Determination of pre and post treatment oxidative status and oxidative DNA damage in diarrheic calves

Mustafa Kabu*, Ibrahim Hakki Cigerci¹, Cangir Uyarlar² and Haci Ahmet Celik³

Faculty of Veterinary Medicine, Department of Internal Medicine, Afyonkarahisar-Turkey.

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ABSTRACT
Increase in production of free radicals causes damage in lipids of cell membrane, weakening in functions of cell proteins and DNA damage. The aim of the present study was to determine the oxidative status, level of oxidative DNA damage and treatment effectiveness on these parameters on diarrheic calves. The study was conducted using 10 clinically holstein calves from 2-3 months of age. Antidiarrheic treatment containing Amonium sulphate (30mg/kg/day, Gabbrocol, Ceva Inc.), a mixture of Bismuth subcarbonate, kaolin, pectin (10g/day, Bismol, Bioteknik Inc.), vitamin and mineral mixture (105g/day, Sky High Energy, Egevet Co.Ltd) as drugs orally administered to all calves for 3 days. Body temperature, feces and blood samples were taken before and after treatment. Dry matter measured (%) in feces, DNA damage level and some hematologic parameters, total oxidant status (TOS), total antioxidant status (TAS) and some biochemical parameters were measured from blood samples. HB, ALT, TP levels did not differ before and after treatment. However, body temperature (p<0.01), WBC (p<0.01), HCT (p<0.05), GGT (p<0.01), TOS (p<0.05), DNA damage (p<0.05) were decreased, dry matter in feces (p<0.01), RBC (p<0.05), AST (p<0.01), Albumin (p<0.01) and TAS (p<0.05) were increased after treatment. The results of the study indicate that oxidative DNA damage increases with degenerative diseases such as diarrhea and decreases to the normal range after effective treatment. It can be suggested from these results that oxidative DNA damage might be a good indicator to reveal the degeneration level of diarrhea in animals and a good parameter to evaluate the effectiveness of the treatment in terms of cellular form.

Key words: Calf, Comet assay, Diarrhea, Oxidative DNA Damage.

INTRODUCTION
Oxidative stress is caused by the increase in the rate of formation of free radicals or decrease in its elimination rate in the organism. These occur due to serious imbalance between free radicals formation and antioxidant defense mechanisms (Serafini and Del Rio, 2004). This ultimately leads to the damage of various cellular and extracellular macromolecules (Dunder and Aslan, 1999). Free radicals of oxygen metabolism may damage structural and functional components of body, such as proteins (enzymes, collagen) neurotransmitters, nucleicacids (DNA and RNA) and fatty acids (Del Maestro., 1980; Cooke et al., 2006). Reactive Oxygen Species (ROS) produced by activated neutrophils during the inflammatory response play an important role in the pathogenesis of inflammatory diseases including hepatitis, gastritis, colitis, chronic renal failure and rheumatoid arthritis (Ho et al., 2000; Karakucuk et al., 2004; Kucukkurt et al., 2014). Among many diseases of animals, Calves diarrhea is one of the most important problems of rearing calves, which are most often seen in the neonatal period. Many infectious and noninfectious factors play pivotal role in the etiology of calf diarrhea. High morbidity and mortality has still been observed in neonatal calf diarrhea even after using chemotherapeutic agents (Blanchard, 2012). It is difficult to find the etiological factors of high mortality in diarrheic calves (Cleek and Phillips, 1981; Hall et al., 1992; Smith, 2012). It is presumed that oxidant-antioxidant imbalance may be the basis of tissue damage in calf’s diarrhea and good treatment therapy can overcome the oxidative stress. Numerous studies to date suggest the importance of chemotherapy in reducing oxidative stress (Block et al., 1992; Keli et al., 1996; Cigerci et al., 2009). So, this study reflects and determinesthe level of oxidative DNA damage and oxidative status,before and after the treatment trial in diarrheic calves.

MATERIALS AND METHODS
Animals: The study was carried out on 10 Holstein calves, at 2-3 months of age and showing the clinical signs of diarrhea. The animals were showing the signs of eye orbital depressions (0.5 cm depression from the skin), skin-tenting time was 2-4 sec and response to the external stimuli was good.

*Corresponding author’s e-mail: mkabu@aku.edu.tr. ¹Faculty of Science and Literatures, Department of Biology, Afyonkarahisar-Turkey. ²Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Afyonkarahisar-Turkey. ³Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Afyonkarahisar-Turkey.
Treatment trial: All infected calves were treated with oral dose of anti-diarrheic drug having ammonium sulphate 30mg/kg/day (Gabbrocol, Ceva Inc.), Bismuth subcarbonate, kaolin and pectin mixture 10 g/day (Bismol, Biotechnology Inc.), vitamin and mineral mixture 105g/day (Sky High Energy, Ltd. Egevet. Co.Ltd.) for three day Body temperature, feces and blood samples were taken before and after treatment.

Measurement of hematologic and biochemical parameters: Fecal dry matter (%) was determined as described by Abe et al. (1999). In hematologic analysis, leukocyte (WBC, x10³/μl), erythrocyte (RBC, x10⁹/μl), hematocrit (HCT,%), hemoglobin (Hb, g/dL) were analyzed using hematologic analyzer. In the blood serum, aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), gamma-glutamyltranspeptidase (GGT, U/L), Total Protein (TP, g/L), albumin (ALB, g/L) levels were measured in ELISA reader by using commercial kits.

