



The Serum Pepsinogen Level of Dairy Cows with Gastrointestinal Disorders

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Abstract

The incidence of abomasal mucosal diseases in dairy cows suffering from gastrointestinal disorders is becoming more frequent in modern intensive production. Clinical signs are often non-specific. In this study, 67 dairy cows with gastrointestinal disorders and 9 healthy dairy cows as the control group were used. In order to make a tentative diagnosis, a complete physical examination was performed, and the fecal samples were taken from each cow for the fecal occult blood (FOB) and the fecal egg count (FEC). Blood samples were taken from the coccygeal vein for WBC, Hematocrit (PCV) evaluations, and serum biochemical analysis. Serum pepsinogen activity and total protein; albumin and globulin were measured using validated standard methods. The statistical analysis was performed using SPSS software. The level of significance was set at $P < 0.05$. A significant increase in serum pepsinogen activity was seen in all the cases of abomasal displacements compared to the control group. Among the abomasal displacement groups a significant increase in serum pepsinogen activity was seen in abomasal displacements with suspected abomasal ulcer in comparison with those without any signs of abomasal ulcer (positive FOB and melena). No considerable differences were observed between WBC, PCV, and total protein and globulin in different gastrointestinal disorders and the control group. In this study, the serum pepsinogen activity in all dairy cows with signs of abomasal ulcer (melena and positive fecal occult blood test) was higher than the control group, since all of the cases had negative abomasal parasites; these increases in the signs of abomasal ulcer could predict abomasal ulcer complication in the cases of displacements.

Introduction

The selection of cattle for high milk production increased the susceptibility of the occurrence of metabolic and digestive diseases. Diagnoses of the gastrointestinal diseases are achieved by invasive and non-invasive methods. The invasive techniques include laparotomy and laparoscopy, while non-invasive techniques are represented by simultaneous auscultation and percussion, sonography, and rectal exploration (Radostits, 2000; Radostits et al., 2007). The prevalence of abomasal ulcers in dairy cows is becoming more commonplace in modern intensive husbandry systems. The various pathological mechanisms of gastric or abomasal ulcers are not obvious. Other concurrent diseases and related factors such as stress, non-steroidal anti-inflammatory drugs and high-grain diets which might decrease mucosal protection or, increase the acidity of ingesta, are proposed; however, the definite roles of these factors remain arguable. Some related factors might affect the mucosa directly, whereas others

might develop mucosal injury by preventing abomasal motility and cause abomasal atony. This could result in acid retention within the abomasum, which has been shown to contribute to gastric ulceration in other species and possible abomasal ulcer in cow with gastrointestinal disorders (Braun et al., 1991; Cebra et al., 2003; Fubini and Divers, 2008). The diagnosis of abomasal ulcers are usually confused with other causes of gastrointestinal disorders with signs of indigestion. Evidences of the abomasal ulcers may range from no clinical signs to haemorrhages and subsequent melena, through to peritonitis if the erosive processes penetrate all layers of the abomasum (Cable, 1998; Katchuik, 1992; Palmer and Whitlock, 1984; Radostits et al., 2007). Clinical signs are often rather diffuse and non-specific and it would be of considerable help to find an association between abomasal ulcers and various clinical parameters. Diagnosis of abomasal ulcers are based on clinical signs and fecal occult blood tests. Gastroscopy has become a superior tool to confirm the diagnosis in

humans, horses, and dogs (Oderda, 1988; Sandin et al., 2000). In cattle practice, this diagnostic method cannot be employed due to the presence of the forestomach and permanent secretion of abomasal juice. In humans, serum pepsinogen is elevated in different diseases including gastric and duodenal ulcers (Samloff et al., 1986). Pepsinogen is a proenzyme produced by parietal cells of the abomasal mucosa. The increased plasma levels of pepsinogen are caused by its leakage into blood vessels from damaged abomasal mucosa (Kataria et al., 2008). The high blood level of pepsinogen can be used in the diagnosis of abomasal parasitism (Berghen et al., 1993). Earlier researchers have reported increased levels of serum pepsinogen with abomasal ulcers in cattle and sheep (Hajimohammadi et al., 2010; Mesaric, 2005; Zadnik and Mesaric, 1999). In the present study, the association of serum pepsinogen activity to diagnose the abomasal ulcers following the gastrointestinal diseases of dairy cattle was investigated.

