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A comparative time-dependent study of hematology, serum gastrin concentrations, and gastroscopic assessment of meloxicam-induced gastric ulceration in dogs

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Abstract

Background: Diagnosis of gastric ulcers by methods other than gastroscopy in dogs has been problematic for many years and biomarkers such as serum gastrin (SG) concentrations have been introduced as a noninvasive way to evaluate gastric diseases.

Objectives: To determine the time course changes in hematology, SG concentrations, and gastroscopic images of meloxicam-induced gastric ulceration in dogs and identify a relationship between SG and gastroscopic image analysis in a clinical setting.

Animals: Fifteen crossbreed dogs.

Methods: Two groups: control (n = 5) and meloxicam-treated (n = 10). The meloxicam-treated group received meloxicam 0.2 mg/kg PO for 15 days. Clinical signs, hematology, SG, and image analysis (PI, pixel intensity; ID, integrated density; RA, relative area; and UI, ulcer index) of the gastroscopic examination were evaluated across time (T5, time 5 day; T10, time 10 day; and T15, time 15 day).

Results: Significant changes were observed among 3 time points and between the 2 groups in terms of SG, hematology, and gastroscopic image analysis. In the meloxicam-treated group, decreases in hemoglobin concentration, red blood cell count and packed cell volume at T10 and T15 ($P = .0001$) were observed, whereas SG, ID, and UI increased over time ($P < .0001$). The PI decreased significantly ($P = .0001$) in the meloxicam-treated group compared to controls. Significant correlations were found between SG and PI, and ID and ulcer area ($r = -0.89, 0.81, 0.64$), respectively.

Abbreviations: GU, gastric ulcer; ID, integrated density; PI, pixel intensity; RA, relative area; SG, serum gastrin; T10, time 10 day of the experiment; T15, time 15 day of the experiment; T5, time 5 day of the experiment; UI, ulcer index.

Conclusion and Clinical Importance: Gastroscopy is the gold standard for early descriptive diagnosis of gastric ulcerations in dogs, and SG is a good indicator for meloxicam-induced gastric ulcers in dogs and can predict the gastroscopic score of the lesion.

KEYWORDS
dog, gastric ulcer, gastrin, gastroscopy

1 | INTRODUCTION

Gastric ulceration (GU) in dogs is a well-recognized condition that usually occurs as a sequela of gastric mucosal barrier dysfunction associated with ingestion of sharp foreign objects[1] or impairment of prostaglandin gastroprotection[2,3] and hypersecretion of gastric acid.[4] Gastric ulceration has been described as a mucosal defect that exposes the submucosa and deeper layers to gastric acid.[5,6] Gastric ulceration commonly is found in young and adult animals, but the prevalence is higher in mature and athletic animals compared to other canine populations. Endoscope studies identified gastric ulceration in 48.5% of canine athletes.[6] Although nonsteroidal anti-inflammatory drugs (NSAIDs), hypovolemic and septic shock, surgery, neurosurgery, neoplasia, hepatic or renal disease, and foreign bodies have been implemented as causes of gastric ulceration and erosion,[7,8] frequent use of NSAIDs is the most common cause of gastrointestinal ulcerations, especially in dogs.[7,9,10]

History, complete physical examination, and clinical signs such as vomiting, melena, and hematemesis can provide valuable information to establish a differential diagnosis with regard to gastric ulceration until a definitive diagnosis by gastroscopy or other confirmatory tools can be carried out.[11,12]

Gastrin is produced by the G-cells in the antrum of the stomach, and plays a central role in the regulation of gastric acid secretion in humans and animals.[13,14] Although gastric acid secretion is monitored and regulated by the serum gastrin (SG) concentration under normal conditions through negative feedback, this mechanism is disrupted during gastric ulceration by impaired acid-mediated inhibitory control of gastrin release.[15] This hormone, the major physiological effect of which is the stimulation of gastric acid secretion, appears to play an important role in gastric inflammatory processes.[16] The relationship between gastrin and NSAIDs-induced gastric ulcers is still unclear. Thus, the use of SG concentration as a biomarker for the prediction and monitoring of the severity of ulceration in meloxicam-associated GU in dogs was evaluated in our study.

