Investigation of the Mechanism of Impaired Skin Barrier Function in Dogs With Malignant Tumors

MIGYEONG GEUM^{1,2} and HA-JUNG KIM^{1,2}

¹Department of Veterinary Internal Medicine, College of Veterinary Medicine,
Chonnam National University, Gwangju, Republic of Korea;

²BK21 Project Team, College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea

Abstract. *Background/Aim:* No study has investigated skin barrier dysfunction with systemic diseases in veterinary medicine. We investigated the mechanism of disturbed skin barrier function in dogs with internal diseases. *Materials and Methods:* Healthy controls and dogs with systemic diseases were enrolled in three different disease groups: malignant tumor, hyperadrenocorticism and kidney disease. Transepidermal water loss (TEWL), serum levels of five selective pro-inflammatory cytokines and claudin-1, and complete blood count were measured. *Results:* TEWL was significantly increased in the malignant tumor group while serum claudin-1 concentrations were significantly lower compared to controls. Tumor necrosis factor- α was also significantly increased in the cancer group. In addition, the malignant tumor group showed significantly higher monocyte chemotactic protein-1 after chemotherapy, but lower interleukin-6 levels, compared to dogs with no chemotherapy. *Conclusion:* Skin barrier function was decreased in dogs with malignant tumors compared to dogs with other systemic diseases by oxidative stress and a reduction in tight junction proteins.

Skin manifestations of internal diseases have been studied both in human and veterinary medicine, and a variety of systemic disorders are associated with impaired skin barrier function (1). The epidermal barrier mediates multiple protective functions against pathogens, allergens, and oxidant stress, and prevents excessive transcutaneous water loss (2). The measurement of transepidermal water loss

(TEWL) is a useful non-invasive method to assess skin barrier function in various systemic diseases as well as primary skin conditions such as atopic dermatitis (3). In clinical studies, TEWL has been utilized to evaluate cutaneous conditions of patients with systemic diseases such as chronic kidney disease or cancer, undergoing chemotherapy (4-8). Skin barrier dysfunction in association with systemic disorders, however, this has not been widely investigated in veterinary medicine. Investigation into epidermal dysfunction in small animal medicine has been used to mostly evaluate impaired skin barrier in canine subjects with atopic dermatitis (9-11).

Assessment of skin barrier function in subjects with naturally occurring systemic diseases has not been reported in veterinary science except for experimental studies of mice with induced intestinal or liver injury, colitis, and colonic tumors (12-16). In a study of defective skin barrier function in mice with induced intestinal tumors, increased oxidative stress and inflammation was associated with impaired skin barrier (16). Pro-inflammatory factors and oxidative stress biomarkers have been measured to investigate the pathogenesis of a variety of systemic diseases both in human and veterinary medicine. The levels of pro-inflammatory cytokines and oxidative markers increased in dogs with mammary carcinomas (17). The overexpression of cytokines in the skin results in disturbed skin barrier, and uncontrolled loss of water and serum levels of cytokines also correlated with epidermal barrier disruption (18).

In this study, the skin barrier function of dogs with systemic diseases including malignant tumors, hyperadrenocorticism, and kidney disease was evaluated by measuring TEWL values and serum claudin-1. The cytokine levels in inflammation and under oxidative stress were also measured to assess the inflammatory condition in each disease group. The aim of this study was to investigate the relationship between disturbed skin barrier function and systemic inflammation as well as high oxidative stress levels in dogs with spontaneously occurring internal diseases.

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Correspondence to: Ha-Jung Kim, College of Veterinary Medicine, Chonnam National University, Yongbong-ro, Buk-gu, Gwangju 61168, Republic of Korea. Tel: +82 625302836, e-mail: kimhj614@jnu.ac.kr

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Materials and Methods

Study population. A total of 33 dogs including 29 privately owned dogs and 3 healthy beagles were enrolled in this study between June 2019 and June 2020 at the Veterinary Teaching Hospital, Department of Veterinary Internal Medicine, Chonnam National University, Gwangju, Republic of Korea. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Chonnam National University (CNU IACUC-YB-2019-23). Owner consent was obtained for all dogs and the beagles were cared for and used in accordance with the IACUC guidelines. Three beagles were enrolled in the healthy control group along with other clinically normal dogs from the Chonnam National University community. All the dogs enrolled in this study were subjected to complete physical examination, complete blood count (CBC) (ProCyt Dx Hematology Analyzer; IDEXX Laboratories, Inc., Westbrook, ME, USA) and biochemistry profile (Catalyst Dx Chemistry Analyzer; IDEXX Laboratories, Inc.).

Twenty-four client-owned dogs were diagnosed and enrolled in three disease groups: 1) malignant tumor; 2) hyperadrenocorticism (HAC); and 3) kidney disease. All dogs with malignant tumors were diagnosed with cancer, regardless of origin. Dogs with Cushing's disease were all diagnosed with naturally occurring, pituitary-dependent HAC, although the exclusion of adrenal-dependent HAC was not intended. The kidney disease group mostly consisted of dogs diagnosed with chronic kidney disease (CKD) in different International Renal Interest Society (IRIS) stages, with the exception of one case of acute kidney injury. Dogs were excluded from the study if they were diagnosed with primary skin disease (e.g., atopic dermatitis, demodicosis).