Materials and Methods

Animals

A total of 67 Holstein dairy cattle suspected of suffering from gastrointestinal diseases having been referred to the Veterinary Clinic of Shiraz University from October 2013 to June 2014 were investigated. The animals were taken from the dairy farms in the outskirts of Shiraz, southern Iran. In addition, 9 clinically healthy Holstein cows in early lactation which belonged to the farm of Shiraz Veterinary Medicine School were used as control group.

Clinical Examination

All the cattle underwent a thorough clinical examination as described previously (Radostits, 2000). The general condition and demeanor, rectal temperature, heart rate, respiratory rate, and lung sounds were determined. Swinging and/or percussion auscultation on both sides of the abdomen, tests for a reticular foreign body, and rectal palpation were also carried out. The displacement diagnosis was based on the presence of the characteristic ping on simultaneous auscultation and percussion, and exclusion of other causes of left- or right-sided pings. To confirm abomasal displacements, the ultrasonography and Liptak tests were helpful. Additionally, the diagnosis was verified surgically when an operation took place. 42 Cows with abomasal displacements were also divided based on signs of suspected abomasal ulcer (melena and positive fecal occult blood test) in 4 groups as follows: left displacement of the abomasum (LDA; n=22), right displacement of the abomasum (RDA; n=6), LDA with suspected abomasal ulcer (n=9), and RDA with suspected abomasal ulcer (n=5).

Moreover, 25 cattle with another common gastrointestinal disease (simple indigestion, vagus indigestion, peritonitis, and ruminal impaction) were diagnosed and added to our study as the other gastrointestinal diseases group.

The control animals were also multiparous and within 1 month of lactation, and were chosen via the same clinical and hematological methods. General condition of the animals, rectal temperature, heart and respiratory rate were determined. Presence of swinging and/or percussion auscultation on both sides, test for foreign body, and rectal palpation were also carried out.

Blood Sampling

Ten (10) mL of blood was collected from the coccygeal vein of each cow and into an evacuated sterile tube without any anticoagulant. Samples were rapidly centrifuged at 3,000 rpm for 15 min. Sera were harvested and aliquots were stored at -20°C until analysis. Beside, 5 mL of blood was also collected into the sterile tube with EDTA as an anticoagulant for WBC count.

Fecal samples were taken from each cow, and fecal occult blood test (FOB) was done by commercial kit (Arj Azma Co.) based on Weber (rapid method). And the fecal egg count (FEC) for abomasal parasites was determined by McMaster technique.

Serum and Blood Biochemical Analysis

The total protein was measured by the Biuret method (Armstrong and Carr, 1964) and the albumin by the Bromocresol green method, while the total globulin concentration was calculated as the difference between the total protein and the albumin.

Serum Pepsinogen Determination

The serum pepsinogen levels in all samples were determined using a micro-method; briefly serum was added to a bovine serum albumin (BSA) substrate solution containing glycine buffer at pH 1.6. During 24 hours incubation at 37°C pepsin hydrolyses the substrate, releasing peptide fragments. After three hours, residual substrate was precipitated with perchloric acid and the peptide fragments remaining in the supernatant were measured using a modified folin reagent. The optical density was then measured at 680 nm with an ELISA-reader (Labsystems, Helsinki, Finland). Activities are calculated by comparison with a series of tyrosine standards, and subtraction of appropriate serum blanks (Dorny and Vercreyusse, 1998).

Statistical Analysis

All data are presented as mean \pm standard deviation (SD). The differences among the mean of concentrations

of different factors in all groups were analyzed by One way ANOVA (95% confidence interval) followed by Tukey's multiple comparison test using SPSS software (SPSS for Windows, version 16, SPSS Inc, Chicago, IL, USA). The level of significance was set at $P < 0.05$.

