = 0,001) aumentaron significativamente, mientras que la razón tiol nativo/tiol total × 100 (P = 0,001) disminuyó significativamente. Aunque se observó una mejora gradual en los parámetros de estrés oxidativo y la homeostasis tiol-disulfuro durante el tratamiento con vincristina, los niveles de malondialdehído permanecieron significativamente más altos que el grupo control al final del tratamiento (P = 0,001), y la homeostasis tiol-disulfuro no volvió completamente a la normalidad. En conclusión, las perras Kangal con tumor venéreo transmisible canino presentan un marcado estado de estrés oxidativo junto con una alteración de la homeostasis tiol-disulfuro. El tratamiento con vincristina mejora parcialmente este desequilibrio redox, pero no logra una normalización completa. Los parámetros de la homeostasis tiol-disulfuro pueden reflejar cambios en el estado redox y ser indicadores útiles para evaluar los cambios bioquímicos relacionados con el tratamiento en la tumor venéreo transmisible canino.

Palabras clave: Tumores venéreos; glutatión; malondialdehído; estrés oxidativo; vincristina.

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INTRODUCTION

Canine transmissible venereal tumor (CTVT), also known as transmissible sarcoma, sexual granuloma, transmissible lymphosarcoma, or sticker tumor, is a contagious sexually transmitted neoplasm in dogs that can be found in genital and extragenital areas depending on its location [1, 2].

It is most seen in the genital area, specifically the posterior part of the vagina, in free-roaming female dogs with uncontrolled sexual activity. It can also occur in extragenital areas because of sniffing, biting, and/or licking of tumor areas [3, 4, 5].

Definitive diagnosis of CTVT is based on physical examination findings and cytological characteristics of shed cells or histopathological evaluation. Treatment options include chemotherapy, radiotherapy, immunotherapy, and surgical excision [2]. The clinical significance of the disease is increased by its ability to easily spread from one dog to another through allogeneic transfer of live cancer cells [6].

Oxidative stress is defined as the imbalance between oxidative and antioxidant defense systems in favor of the oxidant system and is characterized by the accumulation of reactive oxygen species (ROS) [7]. Under physiological conditions, cellular homeostasis depends on the precise regulation of the quantity and activity of antioxidants, which are necessary for maintaining normal biological functions and the vitality of the organism [8].

When ROS production exceeds antioxidant defenses, oxidative damage occurs, leading to structural and functional changes in cellular macromolecules, including lipids, proteins, and deoxyribonucleic acid [9]. This type of oxidative damage plays a role in the development and progression of numerous pathological conditions [10].

For the assessment of oxidative stress, lipid peroxidation products such as malondialdehyde (MDA), enzymatic antioxidants such as glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and non-enzymatic antioxidants such as vitamins, metal ions, thiol-containing compounds, and reduced glutathione (GSH) can be used as biomarkers [7, 11, 12].

The balance between thiols and disulfides in an organism is called thiol-disulfide homeostasis (TDH). The balance between thiols and disulfides, as reflected by TDH, is a critical parameter associated with numerous biochemical processes. TDH has been reported to be affected in various diseases and conditions associated with increased oxidative stress [13, 14, 15].

Thiols are vital for antioxidant defense as they eliminate ROS through both enzymatic and non-enzymatic mechanisms [16]. With a novel method, disulfide levels can be determined quantitatively [14]. In this method, disulfide levels are evaluated together with natural thiol levels, and the sum of these measurements is expressed as total thiol levels. The introduction of this method has significantly contributed to a better understanding of TDH in biological systems [17].

Due to its pathophysiology and metabolic effects, CTVT is an important neoplasm associated with oxidative stress. Its high transmissibility and potential to impair reproductive function may induce significant biochemical alterations at the cellular level, including changes in antioxidant defense mechanisms and disruption of cellular redox homeostasis [1, 18, 19].

Although oxidative stress is known to increase in neoplastic diseases, information regarding thiol-disulfide homeostasis in CTVT, especially under treatment conditions, remains limited [11, 20].

The aim of this study is to reveal the temporal changes in thiol-disulfide homeostasis parameters and MDA-GSH levels during vincristine treatment.