Sample collection and storage. At least 5 ml of blood was drawn by jugular venipuncture from each dog and divided into different tubes for blood tests and sampling. Plasma samples were collected into heparin tubes to determine the biochemistry profiles and ethylenediamine tetraacetic acid tube was used to determine complete blood count (CBC). Blood samples for measuring serum cytokine concentrations were transferred into tubes with serum clot activator and separation gel (VACUETTE® Serum Clot Activator Tubes; Greiner Bio One, Kremsmünster, Austria), centrifuged at 3,000 rpm for 10 minutes, and stored at -80°C until analysis.

Measurement of transepidermal water loss. TEWL was measured using the GPSkin Barrier® (GPOWER Inc, Seoul, Republic of Korea), a portable device using a closed chamber system, which has shown to be reliable for assessing skin barrier function (2). The TEWL value of each anatomical site including concave surface of the pinna, axilla, and inguinal area was repeatedly recorded by lightly placing the probe on the same region. A total of six values for each site were measured. The three sites were selected based on the low hair density, and generally measured in other studies assessing skin barrier functions in dogs (10, 11, 19, 20, 21). All TEWL values were measured in a controlled room at a temperature of 24-26°C and a relative humidity of 40-60%, after the dogs were acclimated for at least 30 min.

Measurement of serum inflammatory cytokines and claudin-1 levels. Canine-specific inflammatory cytokines in each serum sample were simultaneously measured *via* a commercially available multiplex

Table 1. Demographic characteristics of dogs with cancer, HAC, kidney disease and healthy controls.

	Control (n=9)	Cancer (n=10)	HAC (n=6)	Kidney (n=8)
Breeds				
American Cocker Spaniel		1	1	
Beagle	3			
English Cocker Spaniel		1		
German Shepherd	1			
Maltese	1	3	1	2
Miniature Pinscher	1			
Miniature Poodle	1	1	2	
Miniature Schnauzer			1	
Mixed	2			
Old English Bulldog		1		
Pekingese				2
Pomeranian		1		
Shih Tzu		1		1
Toy Poodle			1	
Welshi Corgi		1		
Yorkshire Terrier				3
Age (years) (Mean±SD)	4.11±2.52	9.30±3.37*	9.00±2.19	11.29±4.50**
Gender				
Male (neutered)	7 (4)	5 (5)	3 (3)	4 (4)
Female (neutered)	2 (1)	5 (3)	3 (2)	4 (2)
Weight (kg) (Mean±SD)	11.09±8.44	9.52±6.93	7.31±4.57	4.30±2.14*

* $p < 0.05$, ** $p < 0.01$.

cytokine immunoassay (CCYTOMAG-90K; MILLIPLEX® MAP Canine Cytokine/Chemokine Premixed Magnetic Bead Kit; EMD Millipore, Billerica, MA, USA) utilizing a multiplexing instrument (Luminex® 200; EMD Millipore) and data acquisition and analysis software system (xPONET®; Luminex, Austin, TX, USA). The following cytokines associated with inflammation and oxidative stress were selectively measured: granulocyte-macrophage colony-stimulating factor (GM-CSF), keratinocyte chemotactic-like (KC-like), monocyte chemotactic protein-1 (MCP-1), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α). Serum cytokine concentrations were all measured in duplicate and listed in pg/ml.

Claudin-1, the major component of tight junction in epidermis, was also measured in each dog's serum sample. An enzyme-linked immunosorbent assay (ELISA) kit (ECC0388; Canine CLDN1 ELISA Kit; ABclonal, Woburn, MA, USA) and a spectrophotometer (SpectraMax® 190 Microplate Reader; Molecular Devices, San Jose, CA, USA) were used to determine the serum levels of claudin-1, which were expressed as ng/ml. All tests were performed according to the manufacturer's instructions.

Statistical analyses. Statistical analyses were performed using GraphPad PRISM 8 for Mac (GraphPad Software, San Diego, CA, USA). Data were analyzed using non-parametric tests due to the

Table II. Number of dogs diagnosed with different cancer types.

Cancer type	Number
Lymphoma	4
Mammary gland carcinoma	1
Osteosarcoma	1
Histiocytic sarcoma	1
Multiple primary cancers	3 ^a

^aThree patients diagnosed with multiple primary malignant tumors of different histological origin. 1) Mammary gland carcinoma, leiomyosarcoma, and sebaceous epithelioma. 2) Extraskelatal osteosarcoma, liposarcoma, and malignant peripheral nerve sheath tumor. 3) Lymphoma and mast cell tumor.

small sample size of each group. Kruskal-Wallis test and Dunn's multiple comparison tests were conducted to determine statistically significant differences in age, body weight, TEWL value, serum claudin-1, individual cytokine levels and CBCs between healthy controls and disease groups. The Mann-Whitney test was also performed to investigate the differences in TEWL values, serum claudin-1 and individual cytokine levels between two subgroups of cancer subjects: those who received chemotherapy and those who did not. $p < 0.05$ was considered significant for all tests.

Results

Animal characteristics. The following dog breeds were included in the study: American Cocker Spaniel, Beagle, English Cocker Spaniel, German Shepherd, Maltese, Miniature Pinscher, Miniature Poodle, Miniature Schnauzer, mixed breeds, Old English Bulldog, Pekingese, Pomeranian, Shih Tzu, Toy Poodle, Welshi Corgi and Yorkshire Terrier. The healthy control group consisted of a total of 9 dogs, including 7 males (4 neutered, 3 intact) and 2 female (1 spayed, 1 intact) with a mean age of 4.11 years, which was significantly lower than that of the tumor ($p < 0.05$) or renal ($p < 0.01$) group (Table I). Dogs with kidney disease were mostly small breeds with significantly lower body weight compared to controls ($p < 0.05$). The most common type of malignant tumors was lymphoma (40%) in various stages, followed by multiple primary malignancies (30%) (Table II). All 6 canine subjects with Cushing's disease were diagnosed with spontaneous pituitary-dependent hyperadrenocorticism. The kidney disease group represented CKD patients in all IRIS stages and one case of acute kidney injury IRIS grade IV.

