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Fecal Calprotectin Concentrations and Other Indicators in Dogs with Idiopathic Inflammatory Bowel Disease

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Abstract | Inflammatory bowel disease (IBD) is a disease characterized by chronic gastrointestinal inflammation. Due to the continuous rise in incidence, simple and cost-effective methods of diagnostic and clinical assessment are urgently required. The goal of this study was to look at the hematobiochemical alterations in association with the evaluation of C-reactive proteins (CRP), haptoglobin and fecal calprotectin concentration as prognostic markers in dogs with IBD. After a detailed clinical, laboratory and ultrasonographic examination 21 IBD dogs with symptoms of chronic gastrointestinal diseases were chosen for the study. In addition to 11 healthy dogs served as control group. In comparison to controls, hematological analysis revealed significant variations ($p<0.05$) in total leukocyte count, hemoglobin, and mean platelet volume and significant variation ($p<0.01$) in neutrophils and platelet count. The biochemical analysis revealed a significant rise in serum activity of aspartate aminotransferase ($p<0.05$), and blood urea nitrogen, blood creatinine, lipase and alkaline phosphatase ($p<0.01$) in IBD dogs. The mean values of IBD-related biomarkers (CRP, haptoglobin, and fecal calprotectin concentrations) increased significantly ($p<0.05$ or $p<0.01$) compared to controls. CRP, haptoglobin concentrations, and fecal calprotectin were found to have a strong positive correlation. Furthermore, blood enzymes, glucose and electrolytes were significantly correlated with CRP as an IBD-related biomarker. It is concluded that, hematological indices such as total leukocyte count, neutrophils and platelets count may consider non-invasive hematological markers in IBD dogs as they were increased significantly. In addition, CRP, haptoglobin, and fecal calprotectin were also discovered to be powerful IBD biomarkers and might be used as surrogate measures of disease severity in dogs with IBD.

Keywords | IBD, Fecal calprotectin, CRP, Haptoglobin

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INTRODUCTION

Inflammatory bowel disease (IBD) is a long-term inflammatory illness that affects the intestines. As novel treatment goals become more generally acknowledged accurate disease assessment and therapeutic response prediction have become critical challenges in the clinical veterinary practice. Furthermore diagnostic and clinical assessment procedures that are both accessible and cost-effective are urgently needed. Because serum biomarkers are non-invasive, convenient, and less expensive, they have recently made significant progress and have become a focus in IBD research. (Chen et al., 2020). IBD in dogs is a syndrome defined by gastrointestinal symptoms that are

persistent and recurrent, as well as histologic indicators of mucosal inflammation. (Allenspach et al., 2016; Washabau et al., 2010). Routine clinicopathologic and histopathologic findings in dogs have been reported to be unable to distinguish between different types of GIT illnesses, making invasive diagnostic approaches difficult for evaluating therapeutic responses. Inflammatory markers appear to be useful in the treatment of dogs with IBD, particularly when it comes to predicting therapy response (Estruch et al., 2020). In dogs with IBD, biomarkers that can help with clinical assessment, monitoring, or assessing response to various treatments may be clinically important. Over the last decade, researchers have looked at a variety of relevant, physiological, metabolomic, biological, and histological indicators in dogs with IBD (Heilmann and Steiner, 2018). Despite the fact that routine analysis of certain clinically effective molecules (such as cytokines and chemokines) is limited due to a lack of general availability, high cost, and poor biological specimen stability, a number of inflammatory indicators can be quantified in biological samples (Truar et al., 2018). A few useful markers, such as serum vitamin B12 (cobalamin) and vitamin B9 (folate) concentrations, are already commonly used in the treatment in dogs with suspected IBD, according to Schreiner et al. (2008).

Other inflammatory indicators such as serum C-reactive protein (CRP), haptoglobin (HAP), serum calprotectin, and fecal calprotectin are currently available in assessing the IBD in dogs. Combining data from multiple inflammatory biomarkers, such as serum CRP and fecal calprotectin can improve the precision of any biomarker on its own (Heilmann et al., 2015). Most clinicians recognize CRP as an inflammatory sign. It is one of the most essential proteins produced immediately by hepatocytes in response to interleukins (IL-1- β and IL-6) and tumor necrosis factor (TNF- α) originating at the site of inflammation or disease (Rhodes et al., 2011).

In human medicine, CRP response is linked to a variety of conditions, including infections, inflammations, tissue necrosis, and stress. Because of its short half-life, CRP is a valuable marker for detecting and observing disease activity in Crohn's disease (CD). CRP levels in the blood correspond well with disease activity in CD patients and other inflammatory indicators such as fecal calprotectin (Vermeire et al., 2004).

In veterinary practice, serum CRP levels have a significant biological variance in dogs (Carney et al., 2011) restricts its utility as a diagnostic biomarker in dogs with IBD. Serum CRP appears to be more clinically relevant as a replacement test to assess disease progression and responsiveness to treatment in dogs with IBD (Heilmann and Steiner, 2018; Jergens et al., 2010; Jergens et al., 2003). In veterinary practice, haptoglobin (HAP) is a mild acute phase protein that rises in responds to inflammatory process, infection, or tissue damage (Eckersall and Conner, 1988). A previously reported reference range for canine haptoglobin was 0 to 3 g/l, with concentrations greater than 10 g/l indicating a strong inflammatory response (Eckersall and Conner, 1988). The level of haptoglobin in the dog's blood could provide useful information regarding the severity of the disease and how well it responds to treatment (McGrotty et al., 2003). Fecal calprotectin is a stool biomarker and noninvasive means of evaluating intestinal inflammation that is increasingly being utilized in clinical practice to differentiate between organic and functional gastrointestinal disorders (Heilmann and Steiner, 2018). The concentrations of fecal calprotectin in dogs with persistent gastrointestinal inflammation have been studied and appear to be a valuable indicator of intestinal inflammation in dogs (Grellet et al., 2013; Otoni et al., 2018). Calprotectin is a calcium and zinc binding protein heterocomplex made up of the S100A8 and S100A9 proteins (Foell et al., 2007; Mumolo et al., 2018), that contributes for more than 60% of protein content in neutrophil cytosol, which infiltrates the intestinal mucosa as involved in the inflammatory reflex in IBD (Oyaert et al., 2017). Hyperpermeable intestinal mucosa infiltration of activated neutrophils into the faeces as a result of inflammation, measuring calprotectin levels in stool samples has showed potential in distinguishing patients with IBD (Banerjee et al., 2015; Caviglia et al., 2014). The current study was designed to investigate the changes in the clinical, hematobiochemical, and IBD-related biomarkers as prognostic indicators in dogs with IBD. In addition, determining the relationship between serum CRP, haptoglobin, and changes in fecal calprotectin, as well as the relationship between blood enzymes, electrolytes, and CRP concentration as an IBD biomarker.

