Stress indices and clinico-biochemical alterations in gastroenteritis in dogs

The growing evidences suggest involvement of oxidative stress in several diseases including acquired immunodeficiency syndrome (AIDS), hepatitis and influenza in humans (Semb and Tang 1998), neoplasms like mammary tumor in bitches (Kumaraguruparan et al., 2005), Babesia gibsoni infection (Murase et al., 1996), visceral leishmaniasis (Bildik et al., 2004), lactose intolerance in laboratory animals (Dellan et al., 2005) and diarrhea in calves (Ranjan et al., 2006). Imbalance in redox state as assessed by estimation of activities of superoxide dismutase (SOD) and catalase (CAT), the major antioxidant enzymes in erythrocytes, and level of malonaldehyde (MDA) as the end of lipid peroxidation of polyunsaturated fatty acid-rich erythrocytic membrane, has been reported in canine parvoviral infections (Panda et al., 2009) and bacterial infections in different species (Moral et al., 1977; Fridovich, 1984). Acute cases of gastroenteritis in rats following exposure to lead have been reported to be associated with altered erythrocytic lipid peroxidation and antioxidant enzymes level (Gurer et al., 1998). However, there seems to have no report documenting oxidative stress indices in monogastric animals including dogs suffering from gastroenteritis. No controlled studies have been conducted to evaluate oxidant-antioxidant status in the erythrocytes of dogs suffering from gastroenteritis. Hence, the present clinical study is a novel attempt to investigate the erythrocytic oxidative stress, and compare the clinical score, hematoclinico-biochemical parameters with respect to breed, age, sex and presence or absence of blood in stool in dogs suggestive of gastroenteritis.

MATERIALS AND METHODS

The clinically affected dogs (n=106) with signs of diarrhea with or without vomition, and 21 apparently healthy dogs were recruited for the present investigation. The detailed history including vaccination, deworming, and the management practices and feeding habits of 127 client-owned dogs, was collected before clinical examination. Owners' consent was taken to use the dogs for the study, and guidelines of Institutional Animal Ethical Committee were followed.

Each dog was assigned with the total clinical score (CS) based on degree of dehydration, depression and fecal consistency (Table 1) by modifying the protocol of Walker et al. (1998). Each of the three parameters was given a score ranging from 0-3 and the calculated total CS was used to classify the dogs into three different groups: healthy (total clinical score 0, n=21, group I), moderate (CS = 4 to 6, n=25, group II) and severely affected (CS = 7 to 9, n = 81, group IV). None of the dogs could be categorized under group II as none of the examined dogs had the total clinical score between 1 and 3.

Table 1. Scoring of dogs using three different clinical signs.

<table>
<thead>
<tr>
<th>Score</th>
<th>Fecal consistency</th>
<th>Depression</th>
<th>Dehydration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal and well formed</td>
<td>Normal</td>
<td>Normal eyes and bright</td>
</tr>
<tr>
<td>1</td>
<td>Pasty</td>
<td>Mild</td>
<td>Mild dehydration, slight loss of skin elasticity, skin tent less than 3 sec</td>
</tr>
<tr>
<td>2</td>
<td>Semi-liquid</td>
<td>Moderate</td>
<td>Moderately dehydrated, skin tents greater than 3 sec but less than 10 sec</td>
</tr>
<tr>
<td>3</td>
<td>Watery</td>
<td>Severe</td>
<td>Unable to stand, dehydration, skin tents greater than 10 sec</td>
</tr>
</tbody>
</table>

INTRODUCTION

Gastrointestinal disease with clinical manifestations of vomiting, diarrhea, gastric dilatation or volvulus is the most common presenting complaint in pet dogs (Parr and Otto, 2013). Diarrhea and/ or vomition associated with gastroenteritis is an emergency in small animal practice, and is responsible for many deaths in dogs, particularly in pups (Appel et al., 1979; Radostits et al., 2007). The precise etiologies of gastroenteritis remain unclear in many occasions, and sometimes overlapping (Juckett and Trivedi, 2011; Berset-Istratecru et al., 2013). Recent studies have revealed its association more often with lifestyle factors than specific pathogens (Stavisky et al., 2011). But prospective studies to document the occurrence of diarrhea and vomiting are relatively limited in dogs, and the majority of the literature is based on information from clinical records (Saevik et al., 2012).