TEWL values of cancer, HAC, kidney disease groups and healthy controls. The average TEWL value of all examined body sites in healthy dogs was 7.67 ± 2.81 g/m²/h. All three patient groups had higher mean values than healthy controls without significant differences (Table III). The mean TEWL values of each pinna, axilla, and inguinal area in all the animal groups were mostly higher than those of healthy

Table III. Transepidermal water loss in dogs with cancer, HAC, kidney disease and healthy controls (g/m²/h) (mean $\pm$ SD).

Anatomical sites	Control (n=9)	Cancer (n=10)	HAC (n=6)	Kidney (n=8)
Pinna	8.70 ± 5.68	10.01 ± 9.66	6.86 ± 2.24	8.12 ± 3.26
Axilla	7.70 ± 3.52	$14.24 \pm 4.76^{**}$	9.64 ± 2.03	13.19 ± 7.05
Inguinal	6.59 ± 3.62	8.73 ± 2.10	9.44 ± 9.14	10.40 ± 4.70
Average	7.67 ± 2.81	11.00 ± 4.48	8.65 ± 3.89	10.57 ± 3.80

$^{**}p < 0.01$.

Figure 1. Transepidermal water loss in the pinna, axilla, and inguinal areas and their average values in dogs with cancer, HAC, kidney disease and healthy controls (g/m²/h). $^{**}p < 0.01$. Kruskal-Wallis test and Dunn's multiple comparison tests were used.

controls, with the exception of pinna in the HAC group. Dogs with malignant tumors showed a statistically significant increase in TEWL values in the axillae compared with healthy controls at ($p = 0.01$, Figure 1).

Serum cytokine concentrations in cancer, HAC, kidney disease groups and healthy controls: GM-CSF, KC-like, MCP-1, IL-6, and TNF- α . The mean serum cytokine levels in each group are summarized in Table IV. The mean values of serum GM-CSF, KC-like and IL-6 in all patient groups were higher than those of the healthy group at a level that exceeded statistical significance. Dogs with cancer carried the highest mean concentration of MCP-1 compared to healthy controls and other disease groups, although no significant difference was observed. Levels of serum TNF- α were also elevated in all disease groups, with a significant difference between the cancer and the healthy group ($p < 0.05$).

Table IV. Serum cytokine concentrations of dogs with cancer, HAC, kidney disease and healthy controls (pg/ml) (mean±SD).

Cytokines	Control	Cancer	HAC	Kidney
GM-CSF	16.03±2.08	27.27±12.76	41.61±45.62	27.49±15.84
n	6	9	6	8
p-Value		0.129	0.541	0.648
KC-like	58.18±54.77	491.00±1,010.9	521.84±739.83	284.52±440.12
n	6	10	5	8
p-Value		>0.999	0.331	>0.999
MCP-1	52.95±14.02	76.27±77.25	40.19±15.55	48.42±34.64
n	7	10	6	8
p-Value		>0.999	0.215	0.241
IL-6	39.41±25.46	531.22±1,333.7	47.53±25.20	1,995.1±3,650.2
n	9	9	6	8
p-Value		0.857	>0.999	>0.999
TNF-α	43.65±27.31	117.48±93.82*	55.36±20.30	175.44±222.76
n	7	10	5	8
p-Value		0.023	>0.999	0.196

* $p<0.05$.

Differences in serum cytokine concentrations between disease groups and healthy controls are depicted in Figure 2.

Differences in TEWL values and serum cytokine concentrations between chemotherapy and non-chemotherapy groups. Dogs with malignant tumors were divided into two subgroups: 1) four dogs exposed to chemotherapy at the time of sampling; and 2) six dogs, which were not exposed to chemotherapy. The differences in TEWL values, serum claudin-1 levels, serum cytokine concentrations, and CBC between the two subgroups were investigated. Animals that received chemotherapy carried a higher mean value of claudin-1 and higher mean TEWL values at all the examined sites than those in the non-chemotherapy group without statistical significance (Figure 3). Serum MCP-1 was significantly increased in dogs with cancer undergoing chemotherapy compared with those in the non-chemotherapy group ($p<0.05$), while serum IL-6 values were significantly decreased in the chemotherapy group ($p<0.05$, Figure 4). Results of CBC did not differ significantly between the two subgroups of cancer patients other than the mean value of mean corpuscular hemoglobin (MCH), which was significantly higher in the chemotherapy group (23.73 ± 1.06 ; reference range, 21.2-25.9 pg) compared with the non-chemotherapy group ($p<0.05$); however, both mean values were in a normal reference range.

Complete blood cell counts in cancer, HAC, kidney disease groups and healthy controls. The results of CBC in dogs under cancer, HAC, kidney disease groups and controls are summarized in Table V. Hematocrit (HCT) and red blood cell count (RBC) were low in dogs with cancer and kidney

disease at a level that exceeded statistical significance. Levels of white blood cells (WBC), neutrophils and monocytes were higher in dogs with cancer and kidney disease compared with healthy controls, without significant differences. Dogs with malignant tumors had significantly higher basophils (0.048 ± 0.069 ; reference range, 0.00 - 0.10 K/ μ l) ($p<0.05$) compared with the control group, while the mean values of both groups remained within a normal reference range (Figure 5).