MATERIALS AND METHODS

The present study was carried out on 32 dogs of different ages and breeds (German shepherd, Griffon, and Golden retriever). All dogs included in the current study were collected from a private pet's clinics and shelters in Sharkia governorate. The dogs' owners have given their permission for their canines to be included in the current study. Eleven healthy dogs aged from 5 to 17 years, and of different breeds (German shepherd, Griffon, Golden retriever) were selected as a control group. Dogs had no prior history of GI symptoms based on the results of a routine clinical assessment, CBC, serum biochemical, and faecal examination. Control dogs were used to provide control values for comparing the findings obtained from IBD dogs. Twenty-one dogs aged from 5 to 13 years, of different breeds (German shepherd, Griffon, Golden retriever)

were chosen for this study. Diseased dogs were suffered from varying gastrointestinal abnormalities (mostly IBD) involving chronic diarrhea >3 weeks with no response to dietary and symptomatic therapy. They had not been given any treatment prior to the clinical examination. Physical examination were performed for all dogs and ultrasonography was performed on different abdominal organs especially GIT (Delaney et al., 2003). Clinical disease activity was evaluated based on their divergence from normal dogs in the following: appetite, vomiting, stool (consistency and frequency) and body weight are all factors to consider.

Samples collection and analysis

Serum was obtained from 21 IBD dogs to carry out hematological and biochemical analysis, 7 ml of blood was collected from each dog's cephalic vein, and 1-2 ml of blood was placed in vacuum EDTA coated tubes for hematological analysis. The remaining blood samples were divided into 3-4 ml sterile heparinized tubes and 2 ml plain tubes for plasma and serum sample collection. Plasma and serum were separated immediately after collection by centrifuging for 10 minutes at 3000 rpm.

Hemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC) and total and differential leukocytes counts were determined using Sysmex XN1000 analyzer (Sysmex, USA) using standard methods (Feldman et al., 2010).

Serum samples were harvested and divided into several aliquots used for biochemical analysis (CRP, haptoglobin, pancreatic lipase, alkaline phosphates, and blood parameters (total protein, albumin, globulin and glucose). All biochemical measurements were applied using Beckman AU5800 analyzer (Beckman Coulter, California, USA) using standard methods (Feldman et al., 2010).

Fecal samples (an aliquot of approximately 1 g each) were collected for measuring fecal calprotectin concentrations using the ELISA Test Kit (CALP-0170, Calpro AS, Oslo, Norway) following the standard methods for all the ELISA procedures.

Statistical Analysis

Data were statistically analyzed using SPSS Statistics® 17.0 (Version 17.0 released 2008, SPSS Inc., Chicago). The Shapiro Wilks W Test was used to check for normal distribution, and the data were determined to be normal. Biochemical data were compared using independent samples t-test and all data are listed as mean ± SE. The significance of differences between parameters was assessed at different probability levels of $p<0.05$ and $p<0.01$. For estimating the relationship between the concentration of IBD-related inflammatory markers (CRP, haptoglobin and fecal calprotectin), and for estimating the correlation between CRP and blood enzymes (pancreatic lipase and ALP) and electrolytes concentrations (total Ca, Na, K) in IBD dogs pearson correlation was used.

RESULTS

The current study comprised a total of 32 dogs, from which 21 had inflammatory bowel disease of different ages (5 to 13 years old) and breeds (3 German shepherd, 10 Griffon, 8 Golden retriever), while the remaining 11 were healthy dogs ranging in age from 5 to 17 years old and of various breeds (3 German shepherd, 4 Griffon 4 Golden retriever), all dogs were male. There was significant increase in mean body temperature of 40.3 ± 0.27 °C ($p<0.05$), heart rate of 126.8 ± 2.5 per min. ($p<0.05$), and respiratory rate of 53.4 ± 1.9 breaths per min. ($p<0.01$). The most prevalent clinical indicators in IBD dogs, according to clinical assessment, are vomiting, bloody diarrhea, or watery diarrhea, anorexia, weight loss, and abdominal pain (Table 1).

The mean values of hematological and biochemical indices in dogs with IBD revealed a significant rise in the total leukocytes count (14.26 ± 0.59), neutrophils (69.76 ± 1.7), platelets count (294.6 ± 31.96) and a significant decrease in the mean of platelets volume MPV (20.2 ± 1.47), hemoglobin (10.16 ± 0.52) while, non significant decrease in red blood cell count (4.55 ± 0.14) and PCV (31.19 ± 1.3) in comparison to the controls. As well, the serum activities of AST demonstrate a significant increase (59.23 ± 5.7) and ALT (77 ± 6.5) activity compared to the controls. As well as, there was a highly significant increase in blood urea nitrogen (52.82 ± 3) and blood creatinine (3 ± 0.23). While, the alterations of the mean values of serum total proteins, albumin, globulin was not changed significantly in IBD dogs (Table 1).