Gastroenteritis may be divided into several categories based on etiology. Diarrhea and vomition are prevalent symptoms of gastroenteritis, and are more common in canine parvovirus infections, causing the patients to seek medical attention (Panda et al., 2009). The inflammatory changes in the gastrointestinal tract may result in bloody inflammatory diarrhea, abdominal pain, nausea, inappetence, rectal bleeding, perianal fistulae, weight loss, fever and anemia (Juckett and Trivedi, 2011).

ABSTRACT

The erythrocytic lipid peroxidation and antioxidant enzymes were assessed in 106 dogs with diarrhea and/ or vomition and 21 apparently healthy dogs. The total clinical score (CS) was calculated based on fecal consistency, depression and dehydration. Twenty-five of 106 affected dogs were moderately affected (CS = 4 to 6), and 81 were severely affected (CS = 7 to 9). The dogs' erythrocyte oxidative index (lipid peroxides level, LPO) and antioxidant enzyme activities (superoxide dismutase, SOD and catalase, CAT) were measured with respect to clinical score, ±blood in stool, breed and sex of the dog. The severely affected dogs had significantly (p<0.05) higher LPO (7.16 ±0.20 vs 4.94 ±0.17 nmol of MDA/mg of Hb) and CAT activities (0.33 ±0.0 vs. 0.21 ±0.01 units/mg of Hb) as compared to control dogs with clinical score 0. Dogs with bloody stool (n=39) had significantly lower SOD activity (1.56 ±0.06 vs. 1.93 ±0.09 units/mg of Hb) as compared to dogs without blood in stool (mean CS = 6.73 ±0.17). However, the level of LPO, and activities of SOD and CAT were significantly higher than healthy dogs (CS = 0). It is concluded that increasing CS was associated with increased level of LPO along with alteration in activities of the antioxidant enzymes such as SOD and CAT.

ERYPHTHOXYTIC OXIDATIVE STRESS INDICES AND CLINICO-BIOCHEMICAL ALTERATIONS IN GASTROENTERITIS IN DOGS WITH VARIED CLINICAL SCORES

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Keywords: dogs, catalase, gastroenteritis, lipid peroxides; superoxide dismutase

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Blood samples (3 ml) were collected by venipuncture of either cephalic or recurrent tarsal vein using EDTA (ethylene diamine tetra-acetic acid) as anticoagulant and processed for preparation of RBC hemolysate or further hematological and biochemical studies. Blood samples were centrifuged at 2000 rpm for 5 min in a refrigerated centrifuge to separate plasma. The packed RBC was suspended in PBS (phosphate buffer saline) and was centrifuged at 3000 rpm for 5 min and the supernatant was discarded. This process was repeated three times. Finally, 1 : 20 dilution of RBC hemolysate was prepared using distilled water for estimation of LPO, SOD and CAT.

Erythrocytic lipid peroxides (LPO) level in 1:20 RBC haemolysate was estimated following the methods of Placer et al. (1966). The nmol malonaldehyde (MDA) per mg of RBC haemolysate was calculated by using 1.56 X 10^-10 M cm as molar extinction coefficient (Utley et al., 1967). Lipid peroxides level in the erythrocytes was expressed in nmol of MDA per mg of hemoglobin.

The activity of SOD was measured using nitro blue tetrazolium as substrate (Marklund and Marklund 1974) with certain modifications (Minami and Yoshikawa 1979). One unit of SOD activity was defined as the amount of enzyme which inhibited the auto-oxidation of pyrogallol by 50% under the given laboratory condition and the values were expressed as units/ mg of hemoglobin.

Catalase activity was estimated in the RBC haemolysate after appropriate dilution (Cohen et al. 1970). Briefly, the reaction was initiated by the addition of 50 μl of diluted sample to 2.950 ml of phosphate buffer hydrogen peroxide solution. Initial absorbance at 240 nm was read after 20 sec against reference cuvette in which instead of H₂O₂, the same amount of PBS was added. Time (sec) required for the fall in the initial absorbance by 0.050 was recorded. The activity of the enzyme was expressed as units/ mg of hemoglobin.