Serum claudin-1 concentrations of dogs in the cancer, HAC, kidney disease groups and controls. Dogs with malignant tumors showed significantly lower mean concentrations of serum claudin-1 compared with healthy controls ($p<0.05$) (Table VI). The cancer group also showed the lowest mean value of claudin-1 compared with other disease groups (Figure 6).

Discussion

This is the first study to evaluate skin barrier function in different types of systemic disorders in veterinary medicine. Three prevalent systemic diseases including cancer, HAC, and kidney disease were selected based upon previous studies investigating human diseases frequently associated with skin manifestations. Skin barrier function of each group of animals and controls was evaluated by measuring TEWL, which showed higher average values in all disease groups compared to healthy controls, without statistical significance. Skin barrier function was significantly decreased in dogs with malignant tumors compared to healthy dogs at the axillary sites. The mean TEWL in the axilla of dogs in the cancer group was 14.24 ± 4.76 g/ m^2 /h, which was significantly higher than in healthy controls (7.70 ± 3.52 g/ m^2 /h). This result was comparable to a previous study reporting 6.45 (range, 2.57-18.7) g/ m^2 /h as the median TEWL value in the axilla of healthy dogs (11). However, the mean axillary TEWL of the cancer group (14.24 g/ m^2 /h) was relatively low compared to the value in dogs with atopic dermatitis mostly in 20 other studies (22, 23). This result suggests that skin barrier defects in subjects with cancer can be detected early by measuring TEWL.

Serum claudin-1 was also measured to evaluate the skin barrier function of each dog and the mean value was lowest in the cancer group, with a significant difference when compared with the healthy dogs ($p<0.05$). Claudin-1 is a major transmembrane protein of epidermal tight junctions, and its decrease correlates with inflammation and impairment of skin barrier function in atopic dermatitis (24). Claudins also play a role in cellular growth and adhesion, and upregulated or downregulated expression of claudin-1 has been associated with tumor progression and metastasis in different types of human and canine cancers (25-29). In

Figure 2. Differences in serum cytokine concentrations between dogs with cancer, HAC, kidney disease and healthy controls. * $p < 0.05$. Kruskal-Wallis test and Dunn's multiple comparison tests were used. GM-CSF, Granulocyte-macrophage colony-stimulating factor; KC-like, keratinocyte chemotactic-like (KC-like); MCP-1, monocyte chemotactic protein-1; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α .

this study, most of the dogs with cancer manifested lymphoma or sarcoma except for two dogs with mammary gland carcinomas. Although claudins are mainly expressed in epithelial tumors and absent or reduced expression was reported in lymphomas and sarcomas (25, 30), discrepancies were observed in serum claudin-1 levels between animals manifesting lymphoma in this study (2.96-11.13 ng/ml). Other dogs with sarcoma and carcinoma also showed various serum levels of claudin-1. Therefore, the significantly reduced serum claudin-1 levels in dogs with tumors in this study might reflect both tumor progression and impaired epidermal barrier function. Appropriate therapy may provide a defense against secondary infection and other cutaneous complications in these dogs.

Serum levels of five pro-inflammatory cytokines were mostly increased in the cancer group compared to healthy controls. Serum TNF- α levels were significantly increased ($p < 0.05$) in the cancer group than in the healthy controls. TNF- α is an inflammatory mediator in impaired skin barrier

Figure 3. Transepidermal water loss in the pinna, axilla, and inguinal areas and their average values in two subgroups of dogs with cancer: chemotherapy and non-chemotherapy (g/m²/h). The Mann-Whitney test was used.

Figure 4. Differences in serum cytokine concentrations between dogs with cancer treated with and without chemotherapy. * $p < 0.05$. The Mann-Whitney test was used. GM-CSF, Granulocyte-macrophage colony-stimulating factor; KC-like, keratinocyte chemotactic-like (KC-like); MCP-1, monocyte chemotactic protein-1; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α .

Table V. Complete blood cell counts of dogs with cancer, HAC, kidney disease and healthy controls (mean $\pm$ SD).

Blood cell line	Reference interval	Control (n=9)	Cancer (n=10)	HAC (n=6)	Kidney (n=8)
HCT (%)	37.3-61.7	46.89 $\pm$ 7.25	38.80 $\pm$ 7.89	42.10 $\pm$ 6.46	33.43 $\pm$ 12.88
RBC (M/ μ l)	5.65-8.87	7.25 $\pm$ 1.20	5.99 $\pm$ 1.12	6.60 $\pm$ 0.64	5.48 $\pm$ 2.16
WBC (K/ μ l)	5.05-16.76	10.55 $\pm$ 5.22	14.40 $\pm$ 12.12	8.97 $\pm$ 2.80	17.84 $\pm$ 15.65
Neutrophils (K/ μ l)	2.95-11.64	5.82 $\pm$ 1.87	11.02 $\pm$ 9.29	6.76 $\pm$ 2.64	14.27 $\pm$ 12.45
Monocytes (K/ μ l)	0.16-1.12	0.39 $\pm$ 0.26	1.04 $\pm$ 1.49	0.45 $\pm$ 0.25	1.16 $\pm$ 1.44
Eosinophils (K/ μ l)	0.06-1.23	0.33 $\pm$ 0.25	0.21 $\pm$ 0.11	0.28 $\pm$ 0.12	0.16 $\pm$ 0.25
Basophils (K/ μ l)	0.00-0.10	0.003 $\pm$ 0.005	0.048 $\pm$ 0.069*	0.035 $\pm$ 0.053	0.018 $\pm$ 0.025
Lymphocytes (K/ μ l)	1.05-5.10	2.41 $\pm$ 1.37	2.10 $\pm$ 1.63	1.48 $\pm$ 0.45	2.24 $\pm$ 2.04
Platelets (K/ μ l)	148-484	275.1 $\pm$ 132.8	375.7 $\pm$ 177.6	381.3 $\pm$ 180.9	469.4 $\pm$ 241.7