Recent literature in human medicine has emphasized the importance of endoscopic image analysis, measurements, and color difference to provide a representative and accurate method to diagnose patients suffering from GU.[17,18] Qualitative assessment of histopathology, ultrasound examination findings, and endoscopic images has been used to establish a reliable scoring system for gastric lesions in humans and animals. The score is based on the observations of different individuals, and interobserver variability could limit the applicability of the established score. Computer-assisted diagnosis has been used in medical image analysis for the noninvasive assessment of gastric diseases because it limits interobserver variability and increases the power of statistical significance.[19] A previous study indicated that computer-aided image processing was useful to determine the depth of wall invasion of gastric cancer based on endoscopic images.[20] No previous study has either documented the utility of gastroscopic image processing or investigated the relationship between endoscopic image quantification and diagnostic markers for GU diagnosis in companion animals. Therefore, we aimed to investigate the time course changes in dogs with meloxicam-induced ulcers by hematology and SG concentration assessment and determine if a relationship between SG concentration and gastroscopic image quantification scores would provide detailed descriptive data useful for the diagnosis of GU in dogs.

2 | MATERIALS AND METHODS

2.1 | Animals

Fifteen clinically healthy crossbreed dogs (aged 1-1.8 years, weighing 14.3-21.1 kg for the control group and 1.1-1.8 years, and 12.4-22.1 kg for the meloxicam-treated group) were selected after complete physical examination and observation for 2 consecutive weeks (adaptation period). Dogs were housed in a suitable animal facility and none had any evidence of disease before the study. Feed and water were provided ad libitum. All dogs were vaccinated and treated with an appropriate anthelmintic (praziquantel [Drontal], 5 mg/kg PO) before the experiment. All experimental procedures were approved by the local institutional committee of Animal Ethics, Medicine and Diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt (DMU/VetMed-2019-/0155).

2.2 | Study design

2.2.1 | Induction model of gastric ulceration

After the adaptation period, all dogs were examined by gastroscopy to ensure normal gastric mucosa before starting the experiment. The dogs were randomly divided into 2 groups. The meloxicam-treated group ($n = 10$) received meloxicam at a dosage of 0.2 mg/kg PO for 15 consecutive days according to previous guidelines.[21-23] The control group ($n = 5$) received a placebo.

2.2.2 | Physical examination

All dogs underwent routine general physical examination. Rectal temperature, pulse rate, respiratory rate, and presence or absence of melena, colic, inappetence, anorexia, and vomiting were evaluated daily in all dogs.

2.2.3 | Hematology and SG analysis

Venous blood samples were collected aseptically from the jugular vein with disposable vacutainers. All samples were collected in a time-dependent manner according to meloxicam administration: on T5 (5 days after meloxicam administration), T10 (10 days after meloxicam administration), and T15 (15 days after meloxicam administration) in both the meloxicam-treated and control groups. Whole blood was used for hematological analysis and the following variables were determined using an automated hematology analyzer (Bio Line, BL-6500, China): hemoglobin concentration (Hb), red blood cell count (RBCs), PCV, white blood cell count (WBCs), and platelet count (PLTs). Serum samples were harvested after centrifugation of blood at 3000 rpm for 15 minutes in an Ultra 8F centrifuge, decanted into plastic aliquot tubes and frozen at −20°C. Serum samples were shipped on dry ice to a specialized laboratory for measurement of SG concentration by radioimmunoassay (RIA), with a commercially available kit (Gastrin J-125 RIA kit; Aurica DRG Diagnostics, DRG Instruments GmbH, Marburg, Germany). The SG concentration was measured for each dog in the fasting period before the gastroscopic examination.

2.2.4 | Gastroscopy preparation

Before gastroscopic examination, all dogs were premedicated with atropine sulfate (0.05 mg/kg SC) and xylazine hydrochloride (1 mg/kg IM), and then anesthetized with ketamine hydrochloride (5 mg/kg IV).[21,24] After withholding food and water for 16 and 8 hours, respectively, gastroscopy was performed using a Porta scope Endoscope (PVSM3M, Florida), 110 cm long and 9.8 mm in diameter, following steps described previously.[25] Briefly, the dogs were positioned in left lateral recumbency and the gastroscope was introduced through the cardiac orifice of the stomach and images captured for regions of the stomach (cardiac, fundic, and pyloric).