The serum activities of pancreatic lipase demonstrate a significant increase (244.69 ± 23.27) and alkaline phosphates (310 ± 35.26) activity compared to the controls. There was highly significant decrease in blood glucose (65.6 ± 1.07) and blood electrolytes as sodium (138.7 ± 1.04), potassium (3.01 ± 0.11) and total calcium (8.09 ± 0.4) (Table 2). While, the mean values of IBD-related biomarkers, CRP and HAP concentrations were highly significant increase (4.4 ± 1.07and 164 ± 18.31, respectively). As well as, the fecal calprotectin concentration was highly significant increased (27.6 ± 2.29) in IBD dogs compared to the controls (Table 3).

The relationship between different IBD related biomarkers (CRP, haptoglobin and fecal calprotectin), were performed using Pearson correlation with plotting the results of CRP

Table 1: Dog characteristics, clinical parameters, observations, hematological and biochemical findings in dogs with inflammatory bowel disease and healthy dogs included in the present study. The results are expressed as Mean ± S.E, and/or max., min.

	Healthy controls n=11	IBD dogs n=21	Reference values #
Dog characteristics:			
Age in years, median	8 (5 – 17)	9 (5 – 13)	
Gender, male/female	6 male / 5 female	21 / 00	
Weight in kg, median	22.5 (11 – 38)	15.25 (8 – 25)	
Clinical parameters:			
Anorexia, n (%)		16 (76.00)	
Vomiting, n (%)		8 (38.00)	
Diarrhea, n (%)		21 (100.00)	
Clinical observations:			
Rectal temperature (°C)	38.6 ± 0.18	40.3 ± 0.27 **	38.3 - 39.2
Respiration rate (per min.)	26.4 ± 1.4	53.4 ± 1.9 *	15 - 35
Heart rate (per min.)	77.4 ± 1	126.8 ± 2.5 **	70 - 90
Hematological parameters:			
Hemoglobin (g/dl)	14.65 ± 0.29	10.16 ± 0.52 *	12 - 18
PCV (%)	39.76 ± 1.2	31.19 ± 1.3 ns	37 - 55
Total erythrocyte($\times10^6$/l)	5.68 ± 0.11	4.55 ± 0.14 ns	5.5 - 8.5
Total leukocyte($\times10^3$/l)	9.9 ± 0.38	14.26 ± 0.59 *	6 - 16
Neutrophils (%)	65.09 ± 1.03	69.76 ± 1.7 **	60 - 70
Lymphocytes (%)	27.45 ± 5.66	20.14 ± 1.34 ns	15 - 30
Monocytes (%)	4.36 ± 0.59	4.8 ± 0.38 ns	3 - 8
Eosinophils (%)	2.81 ± 0.32	3.42 ± 0.31 ns	2 - 10
PLT ($\times10^3$/l)	155.74 ± 14.39	294.6 ± 31.96 **	200 - 500
MPV (fl)	23.56 ± 0.44	20.2 ± 1.47 *	>20
Biochemical parameters:			
Total protein (g/dl)	6.3 ± 0.24	6.5 ± 0.18 ns	5 – 7
Albumin (g/dl)	3.6 ± 0.16	2.9 ± 0.14 ns	3.1 - 4.5
Globulin (g/dl)	3 ± 0.13	4.5 ± 0.17 ns	2.8 - 4.5
BUN (mg/dl)	15.46 ± 1.6	52.82 ± 3 **	7 - 25
Creatinine (mg/dl)	0.99 ± 0.1	3 ± 0.23 **	0.4 - 1.8
AST (IU/l)	39.5 ± 2.7	59.23 ± 5.7 *	5 - 55
ALT (IU/l)	36.7 ± 3.1	77 ± 6.5 ns	5 - 60

PCV, packed cell volume; PLT, platelet count; MPV, mean platelet volume; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase. # (Feldman et al., 2010), * statistically significant at P<0.05, ** statistically significant at P<0.01, ns statistically non significant

Table 2: Mean ± S.E values of glucose, blood enzymes and electrolytes in dogs with IBD

Parameters	Control group (n = 11)	IBD group (n = 21)	Reference values #
Glucose (mg/dl)	112.6 ± 2.7	65.6 ± 1.07 **	60 - 125
Alkaline phosphates (IU/l)	43 ± 4.7	310 ± 35.26 **	10 - 150
Lipase (IU/l)	99.14 ± 3.09	244.69 ± 23.27 **	100 - 500
Total Ca (mg/dl)	9.7 ± 0.17	8.09 ± 0.4 **	7.5 - 11.3
Potassium (mmol/l)	4.68 ± 0.21	3.01 ± 0.11 *	4 - 5.6

Sodium (mmol/l)	146.75 ± 0.61	138.7 ± 1.04 [ns]	141 – 156

[#] (Feldman et al., 2010), [*] statistically significant at P<0.05, [**] statistically significant at P<0.01, [ns] statistically non significant

Table 3: Mean ± S.E values of biomarkers of inflammation in dogs with IBD

Parameters	Control group (n = 11)	IBD group (n = 21)	Reference values
Fecal calprotectin (µg/g)	0.2±0.05	27.6±2.29[**]	0.5 – 15.3 [#]
C-reactive protein (mg/dl)	0.7±0.12	4.4±1.07[**]	<1 [#]
Haptoglobin (mg/dl)	78.95±2.8	164±18.31[*]	30 - 250 [##]

[*] statistically significant at P<0.05, [**] statistically significant at P<0.01, [ns] statistically non significant, [#] (Heilmann et al., 2018), [##] (Grobman et al., 2017).

and haptoglobin concentrations against the measurements of the fecal calprotectin in the same samples and the results showed very strong positive correlation (r = 0.733, r = 0.726, respectively) (Figures 1 and 2).