* $p < 0.05$.

function along with IL-6 and oxidative stress mediators during chronic liver injury and colitis in mice (13, 15). The mean values of GM-CSF, KC-like, MCP-1 and IL-6 were all higher than in the control group without significant difference, which might be explained by a high standard deviation due to the small sample size. The cancer group represented dogs with various types of malignant tumors in different stages and body condition, which might have skewed the data distribution. In the current study, half of the dogs with cancer were diagnosed with lymphoma (including a case of multiple primary cancers). In a previous study comparing serum cytokine levels between cases of canine lymphoma and healthy controls, KC-like, MCP-1, and IL-6 (but not GM-CSF and TNF- α) were significantly increased in those with lymphoma (31). GM-CSF is a pro-inflammatory cytokine that promotes the production of granulocytes (neutrophils, eosinophils, and basophils) and

monocytes (32), and was increased in the epidermis of mice after skin barrier disruption (33). KC-like is a canine cytokine similar to murine keratinocyte-derived chemokine (34) and a small number of studies in veterinary medicine suggested that it might be associated with systemic inflammation of infectious origin (34-36). MCP-1 is produced predominantly by dendritic cells and macrophages in response to inflammation and oxidative stress (37). It has been considered a tumor-promoting chemokine and investigated as a poor prognostic factor in human cancer patients (38). In veterinary medicine, previous studies reported the prognostic role of serum MCP-1 in dogs with lymphoma (39), and it was significantly increased in canine disseminated histiocytic sarcoma (40). It was also reported to be a potential skin biomarker that significantly increased in skin barrier damage in humans (41). In the current study, the mean MCP-1 level in the cancer group was significantly

Figure 5. Complete blood cell counts of dogs with cancer, HAC, kidney disease and healthy controls. Horizontal bars represent the mean value and error bars indicate standard deviations. * $p < 0.05$. Kruskal-Wallis test and Dunn's multiple comparison tests were used.

higher compared to the healthy controls. However, it was significantly increased in dogs with cancer treated with chemotherapy ($p < 0.05$) compared with other dogs with

cancer that did not, which was consistent with previous studies in humans, which revealed an increase in circulating MCP-1 after chemotherapy (42, 43). Most cancer

Table VI. Serum claudin-1 concentrations of dogs with cancer, HAC, kidney disease and healthy controls (ng/ml) (mean±SD).

	Control	Cancer	HAC	Kidney
Claudin-1	11.91±2.67	6.34±3.02*	9.10±5.66	8.06±4.81
N	8	9	6	7
p-Value		0.021	0.388	0.112

* $p < 0.05$.

chemotherapeutic agents elevate reactive oxygen species (ROS) in cancer cells *via* mitochondrial ROS generation and inhibition of the antioxidant system, inducing oxidative stress and cell death (44). Exposure to chemotherapeutic drugs leads to increased ROS generation, and may increase MCP-1, which is upregulated by oxidative stress and inflammatory stimuli (37). Circulating levels of IL-6, however, were significantly decreased in the chemotherapy group ($p < 0.05$). This result could also be explained by the response to chemotherapy in that reduced IL-6 expression represents the efficacy of chemotherapeutic agents (45).

In the CBC analysis, the levels of WBC, neutrophils and monocytes were increased in dogs with cancer and kidney disease, suggesting acute and chronic pro-inflammatory state, although significant differences were not observed. Basophils were significantly increased in the dogs with cancer compared with the controls but remained in the normal range. Basophils are rarely observed in the blood of healthy dogs, and their role is mostly associated with hypersensitivity reactions and defense against parasites (46). However, basophils can be quickly recruited in response to inflammation and activated under immunological conditions such as cancer (46). A recent study investigating human cancer suggested a potential prognostic value of activated circulating and tumor-infiltrating basophils (47).

In the current study, the role of parameters assessing skin barrier integrity was limited to TEWL and serum claudin-1. In veterinary medicine, other skin biomarkers such as stratum corneum hydration (SCH) and skin surface pH have also been used to assess skin barrier function in dogs with atopic dermatitis (11, 48). Although TEWL is most commonly used to assess skin barrier function, measuring TEWL requires a strict protocol to minimize data variation, which enhances the utility of additional skin biomarkers in evaluating the abnormalities of skin barrier. The detection of cytokines also needs to be timed because the release of pro-inflammatory cytokines fluctuates due to the short half-lives (40). Another limitation is the small sample size of each group in this study. Larger sample size is desirable to investigate the possibility of correlation between TEWL and pro-inflammatory mediators. Importantly, measuring the mediators of oxidative stress such as malondialdehyde

Figure 6. Differences in serum claudin-1 concentrations between dogs with cancer, HAC, kidney disease and healthy controls. * $p < 0.05$. Kruskal-Wallis test and Dunn's multiple comparison tests were used.

(MDA) enables the evaluation of ROS generated in the system and its effect on skin barrier function given the interdependence of inflammation and oxidative stress (49).