Measurement of the total antioxidant capacity and total oxidant status: Plasma total antioxidant capacity (TAC) and Plasma total oxidant status (TOS) levels were determined using an automated method developed by Erel. (2004) and Erel. (2005) respectively.

DNA damage determination by comet assay: In leukocytes, DNA strand breakage frequency was determined by the Comet assay (single cell gel electrophoresis) as described by Collins et al. (1995). Briefly, slides were coated with low melting agarose gel and then leukocyte suspension was spread on it (Sandwich model). Then slides were immersed in high salt concentration, lysing solution to breakdown entire cell content. After lysing, the electrophoresis in electrophoretic buffer (pH>12.3) was carried out to uncoil the DNA. Subsequent to electrophoresis, the slides were washed and stained with ethidium bromide to examine under a fluorescent microscope. Comet tail was measured in ELISA reader by using commercial kits.

RESULTS AND DISCUSSION

Data regarding body temperature, fecal dry matter and hematological parameters before and after the treatment in calves have been shown in Table 1. There was significant (p<0.05) decrease in the body temperature, WBC and HCT values while significant (p<0.05) increase in fecal dry matter and RBC was observed after the treatment. In current study, there was higher rectal temperature before treatment, which is generally associated with borne infection and inflammation (Risalde et al., 2011). Pre-treatment, fecal dry matter (DM) contents were low due to less intestinal reabsorption of water. In some diarrheic calves, enteritis has been reported that increases the WBC, neutrophil and granulocyes (Sahal et al., 1994; Ocal et al., 2006) due to result of the body’s immune reaction against gastrointestinal infections (Coles, 1986). Moreover, calf diarrhea resulted in decrease of extracellular fluid and increase in hematocrit levels (Vermunt, 1994). In our study, there was no statistical significance (p>0.05) in HB level of calves before and after treatment. In this study, in serum biochemical analysis, significantly (p<0.05) increased concentration of AST was evaluated whereas ALT and GGT (p <0.01) concentrations were significantly (p<0.01) decreased after the treatment. Similarly, total serum proteins and albumin were higher (p<0.05) after treatment as shown in Table 2. Some researchers reported that diarrhea in calves is due to clinical infection and many etiological factors are normally involved. So, serum ALT concentration was raised above to their normal levels as compared to healthy animals (Karademir and Sendil, 2001; Pekcan et al., 2012).

Results after treatment also showed significant decrease (p<0.05) in oxidant and DNA damage level and increase of total antioxidant levels (Table 3). Due to many biochemical metabolic processes, such as signal transduction
and immune function in the body, levels of free radicals fluctuate continuously (Davies, 1995; Alle and Tresini, 2000). Free radicals induce the DNA damage and promotes carcinogenesis (Saravan and Pugal, 2005). Many researchers evaluated the increase of DNA damage and TOS in many diseases. Heaton et al. (2002) stated the DNA damage status as an important biomarker to determine the antioxidant status in the diet of dogs and cats. Altindag et al. (2007) reported the higher TOS and DNA damage in peripheral leukocyte in the patients with osteoarthritis compared to the control group. Similarly, Aslan et al. (2011), demonstrated that DNA damage and TOS levels were significantly higher in patients with Ulcerative Colitis while TAC was significantly lower. These findings correlate to our study, as all diarrheic calves having the higher level of DNA damage in peripheral leukocytes and total oxidant status before treatment. This rise of peripheral leukocyte DNA damage and decreased TAC seems to be associated with stress caused by the disease. Moreover, it was seen that, all abnormal factors in diarrheic calves were came to normal after the treatment.

Up till now, treatment efficacy, clinical examination, hematological and biochemical parameters have been checked in diarrheic calves. The efficacy of the treatment has been determined with the determination of DNA damage status in our study. Moreover, TOS and TAS levels were checked along with the therapeutic activity. One can conclude from these data that, therapeutic efficacy can be assessed by the detection of peripheral leukocyte DNA damage in veterinary medicine and a good treatment trial can reduce the oxidative stress of the body caused by any infectious or non-infectious diseases. But still further studies are required to understand this mechanism.

TABLE 3. DNA damage, Total oxidant status and Total antioxidant capacity levels before and after treatment in diarrheic calves (Mean±S.E.)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Hasarı</td>
<td>Mean: 21,00±2,13</td>
<td>Mean: 17,00±1,71</td>
<td>0,011</td>
</tr>
<tr>
<td>(arbitrary units)</td>
<td>Min:12</td>
<td>Min:10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max:32</td>
<td>Max:26</td>
<td></td>
</tr>
<tr>
<td>Total oxidant status</td>
<td>Mean: 13,47±0,81</td>
<td>Mean: 11,21±0,26</td>
<td>0,017</td>
</tr>
<tr>
<td>(μmol H2O2 equiv./l)</td>
<td>Min:10,79</td>
<td>Min:10,10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max:19,90</td>
<td>Max:12,80</td>
<td></td>
</tr>
<tr>
<td>Total antioxidant capacity</td>
<td>Mean: 0,51±0,02</td>
<td>Mean: 0,55±0,02</td>
<td>0,021</td>
</tr>
<tr>
<td>(mmol Trolox equiv./l)</td>
<td>Min:0,42</td>
<td>Min:0,45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max:0,58</td>
<td>Max:0,65</td>
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</table>

REFERENCES

Cooke MS, Olinski R and Evans MD (2006). Does measurement of oxidative damage to DNA have clinical significance. Clin Chim Acta. 365: 30-49