The relationship between alterations of blood enzymes (ALP and Lipase) and electrolytes (total calcium, sodium and potassium) and CRP (IBD-related biomarker) were performed by plotting the results of serum ALP, Lipase concentrations against the measurements of and CRP in the same samples of IBD dogs and the results showed a moderately positive correlation (r = 0.645, r = 0.537, respectively) (Figures 3 and 4). While, the correlation between serum total calcium, plasma K^+ and CRP illustrated a moderately negative correlation (r = -0.526, r = -0.567, respectively) as well, it was a strongly negative correlation between serum plasma Na^+, glucose and CRP (r = -0.776, r = -0.71, respectively), (Figures 5, 6, 7 and 8).

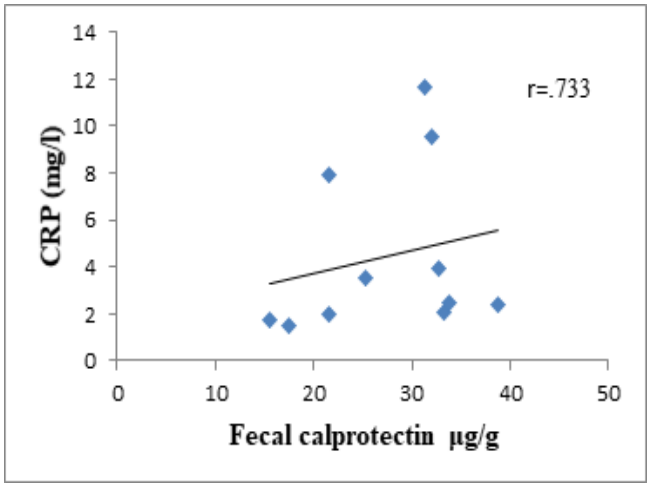

Figure 1: The correlation between serum CRP and fecal calprotectin in IBD dogs. The graph illustrates a strong positive correlation (r = 0.733) between CRP in mg/l and fecal calprotectin µg/g r = Pearson correlation coefficient.

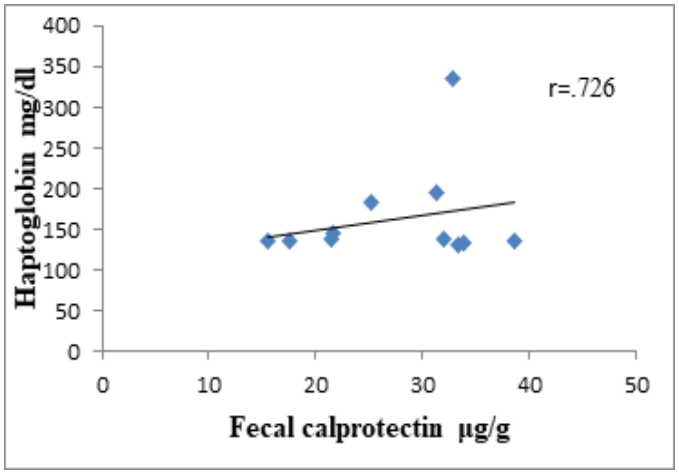

Figure 2: The correlation between serum haptoglobin and fecal calprotectin in IBD dogs. The graph illustrates a strong positive correlation (r = 0.726) between haptoglobin in mg/dl and fecal calprotectin µg/g r = Pearson correlation coefficient.

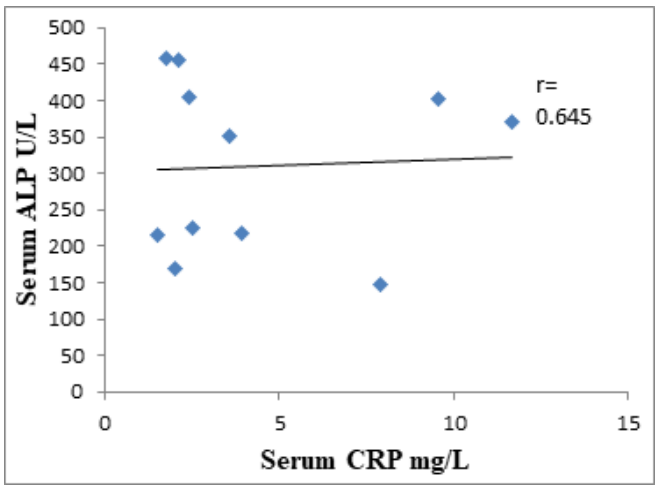

Figure 3: The correlation between serum ALP and CRP in IBD dogs. The graph illustrates a moderately positive correlation (r = 0.645) between ALP U/l and CRP mg/l, r = Pearson correlation coefficient.

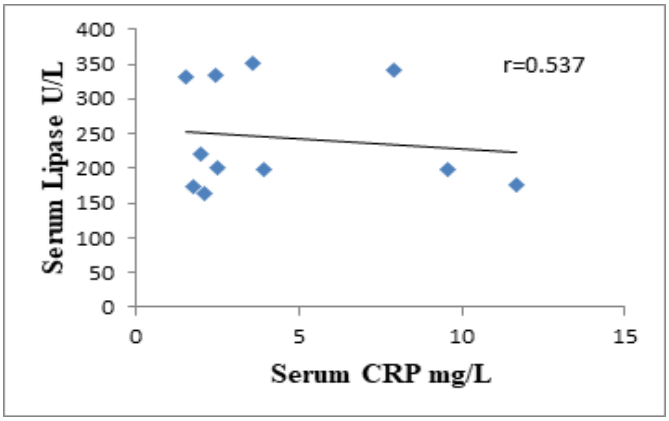

Figure 4: The correlation between serum Lipase and CRP in IBD dogs. The graph illustrates a moderately positive correlation (r = 0.537) between Lipase U/l and CRP mg/l, r = Pearson correlation coefficient.