In conclusion, dogs with spontaneous tumors showed significantly impaired skin barrier function compared to other chronic inflammatory patients (cancer, HAC, and kidney diseases), which was detected early by measuring TEWL. Initiating supportive care may improve the quality of life. Pro-inflammatory mediators including GM-CSF, KC-like, MCP-1, IL-6, and TNF- α showed an increase in dogs with chronic inflammation compared to healthy controls, while TNF- α showed significant difference in cancer dogs. Serum MCP-1 was significantly increased in dogs with tumors undergoing chemotherapy compared with the non-chemotherapy group, which could be associated with the role of MCP-1 in the inflammation and oxidative stress. IL-6 was significantly decreased in dogs exposed to chemotherapy, indicating the chemotherapy response. Decreased serum claudin-1 also showed impaired skin barrier function affected by oxidative stress in dogs with tumors. Further studies with larger sample size are needed to measure multiple parameters of skin barrier function and oxidative stress mediators.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

MG and H-JK designed the experiments and drafted the manuscript. MG carried out the experiments and analyzed the data. H-JK supervised the whole study, reviewed and edited the manuscript. H-JK also acquired the funding for the study.

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References

- Outerbridge CA: Cutaneous manifestations of internal diseases. *Vet Clin North Am Small Anim Pract* 43(1): 135-152, 2013. PMID: 23182329. DOI: 10.1016/j.cvsm.2012.09.010
- Elias PM: The skin barrier as an innate immune element. *Semin Immunopathol* 29(1): 3-14, 2007. PMID: 17621950. DOI: 10.1007/s00281-007-0060-9
- Ye L, Wang Z, Li Z, Lv C and Man MQ: Validation of GPSkin Barrier® for assessing epidermal permeability barrier function and stratum corneum hydration in humans. *Skin Res Technol* 25(1): 25-29, 2019. PMID: 29863296. DOI: 10.1111/srt.12590
- Yosipovitch G, Duque MI, Patel TS, Ishiuiji Y, Guzman-Sanchez DA, Dawn AG, Freedman BI, Chan YH, Crumrine D and Elias PM: Skin barrier structure and function and their relationship to pruritus in end-stage renal disease. *Nephrol Dial Transplant* 22(11): 3268-3272, 2007. PMID: 17595180. DOI: 10.1093/ndt/gfm375
- Topham E: Cutaneous manifestations of cancer and chemotherapy. *Clin Med (Lond)* 9(4): 375-379, 2009. PMID: 19728518. DOI: 10.7861/clinmedicine.9-4-375
- Fabbrocini G, Cameli N, Romano MC, Mariano M, Panariello L, Bianca D and Monfrecola G: Chemotherapy and skin reactions. *J Exp Clin Cancer Res* 31: 50, 2012. PMID: 22640460. DOI: 10.1186/1756-9966-31-50
- Wojtowicz-Prus E, Kilis-Pstrusinska K, Reich A, Zachwieja K, Miklaszewska M, Szczepanska M and Szepietowski JC: Disturbed skin barrier in children with chronic kidney disease. *Pediatr Nephrol* 30(2): 333-338, 2015. PMID: 25127919. DOI: 10.1007/s00467-014-2932-2
- Chandra F, Sandiono D, Sugiri U, Suwarsa O and Gunawan H: Cutaneous side effects and transepidermal water loss to gefitinib: a study of 11 patients. *Dermatol Ther (Heidelb)* 7(1): 133-141, 2017. PMID: 28004308. DOI: 10.1007/s13555-016-0163-0
- Olivry T: Is the skin barrier abnormal in dogs with atopic dermatitis? *Vet Immunol Immunopathol* 144(1-2): 11-16, 2011. PMID: 21835476. DOI: 10.1016/j.vetimm.2011.07.014
- Zajac M, Szczepanik MP, Wilkołek PM, Adamek LR, Pomorski ZJ, Sitkowski W and Gołyński MG: Assessment of the relationship between transepidermal water loss (TEWL) and severity of clinical signs (CADESI-03) in atopic dogs. *Vet Dermatol* 25(6): 503-6, e83, 2014. PMID: 25132586. DOI: 10.1111/vde.12150