Hematological parameters like hemoglobin, total erythrocyte count, total leukocyte count and packed cell volume were estimated using standard protocol. The glucose level in the plasma was estimated by GOD/ POD (glucose oxidase and peroxidase) method using the diagnostics kit (M/s Qualigens Diagnostics, Mumbai, India) with the help of spectrophotometer (M/s ELICO double beam BL 200, Hyderabad, India). The total protein in all the samples was estimated using commercial kits (M/s Qualigens Diagnostics, Mumbai, India). The albumin level was subtracted from the respective total protein level to arrive at globulin level. The ratio of albumin to globulin was represented as the A/ G (albumin/ globulin) ratio.

Data were statistically analyzed using Statistical Package for the Social Sciences. The significance difference (p<0.05) with respect to different parameters were calculated in three different groups (Group I, III and IV) as per the clinical score. The data from all 127 dogs were sorted into another three groups; diarrhea without blood (n=67), diarrhea with blood (n=39) and healthy controls (n=21). Further, the data from 106 affected dogs were re-organized based on sex, breed and age (up to one year, n= 54; 1 to 3 years, n=32; > 3 years, n=20) to assess the alteration in clinical scores, and oxidative stress indices in gastroenteritis.

RESULTS

The mean rectal temperature of dogs belonging to group III (clinical scores 4-6) and group IV (clinical scores 7 and 9) was 38.76 ± 0.21°C and 39.03 ± 0.17°C. The mean temperature in group IV dogs was significantly (p<0.05) higher than the controls (38.4 ± 0.14°C). The mean hemoglobin, PCV, plasma protein and albumin level remained comparable in the three groups of dogs. However, there was a significant difference (p<0.05) in A/G ratio between group-III (0.72 ±0.02) and group-I (0.91 ±0.08), whereas mean values between group-I (0.91 ±0.08) and group-IV (0.93 ±0.04) were comparable. The glucose concentration in the affected dogs was comparable in all the three groups (Gr I – 79.42 ±0.17; Gr III – 71.33 ±1.61; Gr 4 – 73.21 ±13.2mg/dl), but the mean values in the affected dogs were non-significantly (p > 0.05) lower than the control animals.

Erythrocytic LPO level and antioxidant enzyme activity in RBC differed significantly among the three different groups of dogs, classified according to clinical score (Table 2). The mean erythrocytic LPO level in group-III dogs was significantly (p<0.05) higher than group-I (control) and group-IV (severely affected). The erythrocytic SOD activity in group III (2.56±0.16 units / mg Hb) dogs was significantly (p<0.05) higher than that of control dogs (1.32±0.07 units/ mg Hb). The dogs belonging to group IV (1.56±0.05 units / mg Hb) had statistically comparable SOD activity with that of the healthy dogs. The CAT activities of erythrocytes in group III (0.55 ± 0.05 units/ mg Hb) and group IV (0.33 ± 0.01 units/ mg Hb) was significantly (p<0.05) higher than healthy dogs (0.21 ± 0.01 units/ mg Hb).

The mean CS of dogs suffering from diarrhea without blood in stool (6.73 ±0.17, n=67) and that of dogs with blood in stool (8.56±0.08, n=39) were significantly (p<0.05) higher than healthy control dogs (0.00±0.00, n=21). Table 3 shows the mean level of LPO, SOD and CAT in different groups of dog with respect to presence of blood in the stool. The LPO level in dogs with bloody diarrhea was comparable (p> 0.05) to dogs without bloody diarrhea, but both the values were significantly (p< 0.05) higher than that of healthy dogs. SOD activity in erythrocytes from dogs suffering from diarrhea without blood in the stool was significantly higher than that of healthy controls, and dogs with bloody faces. The mean CAT activity in dogs with bloody diarrhea and in dogs without blood in the diarrhoeic stool was significantly (p< 0.05) higher than that of healthy control.

The dogs suffering from gastroenteritis sorted with respect to age, breed and sex, had non- significant variation (p>0.05) in different parameters indicative of oxidative status. Table 4 shows oxidative stress indices in erythrocytes from dogs with respect to age groups suggesting that age group did not have significant effect on LPO level and antioxidant activity such as SOD and catalase in the erythrocytes in dogs.
CAT activities also did not differ significantly among breeds (Table 4).