MATERIALS AND METHODS

Animals

This study was conducted with the permission of Kafkas University Animal Experiments Local Ethics Committee on 08.04.2025 with the number KAÜ–HADYEK/2025–03. All experimental procedures were performed in compliance with both national regulations and internationally accepted guidelines for the use of animals in scientific research. In addition, informed consent was obtained from the owners of all animals prior to their inclusion in the study.

The study population comprised 18 Kangal bitches (*Canis lupus familiaris*) admitted to the Animal Hospital of the Faculty of Veterinary Medicine, Kafkas University, including 9 bitches clinically diagnosed with canine transmissible venereal tumor and 9 clinically healthy control bitches. The animals were aged 3–5 years and weighed (TESS, Animal Scale, Türkiye) 42–57 kg. Bitches assigned to the CTVT group were monitored throughout the treatment period and were re–evaluated at weekly intervals until completion of therapy (FIG. 1).

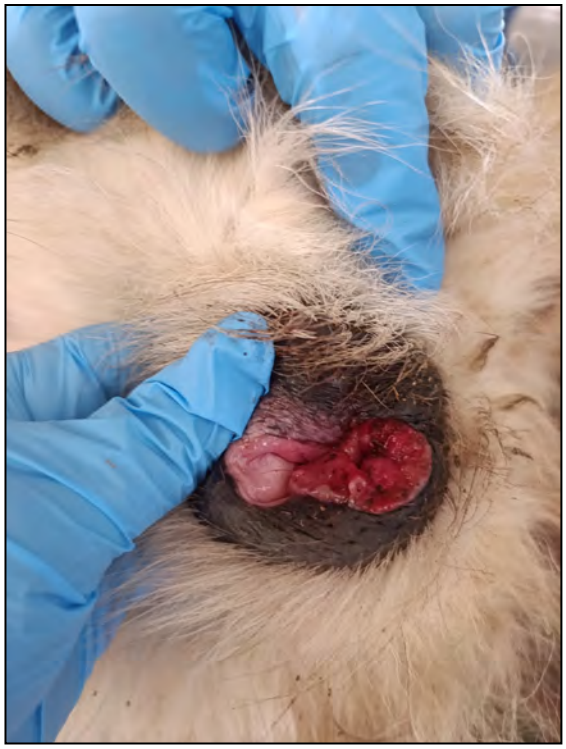

FIGURE 1. A reddish, cauliflower-like tumoral mass with a fragile consistency and hemorrhagic appearance located in the vulvovaginal region of a bitch diagnosed with canine transmissible venereal tumor (CTVT)

Cytological examinations

Genital system examination of female dogs in the CTVT group revealed vulvar swelling, excessive licking of the affected area, and distinct, fragile, cauliflower-like masses. Vaginal cytology was performed for diagnosis and stained using the Diff-Quik method (ChemBio®, Medford, USA). Microscopic examination of the samples (Olympus, CX23, Tokyo, Japan) revealed the presence of TVT cells, confirming the definitive diagnosis.

Experimental design and treatment protocol

The bitches included in the study were divided into two groups: A CTVT group (n = 9) and a control group (n = 9). The control group consisted of bitches presented to the clinic for routine examinations and vaccinations, with no abnormalities detected in their genital systems.

Bitches in the CTVT group received vincristine sulfate as a standard treatment protocol (Vincristine® 2 mg /2 ml IV, Koçak Farma, Türkiye), administered intravenously at a dose of 0.025 mg/kg once weekly [21]. During the treatment period, the clinical status of the bitches and their response to therapy were regularly monitored. For biochemical analyses, blood samples were collected weekly from bitches with CTVT; however, only samples obtained at four predefined stages of the therapeutic process were included in the analyses: prior to the initiation of treatment (Pre–T), during the first week of therapy (T1), at the third week of treatment (T3), and following completion of the treatment protocol (End–T). Treatment continued until the complete disappearance of tumor cells was confirmed by vaginal cytological examination. The duration of treatment varied between 4 and 7 weeks among individuals, depending on the response to vincristine therapy.

Histopathological examinations

Tumoral tissue samples obtained from the bitches were fixed in 10 % formaldehyde solution. Following routine tissue processing, serial sections of 5 µm thickness (Feather A35 microtome blade, Feather, Japan) were cut from paraffin blocks. To evaluate histopathological alterations, the sections were stained with hematoxylin and eosin (H&E). The prepared slides were examined in detail under a light microscope (Olympus, CX23, Tokyo, Japan) by at least two independent pathologists.