- Cobiella D, Archer L, Bohannon M and Santoro D: Pilot study using five methods to evaluate skin barrier function in healthy dogs and in dogs with atopic dermatitis. *Vet Dermatol*, 2019. PMID: 30644144. DOI: 10.1111/vde.12723
- Yokoyama S, Hiramoto K, Koyama M and Ooi K: Skin disruption is associated with indomethacin-induced small intestinal injury in mice. *Exp Dermatol* 23(9): 659-663, 2014. PMID: 25041031. DOI: 10.1111/exd.12499
- Yokoyama S, Hiramoto K, Koyama M and Ooi K: Impairment of skin barrier function *via* cholinergic signal transduction in a dextran sulphate sodium-induced colitis mouse model. *Exp Dermatol* 24(10): 779-784, 2015. PMID: 26014805. DOI: 10.1111/exd.12775
- Yokoyama S, Hiramoto K, Koyama M and Ooi K: Impaired skin barrier function in mice with colon carcinoma induced by azoxymethane and dextran sodium sulfate. *Biol Pharm Bull* 38(6): 947-950, 2015. PMID: 26027838. DOI: 10.1248/bpb.b15-00208
- Yokoyama S, Hiramoto K, Koyama M and Ooi K: Chronic liver injury in mice promotes impairment of skin barrier function *via* tumor necrosis factor- α . *Cutan Ocul Toxicol* 35(3): 194-203, 2016. PMID: 26362357. DOI: 10.3109/15569527.2015.1076433
- Yokoyama S, Hiramoto K and Yamate Y: Impaired skin barrier function caused by reactive oxygen species in mice with colonic tumours. *Cutan Ocul Toxicol* 38(4): 349-355, 2019. PMID: 31137980. DOI: 10.1080/15569527.2019.1622559
- Machado VS, Crivellenti LZ, Bottari NB, Tonin AA, Pelinson LP, Borin-Crivellenti S, Santana AE, Torbitz VD, Moresco RN, Duarte T, Duarte MM, Schetinger MR, Morsch VM, Jaques JA, Tinucci-Costa M and Da Silva AS: Oxidative stress and inflammatory response biomarkers in dogs with mammary carcinoma. *Pathol Res Pract* 211(9): 677-681, 2015. PMID: 26166177. DOI: 10.1016/j.prp.2015.06.011
- Hänel KH, Cornelissen C, Lüscher B and Baron JM: Cytokines and the skin barrier. *Int J Mol Sci* 14(4): 6720-6745, 2013. PMID: 23531535. DOI: 10.3390/ijms14046720
- Oh WS and Oh TH: Measurement of transepidermal water loss from clipped and unclipped anatomical sites on the dog. *Aust Vet J* 87(10): 409-412, 2009. PMID: 19796162. DOI: 10.1111/j.1751-0813.2009.00481.x
- Lau-Gillard PJ, Hill PB, Chesney CJ, Budleigh C and Immonen A: Evaluation of a hand-held evaporimeter (VapoMeter) for the measurement of transepidermal water loss in healthy dogs. *Vet Dermatol* 21(2): 136-145, 2010. PMID: 19961567. DOI: 10.1111/j.1365-3164.2009.00738.x
- Marsella R: Are transepidermal water loss and clinical signs correlated in canine atopic dermatitis? A compilation of studies. *Vet Dermatol* 23(3): 238-e49, 2012. PMID: 22575022. DOI: 10.1111/j.1365-3164.2012.01055.x
- Shimada K, Yoon JS, Yoshihara T, Iwasaki T and Nishifuji K: Increased transepidermal water loss and decreased ceramide content in lesional and non-lesional skin of dogs with atopic dermatitis. *Vet Dermatol* 20(5-6): 541-546, 2009. PMID: 20178492. DOI: 10.1111/j.1365-3164.2009.00847.x
- Szczepanik M, Wilkołek P, Gołyński M, Sitkowski W, Taszkun I and Toczek W: The influence of treatment with lokivetmab on transepidermal water loss (TEWL) in dogs with spontaneously occurring atopic dermatitis. *Vet Dermatol* 30(4): 330-e93, 2019. PMID: 31090122. DOI: 10.1111/vde.12758
- Bergmann S, von Buenau B, Vidal-Y-Sy S, Haftek M, Wladykowski E, Houdek P, Lezius S, Duplan H, Bäsler K, Dähnhardt-Pfeiffer S, Gorzelanny C, Schneider SW, Rodriguez E, Stölzl D, Weidinger S and Brandner JM: Claudin-1 decrease impacts epidermal barrier function in atopic dermatitis lesions dose-dependently. *Sci Rep* 10(1): 2024, 2020. PMID: 32029783. DOI: 10.1038/s41598-020-58718-9
- Ding L, Lu Z, Lu Q and Chen YH: The claudin family of proteins in human malignancy: a clinical perspective. *Cancer Manag Res* 5: 367-375, 2013. PMID: 24232410. DOI: 10.2147/CMAR.S38294