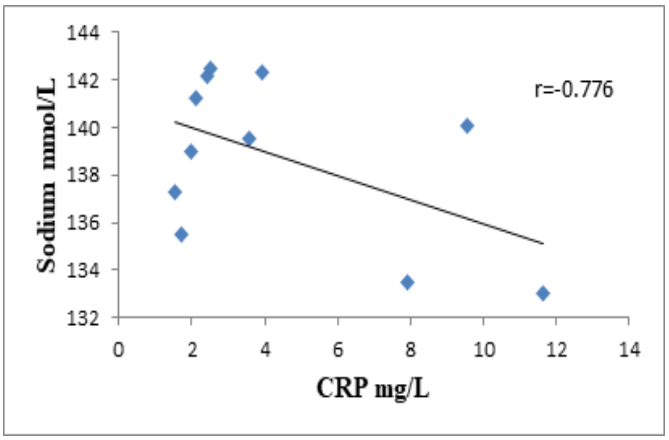

Figure 7: The correlation between serum plasma Na^+ and CRP in IBD dogs. The graph illustrates a strong negative correlation (r = -0.776) between Na^+ mmol/l and CRP mg/l, r = Pearson correlation coefficient.

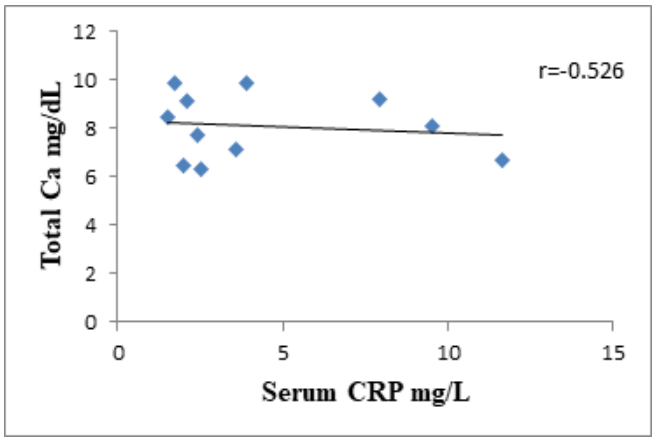

Figure 5: The correlation between serum total calcium and CRP in IBD dogs. The graph illustrates a moderately negative correlation (r = -0.526) between total Ca mg/dl and CRP mg/l, r = Pearson correlation coefficient.

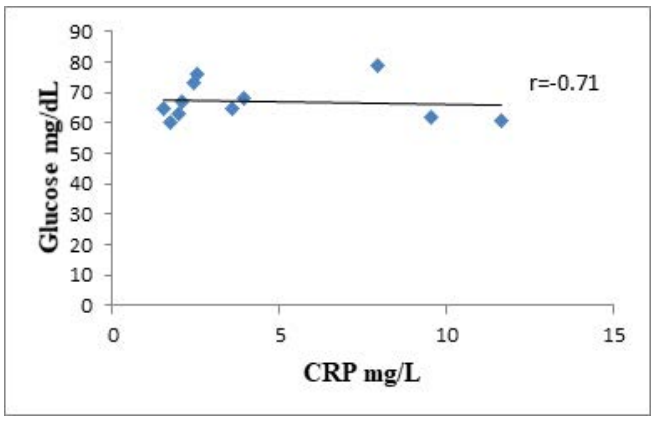

Figure 8: The correlation between blood glucose and CRP in IBD dogs. The graph illustrates a strong negative correlation (r = -0.71) between blood glucose mg/dl and CRP mg/l, r = Pearson correlation coefficient.

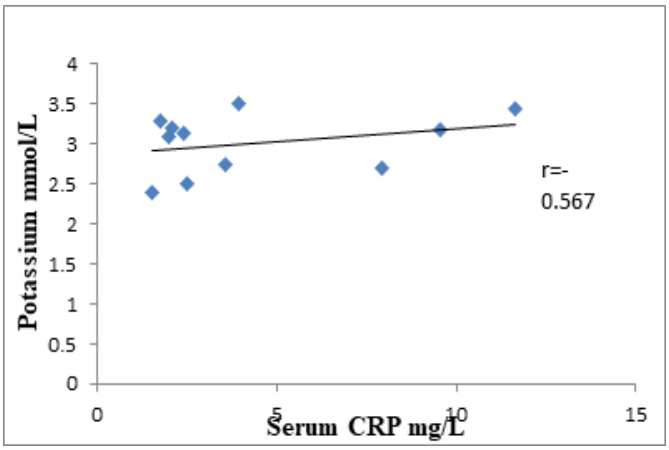

Figure 6: The correlation between serum plasma K^+ and CRP in IBD dogs. The graph illustrates a moderately negative correlation (r = -0.526) between K^+ mmol/l and CRP mg/l, r = Pearson correlation coefficient.

DISCUSSION

In the present study, IBD was most commonly seen in middle-aged (5-13 years) male dogs suffered diarrhea symptoms, where the inflammatory bowel disease is defined by the infiltration of inflammatory cells into the intestinal walls and is the most common cause of persistent diarrhea in dogs. In addition, vomiting, anorexia, weight loss, and abdominal pain were observed, it shows the severity of the disease, and this was similar with previous studies by Bhavani et al. (2021); Volkmann et al. (2017).

In comparison to the control group, hematological examination revealed a significant rise in total leukocytes count and neutrophils, as well as a decrease in red blood cell count, hemoglobin, and PCV. Similar changes were observed by Ristic and Stidworthy (2002), who hypothesized that prolonged gastrointestinal blood loss could lead to non-regenerative iron deficiency anemia

and that neutrophilic leukocytosis was linked to stress and chronic inflammation.