Representative pathological findings were photographed (Olympus Bx53®, Olympus Global, Tokyo, Japan) at different magnifications. The growth patterns of CTVT were classified according to the criteria described by Mukaratirwa *et al*. [22] and Stockmann *et al*. [18].

Blood sampling

Throughout the treatment period, blood samples were obtained from bitches in the CTVT group on a weekly basis immediately prior to each vincristine administration. In contrast, a single blood sample was collected from each animal in the healthy control group.

Venous blood was drawn from the vena *cephalica antebrachii* using a closed–system blood collection device and transferred into serum–separating vacuum tubes (BD Vacutainer®, 8.5 mL, BD, United Kingdom). Following collection, samples were centrifuged (Nuve, NF 400, Türkiye) at 1000 x g for 10 minutes (min) at + 4 °C to allow separation of serum. The resulting serum was carefully aliquoted into sterile microcentrifuge tubes and preserved at –80 °C (Thermo Scientific, USA) until further biochemical analyses were performed.

Biochemical analyses

Glutathione was analyzed spectrophotometrically at 412 nm according to the method of Beutler *et al*. [20]. MDA analysis was performed using the method of Yoshioka *et al*. [23]. The spectrophotometric approach of Erel and Neselioglu [14] was used to measure the total thiol (TT) and natural thiol (NT) amounts. Using the TT and NT values, disulfide (Ds), Ds/TT × 100, Ds/NT × 100 and NT/TT × 100 ratios were calculated [24].

Statistical analysis

Sample size was calculated using G*Power (v3.1.9.7) with assumptions of d=1.88 effect size, 95 % statistical power, and 5 % type-I error margin based on literature data [25]. Normality of continuous variables was examined using the Shapiro-Wilk test; all parameters except GSH and TT were found to meet the assumption of normal distribution ($P > 0.05$).

Statistical analysis of changes during the treatment process was performed using repeated measures analysis of variance (ANOVA) and Tukey multiple comparison test for parametric data; and Friedman and Dunn tests for non-parametric data. Independent t-test or Mann-Whitney U test was applied to compare the CTVT group with healthy controls. Correlations between oxidative stress and homeostasis parameters were evaluated using the Spearman method. A $P < 0.05$ threshold was set for statistical significance, and all calculations were performed using Python and GraphPad Prism (v10.0) software.

RESULTS AND DISCUSSION

Cytological findings

The majority of the tumoral masses were classified as the plasmacytoid type. The neoplastic cells were round to oval in shape and exhibited abundant cytoplasm. Characteristic clear vacuoles were prominent within the mildly basophilic cytoplasm. The nuclei were markedly hyperchromatic and eccentrically located. Another important cytological feature was an increased nucleus–to–cytoplasm ratio in favor of the nucleus. In addition, multinucleation was observed in some tumoral cells (FIG. 2).

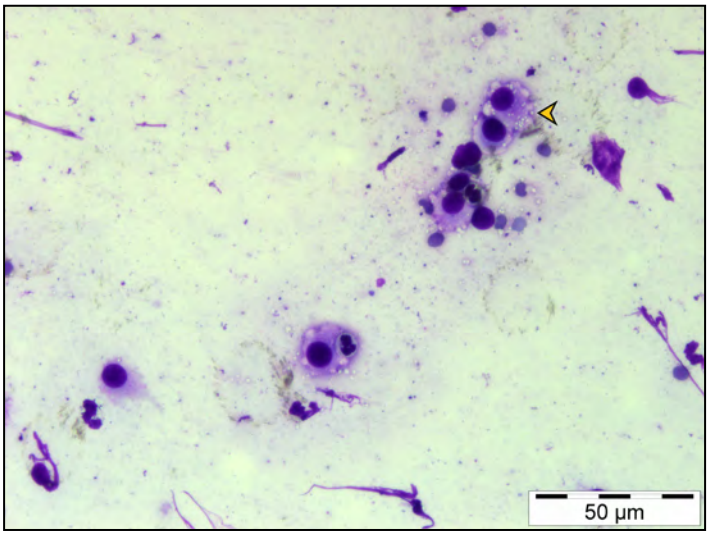

FIGURE 2. Diff–Quik–stained smear from a bitch diagnosed with canine transmissible venereal tumor (CTVT). Tumor cells were characterized by a voluminous cytoplasm, eccentrically positioned nuclei, and prominent intracytoplasmic clear vacuoles (yellow arrowheads)