2.2.5 | Gastroscopic image assessment

Endoscopic examination of the gastric mucosa was monitored at T5, T10, and T15 of the study for the 2 groups and scored according to a previously reported scoring system.[6,26,27] Briefly, the GU was macroscopically evaluated by endoscopy using 4 grades: grade 0, no ulceration; grade 1, small pinpoint erosions in the gastric mucosa; grade 2, more widespread ulceration; and grade 3, ulcerated area deeper, wider and bleeding. For assessment of gastric mucosa echotexture, pixel intensity (PI) and integrated density (ID) were measured. Multiple regions of gastric mucosa were captured for further interpretation by Adobe Photoshop CC software analysis (version 2019). Briefly, endoscopic images were retrieved, copied onto the computer, the area of interest in the endoscopic images selected, and the ulcer area PI and ID measured, excluding artifacts (Figure 1). The PI of the mucosa represents the average pixel values within the selected area of the stomach based on a scale of 1-255: 1 (black) representing inflamed mucosa and severe ulceration and 255 (white) representing normal mucosa. The ID was counted to measure density which represents a consistent relationship between the measured area and the severity of inflammation. An increase in ID indicates that the ulcer has progressed and deepened. Results from the image values of different sites were averaged for analysis.[18,27] For example, dog 3 in the meloxicam-treated group was found to have 12 pinpoint ulcers in the stomach mucosa overall, each 1 of the 12 points was demarcated and measurement performed, exported to an Excel spread sheet, and averaged for the entire stomach. The same procedure was followed for the other dogs, and finally, the mean value for all dogs was used to measure PI and ID. At each time point, endoscopic-assisted biopsy samples were collected for histopathology after capturing images for gastric mucosa assessment. The ulcer index

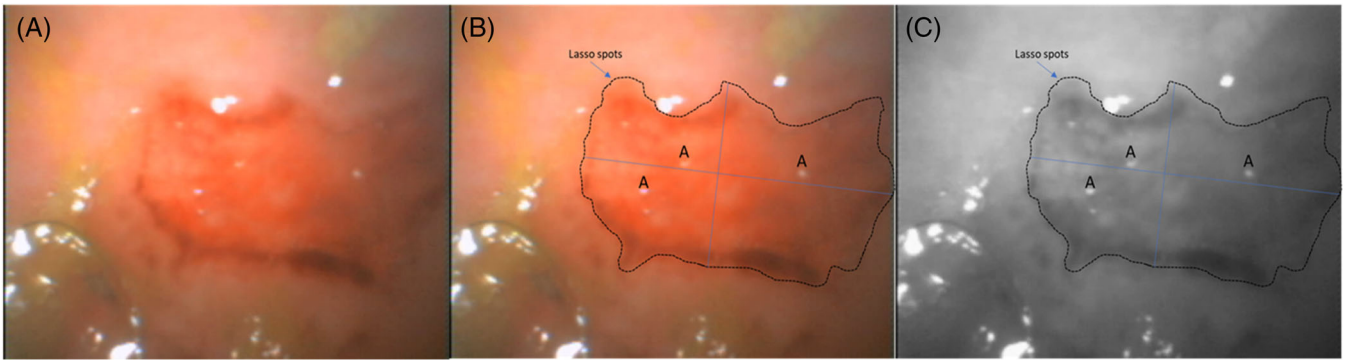

FIGURE 1 Example for preparing the endoscopic image for software and image analysis. A, the original photo retrieved from the gastroscopy of meloxicam-treated group at T15 (15 days after NSAIDs administration). B and C, Two lines and lasso spot technique was applied to measure the area of ulceration in RGB mode and gray scale mode, respectively as well as the PI and ID of the selected area. A, represents the artifacts that was excluded from the measurements. ID, integrated density; NSAIDs, nonsteroidal anti-inflammatory drugs; PI, pixel intensity

(UI) for each animal in the 2 groups was measured by determining the relative area (RA) of the ulcer by the following equation[28]:

$$RA = \frac{\text{total mucosal area}}{\text{total ulcerated area}}.$$

The UI ranged from 0 to 1.0 and the corresponding RA ranged from 100 to 1 (see Table 1 in the Supporting Information).