Our results showed a considerable rise in platelet count and a significant drop in platelet volume average (MPV). Platelet release can be caused by an increase in the pro-inflammatory cytokines concentrations, particularly IL-6, in individuals with persistent inflammation. This is related to IL-6's enhancement of thrombopoietin production. IL-6 increases the megakaryocytic nuclei and the amount of the cytoplasm, resulting in the generation of a significant number of blood platelets (Senchenkova et al., 2013). The fraction of giant platelets increases as the inflammatory process progresses, which is most likely due to intracellular manufacture of pro-inflammatory cytokines, granule degranulation, and activation of the splenic platelet reserve. Simultaneously, these cells migrate promptly to the inflammation site, where they are activated and depleted, explaining why MPV levels are lower in individuals with chronic inflammation (Afsar et al., 2017; Kamath et al., 2001).

The IBD group had significantly higher biochemical alterations, such as blood urea nitrogen and creatinine levels, matching the findings of Bhavani et al. (2021) in diarrheic dogs. Increased blood urea nitrogen suggests prerenal uremia, which is caused by fever-induced tissue catabolism and a decreased glomerulofiltration rate. The levels of ALT and AST in the study group were significantly higher than in the control group, indicating that reactive hepatopathy was to blame (Berghoff and Steiner, 2011). While the mean values of blood total proteins, albumin, and globulin did not or weak changed significantly in IBD dogs, indicating that the dogs in this study did not progress to Protein-Losing Enteropathy (Dossin and Lavoue, 2011).

The serum activities of blood enzymes (pancreatic lipase and alkaline phosphatase) show a considerable increase in IBD dogs. While, blood glucose and blood electrolytes such as sodium, potassium, and total calcium were decreased significantly. Our findings agreed with those of Shinde et al. (2000). Who noticed the same abnormalities and hypothesized that hypoglycemia in the affected dogs was caused by inappetance or anorexia, which was accompanied by intestinal mal-absorption. Furthermore, inflammation increases bowel permeability, resulting in fluid, electrolyte, protein, and cell loss. However, elevated pancreatic lipase and alkaline phosphatase levels were linked to inflammation in idiopathic IBD dogs, as well as systemic disorders, such as pancreatitis and GI inflammation that can cause reactive hepatopathy or physiologic stress. (Ide et al., 2016).

The elevation of the IBD-related biomarkers, CRP and haptoglobin concentrations as well as, the fecal calprotectin concentrations in IBD dogs compared to the controls in the current investigation were similar to previous studies in human and veterinary research field (Grobman et al., 2017). CRP levels were observed to be high in canine patients with inflammatory disorders, including infectious diseases, surgical trauma, acute pancreatitis, glomerulonephritis, and inflammation (Carney et al., 2011; Gommeren et al., 2018). As well, the elevated serum haptoglobin concentration in this study was similar to that previously reported by Eckersall and Conner (1988), who discovered that the reference range for canine haptoglobin was 0 to 3 g/l, with concentrations greater than 10 g/l indicating a strong inflammatory response because haptoglobin is a mild acute phase protein that rises in response to inflammation, infection, or trauma. The increased release of calprotectin from activated macrophages and neutrophils during the inflammatory process is linked to an increase in fecal calprotectin. Calprotectin is a receptor that plays a function in both acute and persistent inflammation. Serum calprotectin levels are elevated in dogs with chronic inflammatory enteropathies, however serum calprotectin is not as selective for the gastrointestinal system as feces calprotectin (Foell et al., 2007; Heilmann et al., 2018; Truar et al., 2018; Wilke et al., 2012).

Fecal calprotectin was found to be substantially correlated with serum CRP and haptoglobin, indicating that intestinal inflammation in dogs with idiopathic IBD is relevant to the systemic inflammatory response. These findings were consistent with previous research that found CRP to be moderately linked with fecal calprotectin in Crohn's disease or ulcerative colitis patients (Ricanek et al., 2011). The results that investigates the relationship between changes in blood glucose, blood enzymes (pancreatic lipase and ALP), and blood electrolytes (total Ca, Na, and K ions) and the concentration of CRP (IBD-related biomarkers), revealed a strong to moderate correlation that indicates the progression of the inflammation. Consequently, the most prevalent conditions related with canine IBD are hypoglycemia and pancreatitis (Ide et al., 2016; Malo et al., 2014).

CONCLUSIONS

In conclusion, when IBD dogs were compared to the control group, hematological indices such as total leukocyte count, neutrophils, and platelet count were considerably higher, showing that IBD dogs have non-invasive hematological markers. CRP, haptoglobin, and fecal calprotectin were also discovered to be powerful IBD biomarkers and might be used as surrogate measures of disease severity in dogs with IBD. The combination of CRP, haptoglobin, and fecal calprotectin measurements has the potential to evaluate canine IBD.

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CONFLICT OF INTEREST

We state that there is no conflict of interest.

NOVELTY STATEMENT

The main objective of this paper is to investigate the changes in the IBD-related biomarkers as prognostic indicators in dogs with IBD. Many researchers studied the fecal calprotectin in human medicine as inflammatory biomarker for IBD diagnosis; this study investigated the fecal calprotectin as novel inflammation biomarkers in dogs with IBD.

AUTHORS CONTRIBUTION

All authors contributed equally to this manuscript including paper writing, editing, and reviewing. In addition, the first and last authors had supervision contribution.