- 26 Bhat AA, Syed N, Therachiyil L, Nisar S, Hashem S, Macha MA, Yadav SK, Krishnankutty R, Muralitharan S, Al-Naemi H, Bagga P, Reddy R, Dhawan P, Akobeng A, Uddin S, Frenneaux MP, El-Rifai W and Haris M: Claudin-1, a double-edged sword in cancer. *Int J Mol Sci* 21(2): 569, 2020. PMID: 31952355. DOI: 10.3390/ijms21020569
- 27 Jakab C, Rusvai M, Gálfi P, Szabó Z, Szabára A and Kulka J: Expression of claudin-1, -3, -4, -5 and -7 proteins in low grade colorectal carcinoma of canines. *Histol Histopathol* 25(1): 55-62, 2010. PMID: 19924641. DOI: 10.14670/HH-25.55
- 28 Flores AR, Rêma A, Amorim I and Gartner F: Altered expression of claudin-1 is related with malignancy in canine thyroid tumors. *In Vivo* 29(1): 23-28, 2015. PMID: 25600525.
- 29 Hammer SC, Nagel S, Junginger J, Hewicker-Trautwein M, Wagner S, Heisterkamp A, Ngezahayo A, Nolte I and Murua Escobar H: Claudin-1, -3, -4 and -7 gene expression analyses in canine prostate carcinoma and mammary tissue derived cell lines. *Neoplasma* 63(2): 231-238, 2016. PMID: 26774145. DOI: 10.4149/208_150924N505
- 30 Soini Y: Expression of claudins 1, 2, 3, 4, 5 and 7 in various types of tumours. *Histopathology* 46(5): 551-560, 2005. PMID: 15842637. DOI: 10.1111/j.1365-2559.2005.02127.x
- 31 Calvalido J, Wood GA, Mutsaers AJ, Wood D, Sears W and Woods JP: Comparison of serum cytokine levels between dogs with multicentric lymphoma and healthy dogs. *Vet Immunol Immunopathol* 182: 106-114, 2016. PMID: 27863540. DOI: 10.1016/j.vetimm.2016.10.009
- 32 Hamilton JA: GM-CSF-dependent inflammatory pathways. *Front Immunol* 10: 2055, 2019. PMID: 31552022. DOI: 10.3389/fimmu.2019.02055
- 33 Wood LC, Jackson SM, Elias PM, Grunfeld C and Feingold KR: Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 90(2): 482-487, 1992. PMID: 1644919. DOI: 10.1172/JCI115884
- 34 Karlsson I, Hagman R, Johannisson A, Wang L, Södersten F and Wernersson S: Multiplex cytokine analyses in dogs with pyometra suggest involvement of KC-like chemokine in canine bacterial sepsis. *Vet Immunol Immunopathol* 170: 41-46, 2016. PMID: 26837616. DOI: 10.1016/j.vetimm.2016.01.005
- 35 Taylor AR, Welsh CJ, Young C, Spoor E, Kerwin SC, Griffin JF, Levine GJ, Cohen ND and Levine JM: Cerebrospinal fluid inflammatory cytokines and chemokines in naturally occurring canine spinal cord injury. *J Neurotrauma* 31(18): 1561-1569, 2014. PMID: 24786364. DOI: 10.1089/neu.2014.3405
- 36 Galán A, Mayer I, Rafaj RB, Bendelja K, Sušić V, Cerón JJ and Mrljak V: MCP-1, KC-like and IL-8 as critical mediators of pathogenesis caused by *Babesia canis*. *PLoS One* 13(1): e0190474, 2018. PMID: 29304171. DOI: 10.1371/journal.pone.0190474
- 37 Deshmane SL, Kremlev S, Amini S and Sawaya BE: Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 29(6): 313-326, 2009. PMID: 19441883. DOI: 10.1089/jir.2008.0027
- 38 Conti I and Rollins BJ: CCL2 (monocyte chemoattractant protein-1) and cancer. *Semin Cancer Biol* 14(3): 149-154, 2004. PMID: 15246049. DOI: 10.1016/j.semcancer.2003.10.009
- 39 Perry JA, Thamm DH, Eickhoff J, Avery AC and Dow SW: Increased monocyte chemotactic protein-1 concentration and monocyte count independently associate with a poor prognosis in dogs with lymphoma. *Vet Comp Oncol* 9(1): 55-64, 2011. PMID: 21303454. DOI: 10.1111/j.1476-5829.2010.00235.x
- 40 Nikolic Nielsen L, Kjelgaard-Hansen M and Kristensen AT: Monocyte chemotactic protein-1 and other inflammatory parameters in Bernese Mountain dogs with disseminated histiocytic sarcoma. *Vet J* 198(2): 424-428, 2013. PMID: 23992872. DOI: 10.1016/j.tvjl.2013.07.030
- 41 Hulshof L, Hack DP, Hasnoe QCJ, Dontje B, Jakasa I, Riethmüller C, McLean WHI, van Aalderen WMC, Van't Land B, Kezic S, Sprickelman AB and Middelkamp-Hup MA: A minimally invasive tool to study immune response and skin barrier in children with atopic dermatitis. *Br J Dermatol* 180(3): 621-630, 2019. PMID: 29989151. DOI: 10.1111/bjd.16994
- 42 Mahoney SE, Davis JM, Murphy EA, McClellan JL, Gordon B and Pena MM: Effects of 5-fluorouracil chemotherapy on fatigue: role of MCP-1. *Brain Behav Immun* 27(1): 155-161, 2013. PMID: 23085145. DOI: 10.1016/j.bbi.2012.10.012
- 43 Geller MA, Bui-Nguyen TM, Rogers LM and Ramakrishnan S: Chemotherapy induces macrophage chemoattractant protein-1 production in ovarian cancer. *Int J Gynecol Cancer* 20(6): 918-925, 2010. PMID: 20683396. DOI: 10.1111/IGC.0b013e3181e5c442
- 44 Yang H, Villani RM, Wang H, Simpson MJ, Roberts MS, Tang M and Liang X: The role of cellular reactive oxygen species in cancer chemotherapy. *J Exp Clin Cancer Res* 37(1): 266, 2018. PMID: 30382874. DOI: 10.1186/s13046-018-0909-x
- 45 Ghandadi M and Sahebkar A: Interleukin-6: a critical cytokine in cancer multidrug resistance. *Curr Pharm Des* 22(5): 518-526, 2016. PMID: 26601970. DOI: 10.2174/1381612822666151124234417
- 46 Rigoni A, Colombo MP and Pucillo C: Mast cells, basophils and eosinophils: From allergy to cancer. *Semin Immunol* 35: 29-34, 2018. PMID: 29428698. DOI: 10.1016/j.smim.2018.02.001
- 47 Bax HJ, Chauhan J, Stavrika C, Khiabany A, Nakamura M, Pellizzari G, Ilieva KM, Lombardi S, Gould HJ, Corrigan CJ, Till SJ, Katugampola S, Jones PS, Barton C, Winship A, Ghosh S, Montes A, Josephs DH, Spicer JF and Karagiannis SN: Basophils from cancer patients respond to immune stimuli and predict clinical outcome. *Cells* 9(7): 1631, 2020. PMID: 32645919. DOI: 10.3390/cells9071631
- 48 Bradley CW, Morris DO, Rankin SC, Cain CL, Mistic AM, Houser T, Mauldin EA and Grice EA: Longitudinal evaluation of the skin microbiome and association with microenvironment and treatment in canine atopic dermatitis. *J Invest Dermatol* 136(6): 1182-1190, 2016. PMID: 26854488. DOI: 10.1016/j.jid.2016.01.023
- 49 Biswas SK: Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid Med Cell Longev* 2016: 5698931, 2016. PMID: 26881031. DOI: 10.1155/2016/5698931

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