2.3 | Statistical analysis

Data were analyzed statistically using computer software. The normality of data distribution was evaluated by the Shapiro-Wilk test. Data were analyzed by 2-way analysis of variance (ANOVA) using GraphPad Prism7 version 7.01 (GraphPad Software, Inc, San Diego, California). Treatment effect (2 levels: ulcer group vs control group) and time effect (3 levels: T5, T10, and T5), as well as treatment × time interaction, were determined followed by Tukey's post hoc test. To quantify the strength of the relationship between groups, omega square (ω^2), a less biased estimator, was calculated to measure effect size.[29] The numbers 0.01, 0.06, and 0.14 were classified as small, medium, and large effect sizes, respectively. Pearson's correlation and linear regression analysis were performed to assess the relationship between SG concentration and values of PI, ID, and area of ulceration in gastroscopic images. In all analyses, $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Clinical findings

Clinical examination findings were recorded every day during the experiment. The most frequently reported clinical signs during

TABLE 1 Clinical variables of dogs for the control group vs meloxicam-treated group

Parameter	Control group (n = 5) (T5, T10, and T15)	Meloxicam-treated group (n = 10)		
		T5	T10	T15
Mild inappetence	(0/5)	(0/10)	(2/10)[a]	(0/10)
Anorexia	(0/5)	(0/10)	(0/10)	(6/10)[a]
Vomiting	(0/5)	(0/10)	(0/10)	(4/10)
Melena	(0/5)	(0/10)	(0/10)	(4/10)
Colic signs	(0/5)	(0/10)	(2/10)	(5/10)

Note: the table shows the clinical signs of the 2 groups of crossbreed dogs; the control group (received placebo) and the meloxicam-treated group (received meloxicam 0.2 mg/kg PO for 15 consecutive days).
Abbreviations: T5, time 5 day of the experiment; T10, time 10 day of the experiment; T15, time 15 day of the experiment.
[a]Means that 2 dogs had a mild inappetence which continued with 4 new dogs and showed complete anorexia at T15.

TABLE 2 Hematological changes and serum gastrin concentration of dogs in the control group and the meloxicam-treated group at 3 different time intervals (T5, T10, and T15)

Group	Control group (n = 5)			Meloxicam-treated group (n = 10)			P-value			
Time	T5	T10	T15	T5	T10	T15	Group	Time	Interaction	Effect size (ω^2)
Hb (mg/dL)	13.68 ± 0.58^{a,A}	13.92 ± 0.29^{a,A}	13.36 ± 0.87^{a,A}	13.72 ± 0.62^{a,A}	9.48 ± 0.72^{b,B}	8.1 ± 0.77^{c,B}	.0001	.0001	.0001	0.923
RBCs (10^6)	4.84 ± 0.32^{a,A}	4.54 ± 0.63^{a,A}	4.6 ± 0.26^{a,A}	4.66 ± 0.20^{a,A}	3.34 ± 0.20^{b,B}	3.34 ± 0.29^{b,B}	.001	.0001	.0005	0.116
PCV %	51.4 ± 4.97^{a,A}	48.8 ± 2.38^{a,A}	49.4 ± 1.51^{a,A}	47 ± 4.52^{a,A}	35.4 ± 2.07^{b,B}	25.6 ± 1.14^{c,B}	<.0001	<.0001	<.0001	0.166
WBCs (10^3)	15.36 ± 0.87^{a,A}	14.29 ± 1.99^{a,A}	14.03 ± 2.34^{a,A}	17.53 ± 1.28^{a,A}	27.25 ± 5.81^{b,B}	40.64 ± 6.22^{c,B}	<.0001	<.0001	<.0001	0.155
PLTs (10^4)	32.48 ± 3.0^{a,A}	32.54 ± 2.75^{a,A}	31.22 ± 2.36^{a,A}	31.26 ± 1.74^{a,A}	27.32 ± 1.64^{b,B}	24.02 ± 1.54^{c,B}	.005	<.0001	.0009	0.079
SG (ng/L)	39.06 ± 1.55^{a,A}	40.56 ± 2.98^{a,A}	38.9 ± 3.26^{a,A}	55.56 ± 4.40^{a,B}	225 ± 11.1^{b,B}	319.6 ± 6.58^{c,B}	<.0001	<.0001	<.0001	0.199