REFERENCES

Afsar N, Afroze IA, Tahniath H, Abid Z (2017). Role of Mean platelet Volume as an adjunct in evaluation of acute inflammation. Annals of Pathology and Laboratory Medicine, Vol. 4, Issue 4, July-August, 2017 4, A466-A469. https://doi.org/10.21276/APALM.1486

Allenspach K, Culverwell C, Chan D (2016). Long-term outcome in dogs with chronic enteropathies: 203 cases. Vet. Rec. 178: 368. https://doi.org/10.1136/vr.103557

Banerjee A, Srinivas M, Eyre R, Ellis R, Waugh N, Bardhan KD, Basumani P (2015). Faecal calprotectin for differentiating between irritable bowel syndrome and inflammatory bowel disease: a useful screen in daily gastroenterology practice. Frontline Gastroenterol. 6: 20-26. https://doi.org/10.1136/flgastro-2013-100429

Berghoff N, Steiner JM (2011). Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. Vet. Clin. North Am. Small Anim. Pract. 41: 311-328. https://doi.org/10.1016/j.cvsm.2011.01.001

Bhavani MS, Kavitha S, Vairamuthu S, Vijayarani K, Bhat A (2021). Clinical Signs, Activity Indices and Prognostic Indicators in Dogs with Idiopathic Inflammatory Bowel Disease. Indian J. Anim. Res. 1-6. https://doi.org/10.18805/IJAR.B-4399

Carney PC, Ruaux CG, Suchodolski JS, Steiner JM (2011). Biological variability of C-reactive protein and specific canine pancreatic lipase immunoreactivity in apparently healthy dogs. J. Vet. Intern Med. 25: 825-830. https://doi.org/10.1111/j.1939-1676.2011.0729.x

Caviglia GP, Pantaleoni S, Touscoz GA, Adriani A, Rosso C, Smedile A, Pellicano R, Astegiano M, Bresso F (2014). Fecal calprotectin is an effective diagnostic tool that differentiates inflammatory from functional intestinal disorders. Scand. J. Gastroenterol. 49: 1419-1424. https://doi.org/10.3109/00365521.2014.934913

Chen P, Zhou G, Lin J, Li L, Zeng Z, Chen M, Zhang S (2020). Serum Biomarkers for Inflammatory Bowel Disease. Front Med. (Lausanne). 7: 123. https://doi.org/10.1093/ibd/izaa177

Delaney F, O'Brien RT, Waller K (2003). Ultrasound evaluation of small bowel thickness compared to weight in normal dogs. Vet. Radiol. Ultrasound. 44: 577-580. https://doi.org/10.1111/j.1740-8261.2003.tb00510.x

Dossin O, Lavoue R (2011). Protein-losing enteropathies in dogs. Vet. Clin. North Am. Small Anim. Pract. 41: 399-418 https://doi.org/10.1016/j.cvsm.2011.02.002.

Eckersall PD, Conner JG (1988). Bovine and canine acute phase proteins. Vet. Res. Commun. 12: 169-178. https://doi.org/10.1007/BF00362798

Estruch JJ, Barken D, Bennett N, Krawiec DK, Ogilvie GK, Powers BE, Polansky BJ, Sueda MT (2020). Evaluation of novel serological markers and autoantibodies in dogs with inflammatory bowel disease. J. Vet. Intern Med. 34: 1177-1186. https://doi.org/10.1111/jvim.15761

Feldman BF, Zinkl JG, Jain NC, Schalm OW (2010). Schalm's veterinary hematology. Wiley-Blackwell, Ames, Iowa.

Foell D, Wittkowski H, Vogl T, Roth J (2007). S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. J. Leukocyte Biol. 81: 28-37. https://doi.org/10.1189/jlb.0306170

Gommeren K, Desmas I, Garcia A, Bauer N, Moritz A, Roth J, Peeters D (2018). Inflammatory cytokine and C-reactive protein concentrations in dogs with systemic inflammatory response syndrome. J. Vet. Emerg. Crit. Care. (San Antonio) 28: 9-19. https://doi.org/10.1111/vec.12685

Grellet A, Heilmann RM, Lecoindre P, Feugier A, Day MJ, Peeters D, Freiche V, Hernandez J, Grandjean D, Suchodolski JS, Steiner JM (2013). Fecal calprotectin concentrations in adult dogs with chronic diarrhea. Am. J. Vet. Res. 74: 706-711. https://doi.org/10.2460/ajvr.74.5.706

Grobman M, Outi H, Rindt H, Reinero C (2017). Serum Thymidine Kinase 1, Canine-C-Reactive Protein, Haptoglobin, and Vitamin D Concentrations in Dogs with Immune-Mediated Hemolytic Anemia, Thrombocytopenia, and Polyarthropathy. J. Vet. Intern Med. 31: 1430-1440. https://doi.org/10.1111/jvim.14787

Heilmann RM, Berghoff N, Mansell J, Grutzner N, Parnell NK, Gurtner C, Suchodolski JS, Steiner JM (2018). Association of fecal calprotectin concentrations with disease severity, response to treatment, and other biomarkers in dogs with chronic inflammatory enteropathies. J. Vet. Intern Med. 32: 679-692. https://doi.org/10.1111/jvim.15065

Heilmann RM, Steiner JM (2018). Clinical utility of currently available biomarkers in inflammatory enteropathies of dogs. J. Vet. Intern Med. 32: 1495-1508. https://doi.org/10.1111/jvim.15247

Heilmann RM, Thieman-Mankin KM, Cook AK (2015). Bilateral renal sclerotherapy as a treatment option in a mixed breed male dog with idiopathic renal haematuria. Tierarztl Prax Ausg K Kleintiere Heimtiere. 43: 253-259. https://doi.org/10.15654/TPK-140861

Ide K, Kato K, Sawa Y, Hayashi A, Takizawa R, Nishifuji K (2016). Comparison of the expression, activity, and fecal

concentration of intestinal alkaline phosphatase between healthy dogs and dogs with chronic enteropathy. Am. J. Vet. Res. 77: 721-729. https://doi.org/10.2460/ajvr.77.7.721