Histopathological findings

In CTVTs at the progression stage, round tumor cells were found to be surrounded by an almost imperceptibly thin fibrovascular stroma. At this stage, cellularity was markedly high, and the cells were arranged in solid masses or cord–like patterns. These cells exhibited eosinophilic cytoplasm with prominent nuclei and nucleoli. In different tumor foci, the average number of typical and atypical mitotic figures ranged between 3 and 5 per high–power field at × 40 magnification.

Numerous blood vessels were observed within the tumor stroma. Some tumor areas, particularly in the central regions, showed pronounced necrosis and hemorrhage. Cellular pleomorphism was minimal. In CTVTs at the early regression or stable stage, cellularity and stromal thickness were similar to those observed in the progression stage.

The main distinguishing feature between these two developmental patterns was the presence of marked lymphocytic infiltration surrounding the tumor foci in the stable stage. In contrast, CTVTs at the late regression (R) stage exhibited decreased cellularity and a markedly thickened stroma surrounding the tumor cells compared with the progression and stable stages. Additionally, a pronounced reduction in mitotic figures was observed, and apoptotic bodies were occasionally detected (FIG. 3).

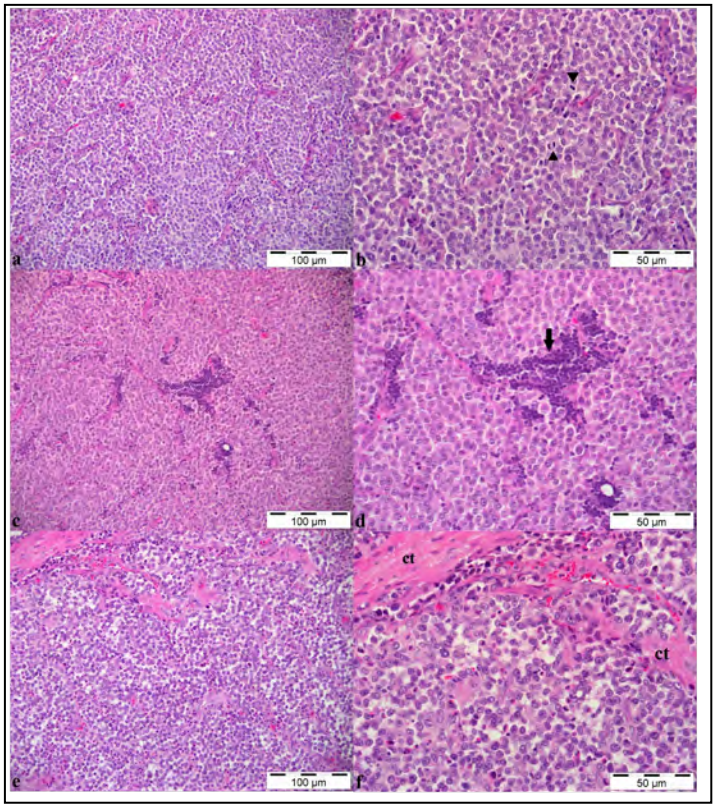

FIGURE 3. Canine transmissible venereal tumor (CTVT), H&E staining, different magnifications. (a–b) Progression stage: round tumor cells separated by thin fibrovascular stroma, mitotic figures (arrowheads), and marked increase in cellularity. (c–d) Stable stage: dense lymphocytic infiltrates surrounding the tumor tissue (arrow). (e–f) Regression stage: increased connective tissue (ct) accompanied by reduced cellularity

Oxidative stress findings

Changes in oxidative stress markers and thiol–disulfide homeostasis parameters during vincristine sulfate treatment in bitches diagnosed with CTVT are presented in FIG. 4.