Note: Values are expressed as mean ± SD. Hematological variables and serum gastrin concentration in the control group and meloxicam-treated group at 3 time intervals (T5, T10, and T15) were analyzed by 2-way ANOVA. In the same row, the small superscript letters are used to compare means within the same group (eg, T5 and T10 of control), whereas the large superscript letters are used to compare the means between 2 groups at the same time (eg, T5 of the control group and T5 of the meloxicam group). The different letters (small and large) show the statistical significance. *P* value was described the significant group × time interaction. Effect size was expressed in the form of omega square (ω^2) to show the amount of significance for each variable between both groups.
Abbreviations: ANOVA, analysis of variance; Hb, hemoglobin; PLTs, platelet count; RBCs, red blood cell count; SG, serum gastrin concentration; T5, time 5 day of the experiment; T10, time 10 day of the experiment; T15, time 15 day of the experiment; WBCs, white blood cell count.

the study are presented in Table 1. Mild inappetence was first observed at T10 (2 dogs) after repeated meloxicam administration and progressed to anorexia in the meloxicam-treated group at T15 (6 dogs), with no changes in the control group. Nonsignificant change in body weight was recorded in both groups. Two dogs from the meloxicam-treated group showed signs of abdominal pain manifested as arching of the back and sitting on the hindquarters at T10 that continued until T15 to include 5 dogs. Melena (4 dogs) and vomiting (4 dogs) appeared only at T15 of administration. No remarkable changes occurred in the control group. Rectal temperature, pulse, and respiratory rates were within the reference range in both groups throughout the experiment.

3.2 | Hematological and SG analysis

The hematology and SG results were expressed as mean ± SD between the control group and meloxicam-treated group as well as at the 3 time points within the same group (Table 2). In the meloxicam-treated group, at T10 and T15, Hb, RBCs, PCV, and PLTs were decreased ($P < .0001$, .0001, .0001, and .003, respectively) whereas WBCs increased ($P < .0001$). No significant changes were observed in the hematological data at T5 ($P > .05$). Multiple comparisons for all hematological variables within the same group identified a significant effect of time (T5, T10, and T15) as a factor within the meloxicam-treated group.

The SG concentration increased markedly in the meloxicam-treated group compared to the control group at 3 time points. Also, SG concentration was increased 4.5 and 5.8-fold, respectively, at T10 and T15 compared with T5 ($P < .0001$, <.0001). A significant group × time interaction was identified. The magnitude of these differences was clinically relevant and associated with a large effect size on Hb, PCV, WBCs, and SG ($\omega^2 > 0.14$) and a moderate effect size was observed on RBCs and PLTs (ω^2 between 0.06 and 0.14). Detailed comparisons of the obtained data within the groups throughout the time course are illustrated in Table 2 in the Supporting Information.

3.3 | Endoscopic images analysis

Endoscopic image analysis was performed to evaluate changes in gastric mucosa. The time analysis of the meloxicam-treated group was graded by 3 different criteria after ulcer induction (Table 3). No changes were observed in the gastric mucosa throughout the experiment in the control group. The gastric mucosa appeared as normal reddish pink before gastric insufflation and turned glistening white after insufflation, with overdistended rugal folds. On the other hand, the gastric mucosa of 9/10 dogs in the meloxicam-treated group showed mild gastric inflammation at T5 (see Supporting Information for full description of histopathological data, Figure 1) which appeared as localized spots in the gastric wall, mainly the cardia and fundus. At T10, these minor lesions became prominent in all dogs in the group (10/10) and turned into superficial erosions, mucosal disruptions, and typically were dark red in color. At T15, the superficial lesions in all dogs had progressed to deep ulceration and extensive mucosal damage that extended into the submucosa and central lesions with dark brown color representing dried clotted blood. The ulcers at T10 and T15 more well developed in the fundic and pyloric regions of the stomach than the cardiac region.

Results of software analysis of the endoscopic images of the gastric mucosa of dogs in both groups (PI, ID, ulcer area, RA, and UI) are shown in Table 4. In the meloxicam-treated group, the values of PI were significantly lower at T5, T10, and T15 compared to the control group ($P = .02$, <.0001, <.0001, respectively) whereas the ID values were increased ($P = .03$, .02, <.0001, respectively). Multiple comparisons within the same group indicated no significant change in PI and ID values in the control group. On the other hand, significant changes were present in the meloxicam-treated group at the different time intervals (T5, T10, and T15). At T5, T10, and T15, the RA of the control group was 0 (no lesions detected), whereas the RA values of the meloxicam-treated group were 90.39, 55.99, and 24.08, which corresponded to UI values of 0.2, 0.5, and 0.8, respectively (Table 4). The amount of significance for endoscopic image analysis between the 2 groups was large for PI and ID ($\omega^2 > 0.14$).