Erythrocytic lipid peroxidation and antioxidant enzymes like SOD and catalase (Figure 1) seems to have no report in dogs on erythrocytic lipid peroxide level in gastroenteritis with or without blood in stool. All the affected dogs (n=106), classified into two groups, male (n=66) and female (n=40), irrespective of final clinical score and breed, showed statistical similarity between the two groups with respect to LPO (6.85 ±0.18 vs 7.29 ±0.32 nmol of MDA/mg of Hb), SOD (1.51 ±0.07 vs 1.52 ±0.07 units /mg of Hb) (Figure 1).

Among all the affected dogs with gastroenteritis, the Spitz breed was the most affected (n=36). Erythrocytic lipid peroxidation and antioxidant enzymes like SOD and CAT activities also did not differ significantly among breeds (Table 4).

**Table 3. The oxidative stress indices in dogs with gastroenteritis with or without blood in stool.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Control (n=21)</th>
<th>Stool without blood (n=67)</th>
<th>Stool with blood (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean clinical score</td>
<td>0.00±0.00 (0.0-0.0)</td>
<td>6.73±0.17 (4-9)</td>
<td>8.56±0.08 (8-9)</td>
</tr>
<tr>
<td>LPO (nmol of MDA/mg of Hb)</td>
<td>4.94±0.17 (3.21-8.01)</td>
<td>7.53±0.22 (3.02-15.01)</td>
<td>7.35±0.32 (3.02-15.84)</td>
</tr>
<tr>
<td>SOD (units/mg of Hb)</td>
<td>1.32±0.07 (0.56-2.45)</td>
<td>1.93±0.09 (0.7-5.24)</td>
<td>1.56±0.06 (0.44-3.14)</td>
</tr>
<tr>
<td>Catalase (units/mg of Hb)</td>
<td>0.21±0.01 (0.13-0.35)</td>
<td>0.40±0.02 (0.11-1.56)</td>
<td>0.35±0.02 (0.12-0.79)</td>
</tr>
</tbody>
</table>

LPO- Lipid peroxides, SOD - Superoxide dismutase. Values are expressed as Mean± S.E; Values in parenthesis denote range. Mean values with different superscripts (a, b and c) in a row differ significantly at P < 0.05.

Fig 1. Sex-wise erythrocytic LPO level and activity of SOD and catalase activity in dogs with clinical signs of diarrhea (n=106).

**Discussion**

The etiological categorization of gastroenteritis is a major challenge to the clinicians in primary veterinary healthcare centers for therapeutic management and the prognosis of ailng dogs. Various etiological agents such as bacteria, viruses, parasites, fungi and an adverse reaction to dietary factors or drug reaction have been incriminated to cause gastrointestinal inflammatory changes, and associated clinical signs (Tams 2000; Sokolow et al., 2005). Most cases of diarrhea caused by dietary imprudence, parasites and drugs, unlike pathogen-induced diarrhea, are short in duration, mild in severity, and readily respond to symptomatic therapy. Very limited studies have been carried out in the past to define the incidence of different causes of gastroenteritis (Jani et al., 1992; Banja et al., 2002; Squires, 2003; Sokolow et al., 2005) and to assess the clinical score, clinico-hemato-biochemical alterations (Dharmadeeran et al., 2003; Biswas et al., 2005) during the course of gastroenteritis in dogs. Out of 127 cases selected for this study, 81 dogs were severely affected and 25 were moderately affected according to the clinical score. The major clinical signs were lethargy, vomition, inappetence, persistent diarrhea and/ or rise of temperature. Macartney et al. (1984) recorded similar clinical signs in parvovirus gastroenteritis. Dogs with blood in the stool had significantly higher rectal temperature than dogs without blood in feces, indicating that gastrointestinal bleeding elevates body temperature. Several authors have reported significantly higher packed cell volume in dogs with diarrhea and the same was attributed to dehydration (Jani et al., 1992; Jani, 2004). The dogs with blood in stool had significantly higher plasma protein level than their counterparts without blood in feces. Hypoalbuminemia and hyperglycemia have been reported in dogs suffering from parvoviral gastroenteritis (Jacobs et al., 1980).