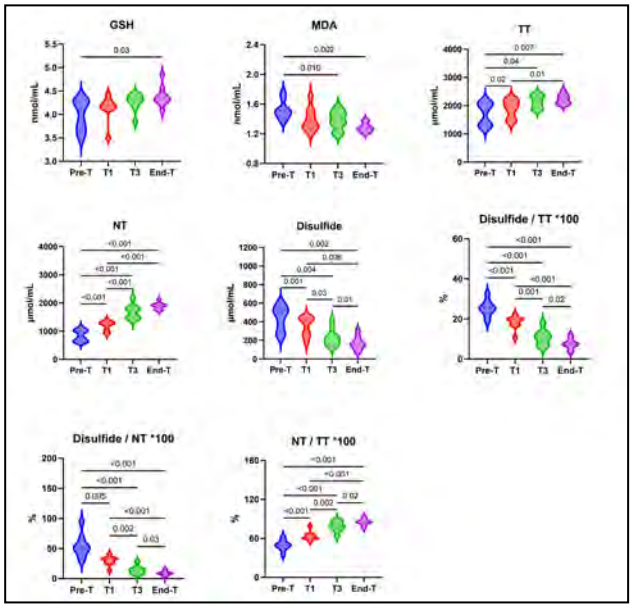

FIGURE 4. Time–dependent alterations in oxidative stress and thiol–disulfide homeostasis parameters during vincristine sulfate therapy in bitches with canine transmissible venereal tumor (CTVT). (GSH, glutathione; MDA, malondialdehyde; TT, total thiol; NT, native thiol; Pre–T, pre–treatment; T1, first week of treatment; T3, third week of treatment; End–T, end of treatment)

In the current study, temporal changes in oxidative stress markers and thiol–disulfide homeostasis during vincristine treatment were comprehensively evaluated in Kangal bitches diagnosed with CTVT. Consistent with recent literature highlighting the severe systemic oxidative burden intrinsically induced by this tumor [26], the present findings explicitly demonstrate that CTVT is characterized by a pronounced oxidative stress state accompanied by redox imbalance. Furthermore, the present data indicate that while vincristine treatment gradually attenuates this disturbance, it does not achieve complete normalization. This observation strongly aligns with contemporary evidence showing that chemotherapeutic interventions significantly modulate systemic redox dynamics and can sustain certain oxidative stress biomarkers in canine oncology patients [25, 27].

During the Pre–T period, GSH levels were relatively low and showed a gradual increase throughout the treatment period. In particular, GSH levels at the End–T time point were statistically significantly higher than those in the Pre–T period (P = 0.030).

Malondialdehyde levels decreased significantly during the treatment course. A reduction in MDA levels was observed starting from the T1 time point, and this decrease became more pronounced during the T3 and End–T periods. Pre–T MDA values were significantly higher than those measured at the T3 and End–T time points (P = 0.010 and P = 0.002, respectively).

Reactive oxygen species play a critical role in cancer development by affecting many stages of tumor growth. Their impact has been documented from early preneoplastic alterations associated with chronic inflammation and oxidative DNA damage to later events, including genomic instability, tumor cell proliferation, invasion, and metastatic spread [28, 29]. Nevertheless, the role of oxidative stress in tumor is not unidirectional. Although excessive ROS production may facilitate tumor progression, ROS can also exert antitumoral effects by triggering regulated cell death pathways, including apoptosis and ferroptosis [28, 30].

Glutathione is a key molecule at the center of cellular antioxidant defense systems and plays a direct role in ROS detoxification. Through the action of Glutathione Peroxidase, hydrogen peroxide and lipid peroxides are neutralized, while GSH itself is converted to its oxidized form, a process that is critical for maintaining cellular redox balance [16]. Increased metabolic activity, mitochondrial dysfunction, and chronic inflammation within the tumor microenvironment enhance ROS production in neoplastic tissues, thereby accelerating GSH consumption [31].

Due to the high reactivity and short half–life of ROS, their direct measurement is impractical; therefore, indirect biomarkers such as MDA, GSH, and thiol–disulfide homeostasis parameters are widely used to assess oxidative stress [14, 32]. MDA, one of the end products of lipid peroxidation, is considered a reliable indicator of oxidative stress [8, 33].

Numerous studies in Veterinary oncology have reported that increased oxidative stress is associated with tumor progression and elevated cancer risk [10, 34]. In a previous study, serum MDA levels were significantly higher in bitches with CTVT than in healthy bitches [35], and similar results were reported in dogs with different cancer types [36]. The higher MDA values observed in the present study compared with the control group further suggest that CTVT represents a strong source of oxidative stress.