TABLE 3 Gastroscopic description of the meloxicam-treated group of dogs graded at various times (T5, T10, and T15)

		Description	
Grade	Time	Meloxicam-treated group (n = 10)	Control group (n = 5)
1	T5	Petechial hemorrhage and pinpoint ulceration in the gastric wall	ND
2	T10	Superficial erosion in the mucosa appeared in form of tiny spots of erosion	ND
3	T15	Deep ulcer in the gastric mucosa appeared in the form of either single, large, and deep ulcer or multiple, small, deep ulcers	ND

Note: Macroscopic evaluation of the gastric mucosa with gastroscopy at the 3 time points between both groups.

Abbreviations: ND, not detected; T5, time 5 day of the experiment; T10, time 10 day of the experiment; T15, time 15 day of the experiment.

TABLE 4 Endoscopic images measurements (pixel intensity, integrated density, relative area, and ulcer index) for the 2 groups of dogs; control and meloxicam-treated groups

Group	Control group (n = 5)			Meloxicam-treated group (n = 10)			*P*-value			Effect size (ω^2)
Time	T5	T10	T15	T5	T10	T15	Group	Time	Interaction	
PI	203.45 ± 12.9^{a,A}	215.74 ± 10.7^{a,A}	192.50 ± 19.2^{a,A}	176.61 ± 10.9^{a,B}	146.78 ± 11.3^{b,B}	109.20 ± 18.4^{c,B}	<.0001	.0001	.003	0.153
ID (10^3)	0.547 ± 0.11^{a,A}	0.424 ± 0.13^{a,A}	0.567 ± 0.16^{a,A}	10.60 ± 1.1^{a,B}	1107.194 ± 177^{b,B}	8097.193 ± 655.2^{c,B}	<.0001	.001	.001	0.197
Ulcer area/ mm^2	ND	ND	ND	1299.4 ± 35.3^{a}	4883.14 ± 141.4^{b}	23 487.8 ± 301^{c}	–	–	–	
RA/mm^2	ND	ND	ND	90.39	55.99	24.08	–	–	–	
UI	0	0	0	0.2	0.5	0.8	–	–	–	

Note: Values of PI and ID are expressed as mean ± SD. PI and ID in the control group and the meloxicam-treated group at 3 time point (T5, T10, and T15) were analyzed by 2-way ANOVA. In the same row, the small superscript letters are used for comparing means within the same group (eg, T5 and T10 of control), whereas the large superscript letters are used to compare the means between 2 groups at the same time (eg, T5 of the control group and T5 of meloxicam-treated group). The effect size is expressed in the form of omega square (ω^2) to show the clinical relevance of endoscopic image analysis between both groups.

Abbreviations: ANOVA, analysis of variance; ID, integrated density; ND, not detected; PI, pixel intensity; RA, relative area; T5, time 5 day of the experiment; T10, time 10 day of the experiment; T15, time 15 day of the experiment; UI, ulcer index.

3.4 | Relationship between SG and gastroscopic image analysis

The relationship between SG and different measurements obtained from endoscopic image analysis is presented in Figure 2. A positive correlation was found between SG concentration and ID ($r = .81$, $P = .0002$) and the area of ulceration ($r = .64$, $P = .02$). A strong negative correlation was found between SG concentration and PI values of the gastric mucosa ($r = -.89$, $P = .0001$). Also, SG had a significant effect on PI, ID, and ulcer area based on regression analysis ($R^2 = 0.972$, 0.891, and 0.921, respectively).

4 | DISCUSSION

In companion animals, GU is a complex problem because different causes require a combination of diagnostic approaches for accurate detection. Although gastroscopy is a precise and more sensitive method for GU evaluation, it requires anesthesia, which remains an obstacle. Recent studies have incorporated computer-aided approaches as noninvasive tools for scoring of stomach diseases, especially gastric lesions and cancers.[18,20,30] In our study, we evaluated the combination of endoscopically-derived image scores and a laboratory marker, SG concentration, in a canine model of meloxicam-induced GU.