Jergens AE, Crandell JM, Evans R, Ackermann M, Miles KG, Wang C (2010). A clinical index for disease activity in cats with chronic enteropathy. J. Vet. Intern Med. 24: 1027-1033. https://doi.org/10.1111/j.1939-1676.2010.0549.x

Jergens AE, Schreiner CA, Frank DE, Niyo Y, Ahrens FE, Eckersall PD, Benson TJ, Evans R (2003). A scoring index for disease activity in canine inflammatory bowel disease. J. Vet. Intern Med. 17,: 291-297. https://doi.org/10.1111/j.1939-1676.2003.tb02450.x

Kamath S, Blann AD, Lip GY (2001). Platelet activation: assessment and quantification. Eur. Heart. J. 22: 1561-1571. https://doi.org/10.1053/euhj.2000.2515

Malo MS, Moaven O, Muhammad N., Biswas B, Alam SN, Economopoulos KP, Gul SS, Hamarneh SR, Malo NS, Teshager A, Mohamed MM, Tao Q, Narisawa S, Millan JL, Hohmann EL, Warren HS, Robson SC, Hodin RA (2014). Intestinal alkaline phosphatase promotes gut bacterial growth by reducing the concentration of luminal nucleotide triphosphates. Am. J. Physiol. Gastrointest. Liver Physiol. 306: G826-838. https://doi.org/10.1152/ajpgi.00357.2013

McGrotty YL, Knottenbelt CM, Ramsey IK, Reid SW, Eckersall PD (2003). Haptoglobin concentrations in a canine hospital population. Vet. Rec. 152: 562-564. https://doi.org/10.1136/vr.152.18.562

Mumolo MG, Bertani L, Ceccarelli L, Laino G, Di Fluri G, Albano E, Tapete G, Costa F (2018). From bench to bedside: Fecal calprotectin in inflammatory bowel diseases clinical setting. World J. Gastroenterol. 24: 3681-3694. https://doi.org/10.3748/wjg.v24.i33.3681

Otoni CC, Heilmann RM, Garcia-Sancho M, Sainz A, Ackermann MR, Suchodolski JS, Steiner JM, Jergens AE (2018). Serologic and fecal markers to predict response to induction therapy in dogs with idiopathic inflammatory bowel disease. J. Vet. Intern Med. 32: 999-1008. https://doi.org/10.1111/jvim.15123

Oyaert M, Boel A, Jacobs J, Van den Bremt S, De Sloovere M, Vanpoucke H, Van Hoovels L (2017). Analytical performance and diagnostic accuracy of six different faecal calprotectin assays in inflammatory bowel disease. Clin. Chem. Lab Med. 55: 1564-1573. https://doi.org/10.1515/cclm-2016-1012

Rhodes B, Fürnrohr BG, Vyse TJ (2011). C-reactive protein in rheumatology: biology and genetics. Nat. Rev. Rheumatol. 7: 282-289. https://doi.org/10.1038/nrrheum.2011.37

Ricanek P, Brackmann S, Perminow G, Lyckander LG, Sponheim J, Holme O, Hoie O, Rydning A, Vatn MH (2011). Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. Scand. J. Gastroenterol. 46: 1081-1091. https://doi.org/10.3109/00365521.2011.584897

Ristic JM, Stidworthy MF (2002). Two cases of severe iron-deficiency anaemia due to inflammatory bowel disease in the dog. J. Small Anim. Pract. 43: 80-83. https://doi.org/10.1111/j.1748-5827.2002.tb00034.x

Schreiner NM, Gaschen FAG (2008). Clinical signs, histology, and CD3-positive cells before and after treatment of dogs with chronic enteropathies. J. Vet. Intern Med. 22: 1079-1083. https://doi.org/10.1111/j.1939-1676.2008.0153.x

Senchenkova EY, Komoto S, Russell J, Almeida-Paula LD, Yan L.S., Zhang, S., Granger DN (2013). Interleukin-6 mediates the platelet abnormalities and thrombogenesis associated with experimental colitis. Am J Pathol 183, 173-181. https://doi.org/10.1016/j.ajpath.2013.03.014

Shinde SS, Rajguru DN, Anantwar LG, Saleem M, Bawaskar SS (2000). Prevalence and haematological changes in canine gastroenteritis in Parbhani district. Intas. Polivet. 1: 129-131.

Truar K, Nestler J, Schwarz J (2018). Feasibility of measuring fecal calprotectin concentrations in dogs and cats by the fCAL® turbo immunoassay. J. Vet. Intern. Med. 32: 580.

Vermeire S, Van Assche G, Rutgeerts P (2004). C-reactive protein as a marker for inflammatory bowel disease. Inflamm Bowel Dis 10, 661-665. https://doi.org/10.1097/00054725-200409000-00026

Volkmann M, Steiner JM, Fosgate GT, Zentek J, Hartmann S, Kohn B (2017). Chronic Diarrhea in Dogs - Retrospective Study in 136 Cases. J. Vet. Intern. Med. 31: 1043-1055. https://doi.org/10.1111/jvim.14739

Washabau RJ, Day MJ, Willard MD, Hall EJ, Jergens AE, Mansell J, Minami T, Bilzer TW (2010). Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. J. Vet. Intern. Med. 24: 10-26. https://doi.org/10.1111/j.1939-1676.2009.0443.x

Wilke VL, Nettleton D, Wymore MJ, Gallup JM, Demirkale CY, Ackermann MR, Tuggle CK, Ramer-Tait AE, Wannemuehler MJ, Jergens AE (2012). Gene expression in intestinal mucosal biopsy specimens obtained from dogs with chronic enteropathy. Am. J. Vet. Res. 73: 1219-1229. https://doi.org/10.2460/ajvr.73.8.1219