During the Pre–T period, serum GSH (P = 0.002), TT (P = 0.001), and NT (P = 0.001) levels were found to be significantly lower in CTVT–affected bitches compared with the healthy control group, whereas MDA (P = 0.001) and disulfide (P = 0.001) levels, as well as disulfide–based ratios, were significantly higher.

In the present study, serum GSH levels were significantly lower in CTVT–affected Kangal bitches during the Pre–T period compared with healthy controls. Although a gradual increase in GSH levels was observed during vincristine treatment, these levels did not fully reach control values even at the end of treatment. This finding suggests that CTVT is associated with marked GSH depletion and that chemotherapy only partially corrects this imbalance [36].

Vincristine and other chemotherapeutic agents are known to disrupt mitochondrial function and increase ROS production [37]. This chemotherapy–induced secondary oxidative stress may contribute to sustained GSH consumption even when tumor burden is reduced. Moreover, GSH synthesis is closely linked to cysteine bioavailability and cellular energy status; subclinical metabolic stress and tissue damage during chemotherapy may limit GSH synthesis capacity and prevent complete restoration [8].

At the T1 time point, the differences between the control and CTVT groups remained statistically significant for all parameters (GSH, P = 0.009; MDA, P = 0.001; TT, P = 0.002; NT, P = 0.001); however, the mean differences showed a decreasing trend compared with the Pre–T period. Similarly, at the T3 time point, although statistically significant differences persisted between the control group and CTVT–affected bitches for most oxidative stress and thiol–disulfide homeostasis parameters, the magnitude of the control–CTVT differences was further reduced.

At the End–T period, MDA (P = 0.001) and NT (P = 0.001) levels continued to differ significantly from those of the control group, whereas the differences between the control group and CTVT–affected bitches for GSH (P = 0.095), disulfide (P = 0.270), Disulfide/TT*100 (P = 0.090) and Disulfide/NT*100 (P = 0.094) were no longer statistically significant.

Total thiol concentrations showed a significant increase in response to treatment, with values at End–T being significantly higher than those at Pre–T (P = 0.007). Increased ROS production in tumors accelerates oxidative modification of thiol groups, leading to impairment of protein function. This not only weakens antioxidant defenses but also affects critical biological processes such as cellular signaling, enzyme activity, and cell cycle regulation [16, 31].

In the present study, the marked decreases in TT and NT levels during the Pre–T period suggest that CTVT is a potent source of oxidative stress leading to systemic thiol consumption.

The significantly elevated disulfide levels and disulfide/TT × 100 and disulfide/NT × 100 ratios observed in the Pre–T period indicate that thiol–disulfide homeostasis is characterized not only by a reduction in absolute thiol levels but also by a shift in redox balance toward disulfide formation.

These ratios are considered sensitive indicators of the organism's adaptive capacity to oxidative stress [13, 24]. Accordingly, the observed alterations in CTVT–affected bitches demonstrate a profound disruption of redox homeostasis.

Native thiol levels exhibited a marked and progressive increase throughout the treatment period. NT levels increased significantly at each successive time point, reaching the highest values at the end of treatment, and a statistically significant difference was observed compared with the Pre–T period (P = 0.001).

A significant decrease in disulfide levels was detected during the treatment period. Disulfide concentrations were found to be significantly lower at the End–T period compared with the Pre–T and T1 periods (P = 0.002 and P = 0.006, respectively).

In analyses evaluating thiol–disulfide balance based on ratios, disulfide/TT×100 and disulfide/NT×100 ratios showed significant decreases throughout the treatment period. The lowest values for both ratios were recorded at the end of treatment. Conversely, the NT/TT×100 ratio showed a significant increase during treatment and was found to be statistically significantly higher at the End–T periods compared with the Pre–T period (P = 0.001).

During vincristine treatment, gradual increases in TT and NT levels accompanied by decreases in disulfide levels and disulfide–based ratios suggest that oxidative stress pressure is partially alleviated as tumor burden decreases [24].

However, the failure of these parameters to fully normalize by the end of treatment indicates incomplete restoration of thiol–disulfide balance. This may be explained by the additional oxidative burden imposed by the chemotherapeutic agents themselves, which can enhance ROS production further and affect thiol systems [31, 37].