Administration of NSAIDs remains a major cause of GU in companion animals.[2,3,31] However, many studies suggest that the cyclooxygenase 2 (COX-2) inhibitors[32-34] are slightly safer for the gastric mucosa than are COX-1 inhibitors,[35-37] but it is still unclear why gastric lesions become predominant, and sometimes fatal, after frequent usage of COX-2 inhibitors for pain relief and after surgery. We used the COX-2 inhibitor meloxicam to investigate the time course of changes in the gastric mucosa of pharmacologically-induced GU using hematology variables, SG, and gastroscopic imaging for assessment.

In general, preliminary GU diagnosis is suggested by patient history and clinical examination followed by hematological analysis and confirmed by endoscopy or other diagnostic imaging. In our study, repeated meloxicam administration in dogs resulted in clinical signs of vomiting, anorexia, hematemesis, melena, weakness, and anemia.[38,39] A few dogs seemed to suffer from abdominal pain based on assuming a sitting position and rolling.[4,40,41] The number of studies presenting the time course of changes in the hematological findings and SG concentration profile in dogs suffering from GU is limited. Our longitudinal study provides descriptive data about the hematological changes in dogs suffering from meloxicam-induced GU. In this regard, a significant decrease in Hb, RBCs, PCV and PLTs count, and increase in WBCs was observed after 10 and 15 days of meloxicam administration. These variables serve as indicators of the severity of clinical abnormalities, especially anemia, dehydration, and sepsis, and were the most common hematological findings in the meloxicam-treated group.[11,42,43] Thrombocytopenia was observed on day 10 after ulcer induction and became prominent on day 15. Previous studies have discussed the likelihood that NSAIDs will cause bleeding

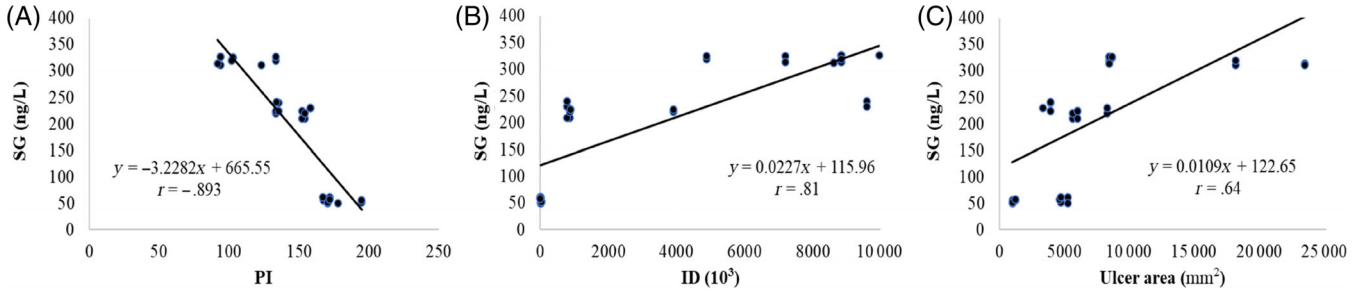

FIGURE 2 Correlation and linear regression between SG and PI, ID, and the area of ulceration in the meloxicam-treated group (n = 10): A, Negative correlation between SG concentration and PI of gastroscopic image analysis. B and C, The positive correlation between SG concentration and ID and ulcer area, respectively. ID, integrated density; PI, pixel intensity; SG, serum gastrin

complications,[11,44,45] which could be attributed to decreased thromboxane production and enhanced bleeding tendency. The WBC count in the meloxicam-treated group increased significantly after 10 days of administration and was markedly high at day 15. This significant increase was probably in response to the inflammatory reaction in the gastric wall that occurred as a result of the destruction of the bicarbonate layer of the mucosa after inhibition of prostaglandin synthesis, and confirms the increase in leukocyte infiltration at the site of injury (see Supporting Information) when a COX-2 inhibitor is administered.[4,44,46,47]