Lipid peroxidation is a major deteriorating change in the cellular ageing process, bacterial and viral infection, following xenobiotic insults on unsaturated fatty acids, due to excessive generation of free radicals that cause injury to cells (Halliwell et al., 1992; Somashekariaish et al., 1992). Measurement of MDA continues to be a useful method for determination of the extent of lipid peroxidation, as it is the most abundant aldehyde formed as a by-product during this process (Gurer et al., 1998). The erythrocyte membrane is rich in polyunsaturated fatty acids and thus, is prone to oxidative damage by pro-oxidants (Clemens and Waller, 1987). Significantly (p<0.05) higher level of erythrocytic lipid peroxides in dogs with signs of diarrhea and vomition as compared to healthy controls might be due to excess production of free radicals (Ranjan et al., 2006). There seems to have no report in dogs on erythrocytic lipid peroxide level in gastroenteritis.

**Table 4: Oxidative stress indices and antioxidant activity changes with respect to age in dogs suffering from gastroenteritis.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Up to 1 year (n=54)</th>
<th>1-3 years (n=32)</th>
<th>More than 3 years (n=20)</th>
<th>Mean ± SE of all affected dogs (n=106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean clinical score</td>
<td>7.30±0.26</td>
<td>7.31±0.34</td>
<td>7.57±0.035</td>
<td>7.35±0.36</td>
</tr>
<tr>
<td>LPO (nmol of MDA/mg of Hb)</td>
<td>7.78±0.28</td>
<td>7.09±0.34</td>
<td>7.21±0.18</td>
<td>7.46±0.18</td>
</tr>
<tr>
<td>SOD (units/mg of Hb)</td>
<td>1.79±0.09</td>
<td>1.75±0.07</td>
<td>1.86±0.22</td>
<td>1.79±0.06</td>
</tr>
<tr>
<td>Catalase (units/mg of Hb)</td>
<td>0.39±0.02</td>
<td>0.41±0.04</td>
<td>0.34±0.02</td>
<td>0.38±0.01</td>
</tr>
</tbody>
</table>

LPO- Lipid peroxides; SOD- Superoxide dismutase; Values are expressed as Mean ± S.E.
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It was expected to have reduced SOD activity than CAT. The activity of these enzymes gets elevated following infection or xenobiotic insult to counter the ROS as a self regulatory correcting mechanism. However, continual assault beyond the auto-regulatory mechanism causes a decline in enzyme activities leading to a state of oxidative damage. In the present finding, SOD and CAT activity in moderately affected dogs remained significantly higher than in the controls. In severely affected dogs, the SOD activity dropped down and became comparable to healthy controls. Although the CAT activity was also reduced in severely affected dogs, it continued to remain significantly higher than healthy animals, suggesting that SOD serves as first line of defense followed by CAT in oxygen toxicity. SOD catalyzes superoxide radicals producing H$_2$O$_2$ and the latter is acted upon by CAT to produce water and oxygen (Halliwell, 1984).

Superoxide dismutase, CAT and glutathione peroxidase are the major enzymes present in RBC to counteract the toxic effects of reactive oxygen species such as superoxide radicals and hydrogen peroxides. SOD has proven to be a useful probe to study the participation of free radicals in reactions involving oxygen, since it acts as a defense against oxidative tissue damage by the dismutation of superoxide radicals (Fridovich, 1984). The activity of these enzymes helps to determine the biochemical environment and the pathophysiology of gastroenteritis may ameliorate the disease severity.

It is concluded that increasing clinical score in gastroenteritis in dogs was associated with increased oxidative stress. Age, sex and breed has little predisposition on the severity of clinical score, lipid peroxide level and antioxidant enzymes in gastroenteritis in dogs. Elevated levels of lipid peroxides and significant variation in the antioxidant enzyme profile like SOD and CAT with statistically comparable clinical haematological parameters indicates that alteration in oxidative stress indices can serve as a better tool to assess the severity of the disease and to monitor the disease progression. Therefore, incorporation of antioxidant in conventional therapeutics in view of involvement of redox imbalance in the pathophysiology of gastroenteritis may ameliorate the disease severity.

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References


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