Comparisons of oxidative stress and thiol–disulfide homeostasis parameters between the control group and CTVT–affected bitches at different time points were evaluated using pairwise analyses (SUPPLEMENTARY TABLE I).

Associations between oxidative stress indices and thiol–disulfide homeostasis parameters were examined using Spearman's rank correlation analysis, and the resulting correlation matrix was visualized as a heat map (FIG. 5). MDA levels demonstrated strong inverse correlations with both reduced GSH and NT concentrations (P = 0.001).

In contrast, MDA showed significant positive correlations with disulfide levels and disulfide–derived ratios (P = 0.001). Furthermore, GSH and NT concentrations were negatively associated with disulfide content and disulfide–based indices, indicating a shift toward an oxidized thiol state. A pronounced positive correlation was observed between NT levels and the NT/TT × 100 ratio (r = 0.903, P = 0.001).

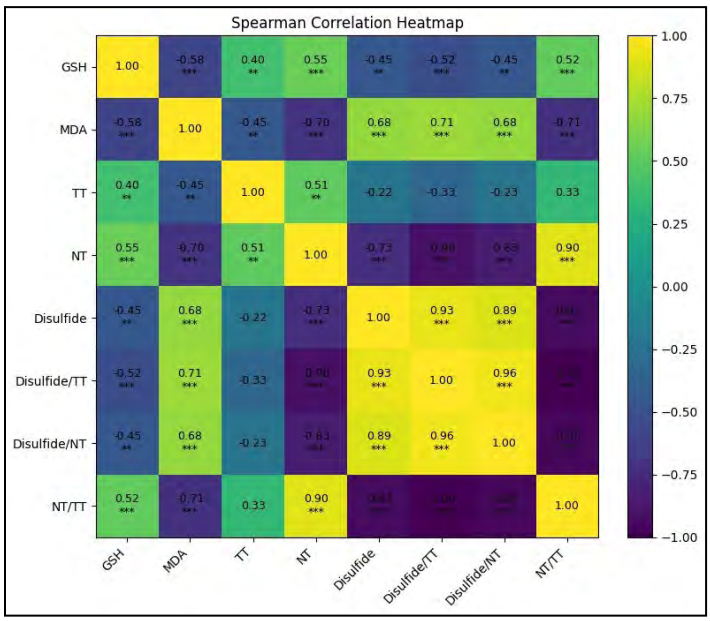

FIGURE 5. Correlations between oxidative stress markers and thiol–disulfide homeostasis parameters. (GSH, glutathione; MDA, malondialdehyde; TT, total thiol; NT, native thiol). (*P < 0.05, **P < 0.01, ***P < 0.001)

The strong negative correlations observed between MDA levels and GSH, TT, and NT indicate that increased lipid peroxidation is closely associated with depletion of the antioxidant thiol pool. Conversely, strong positive correlations between MDA levels and disulfide concentrations, as well as disulfide/TT×100 and disulfide/NT×100 ratios, demonstrate that increased oxidative stress parallels the conversion of thiol groups to disulfide forms.

Furthermore, the strong positive association between NT and the NT/TT×100 ratio supports the role of native thiols as sensitive indicators of redox balance. Overall, this correlation profile suggests that oxidative stress in CTVT is not limited to lipid peroxidation but also induces a systemic redox imbalance by shifting thiol–disulfide homeostasis toward disulfide formation. Therefore, thiol–based parameters may serve as functional and complementary biomarkers for evaluating CTVT pathophysiology [13, 14, 15].

CONCLUSION

This study demonstrates that Kangal bitches diagnosed with CTVT exhibit a pronounced oxidative stress state accompanied by impaired thiol–disulfide homeostasis. Elevated MDA levels together with decreased GSH, TT, and NT levels and increased disulfide–based ratios indicate a redox shift toward disulfide formation.

Although vincristine treatment led to a gradual improvement in oxidative stress markers and thiol–disulfide parameters, complete normalization was not achieved by the end of treatment. This study is one of the few to evaluate thiol-disulfide homeostasis in CTVT within a longitudinal treatment framework.

By assessing dynamic changes during vincristine therapy, the present study provides additional insight into treatment–related redox alterations. These findings may contribute to a better understanding of oxidative mechanisms in CTVT and support the use of redox–based parameters in future studies.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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