Gastrin is an important regulator hormone of gastric acid secretion released mainly by G cells. Because the relationship between SG concentration and NSAIDs inducing GU is unclear, we measured the SG concentration at the 3 time points of the experiment. The SG concentration was markedly increased longitudinally with the coexistence of GU at T5, T10, and T15. In previous studies, investigators tried to evaluate the mechanism of increase in gastrin release with the increase in acid secretion from the stomach and indicated that this increase was related to gastric acid and the food provided to the patients.[15,48,49] To avoid confusion with the transient postprandial increase of gastrin, we measured the SG concentration during the period of fasting before gastroscopy. The marked increase in SG concentration in dogs treated with meloxicam likely was a result of impairment in the feedback mechanism between gastric acid and antral gastrin secretion. Another explanation is the direct relationship between the lesions found in the stomach and G cell stimulation.[50] The number of studies correlating SG concentration and gastric lesions is limited and the effect has been seen more often in humans than in dogs, but the correlation supports our finding of a relationship between both factors.[50-53]

The efficacy of endoscopic diagnosis has improved as a result of advances in imaging techniques in the last few decades.[54] The gastric mucosa was evaluated macroscopically by endoscopy in both groups at 3 time points and no ulceration was found in the control group. In the treatment group, grade 1 was expressed as small pinpoint erosions, and petechial hemorrhages in the gastric mucosa were observed at T5. At T10, the ulcers became more widespread, mainly in the fundus and pylorus, and the damaged mucosa turned to a brown color (grade 2). Finally, at T15, the ulcerated area became deeper, wider and the tendency of hemorrhage was more obvious in response to the decrease in PLTs and the rupture of microvascular capillaries in the mucosa as reported previously,[44] with the mucosa turning to a blackish color as a result of the formation of clotted blood in the disrupted area (grade 3). This gradual increase in ulcer grade confirms gastric lesions and mucosal disruption after frequent meloxicam administration in dogs, likely attributable to inhibition of COX-2 enzyme and prostaglandin synthesis.[11,12]

The RA of the ulcer was measured and gave a reliable indication of the UI in the meloxicam-treated group. The increase in UI after meloxicam administration in this group may be attributed to the inhibition of prostaglandin synthesis and increase in gastrin hormone secretion that in turn increased the production of hydrochloric acid in the stomach and impaired gastroprotection.[55-57] As reported in recent studies,[17,18] the pixel difference and density can be measured to provide an accurate assessment of gastrointestinal lesions. The values of PI and ID in various parts of gastric mucosa were calculated using computer software analysis and showed a statistical difference at the same time interval between both groups as well as in the mucosa of the meloxicam-treated group at different time points (T5, T10, and T15), unlike hematological results which showed differences starting at T10 except for SG concentration. The latter result confirms the practicality of gastroscopy for early detection of GU in dogs.[58-60]

We evaluated the relationship between the SG concentration in the blood of dogs suffering from meloxicam-induced GU and quantitative analysis of the gastroscopic images of the ulcerated gastric mucosa in terms of PI and ID differences. Although endoscopy is the standard tool for detection of GU, its repeatability and invasiveness remain issues for the patient and veterinarian. The magnitude of the differences between the 2 groups is clinically relevant for each variable at the specific time point which indicates the value of these variables in the clinical setting. The effective correlation and regression among SG, PI, ID, and ulcer area could predict GU in dogs from SG concentration with the assistance of physical and laboratory diagnosis.

The PI and ID derived from image analysis of the stomach mucosa cannot differentiate among causes of GU, but their detection makes GU scoring more accurate because it increases the statistical power. The quantification of scores will be more accurate than the subjective or qualitative scoring that is commonly used in literature.[30] This

approach will decrease interobserver variability for scoring assessment. We focused on meloxicam-induced GU, and image processing of other causes of GU could produce a reliable quantitative scale that could be used in the future for diagnosis. If image analysis indices from different causes of GU can be correlated with a biochemical marker of diagnostic power (eg, gastrin), a reliable scale may be produced for the clinical setting. In conclusion, clinical signs, hematology, SG concentration, and gastroscopy are complementary tools for the diagnosis of GU in dogs. The SG concentration is helpful to predict the status of GU caused by meloxicam administration without the necessity of gastroscopy.

Our study had some limitations. The number of dogs was small and could affected our results. The results of endoscopic image analysis should be extrapolated individually for each dog, and not collectively. Our results were focused on meloxicam-induced GU and other NSAIDs should be evaluated in further studies. The veterinarian should keep in mind any history of medication with proton pump inhibitors and H2 receptor antagonists when using SG concentration to assess GU because they may adversely affect SG concentration.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This study was approved by the institutional committee of Animal Medicine and Diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt (DMU/VetMed-2019−/0155).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

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