Results

The changes in serum pepsinogen activities and other hematological parameters in dairy cows with the common gastrointestinal disorders are presented in table 1. The serum pepsinogen activity was significantly higher in LDA, RDA and LDA, and RDA with suspected abomasal ulcer groups than control group in comparison with other gastrointestinal diseases group in which there was no significant difference in serum pepsinogen activity compared to the control group. Furthermore, a

significant increase in serum pepsinogen activity was seen in abomasal displacement groups with suspected abomasal ulcer in comparison with LDA and RDA groups without any signs of abomasal ulcer (melena and positive fecal occult blood test). There was no significant alteration in hematological parameters (WBC, PCV, and total protein and globulin) between groups (Table 1). A significant decrease in total albumin was observed in the other gastrointestinal diseases group compared to the control group, and there was a significant difference in total albumin between LDA and the other gastrointestinal diseases group.

In all of cases analysed, there was no abomasal parasites detected via McMaster technique.

Table 1. Mean \pm SD of serum pepsinogen activity, WBC, hematocrit, total protein, albumin and globulin in the common gastrointestinal diseases in cattle.

Different diseases	Pepsinogen (IU/L)	WBC (per/ μ L)	Hematocrit (%)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
LDA (n= 22)	4.51 \pm 1.26 ^{*a}	7340 \pm 2438	35.17 \pm 5.29	6.65 \pm 0.91	3.08 \pm 0.31 ^a	3.57 \pm 0.94
RDA (n=6)	5.68 \pm 1.21 ^{*b}	8650 \pm 842	35.40 \pm 5.41	6.75 \pm 0.98	3.13 \pm 0.56	3.61 \pm 0.75
LDA and suspected Abomasal ulcer(n=9)	8.23 \pm 1.27 ^{*ac}	9827 \pm 4027	32.05 \pm 3.19	5.64 \pm 1.42	2.66 \pm 0.36	3.17 \pm 1.46
RDA and suspected Abomasal ulcer (n=5)	8.29 \pm 1.36 ^{*b}	10070 \pm 4286	28.06 \pm 7.12	5.92 \pm 2.26	2.84 \pm 0.67	3.08 \pm 1.61
other gastrointestinal diseases (n= 25)	3.58 \pm 1.21 ^{bc}	8604 \pm 4147	33.43 \pm 6.71	6.54 \pm 1.68	2.58 \pm 0.61 ^{*a}	3.95 \pm 1.50
Control (n=9)	2.43 \pm 0.32 [*]	6922 \pm 1252	33.88 \pm 3.25	7.06 \pm 0.29	3.32 \pm 0.15 [*]	3.73 \pm 0.28

Values with the superscript * in a column are significant difference with respect to the control ($P < 0.05$).

For each parameter, same small superscription shows significant different between various diseases ($P < 0.05$)

Discussion

In ruminant blood, a certain physiological activity of pepsinogen is present. The serum pepsinogen value in healthy cows does not significantly change with the lactating stage or age (Ohwada et al., 2002). Harvey-White et al. (1983) and Hilderson et al. (1989) found that serum pepsinogen values of zero to 5.0 IU/L are normal for young and mature cattle and are not correlated with any clinically significant damage to the abomasal mucosa. Paynter (1994) reported that the reference range for the mean pepsinogen activity in cattle is 2.54–3.54 IU/L. There are conflicting findings on how serum pepsinogen passes from peptic cells to the blood in cattle and two main theories to describe increasing serum pepsinogen levels. The first involves increased epithelial and vascular permeability allowing pepsinogen to leakage into the blood (Scott et al., 1999; Tanaka et al., 1991), and the second affects the direct hyper-

secretion of pepsinogen into the blood from zymogenic cells in a retrograde direction (Fox et al., 2002; McKellar et al., 1990). An increase in pepsinogen levels indicates mucosal damage as an effect of *Ostertagia* infection and abomastitis in cows (Berghen et al., 1993; Scott et al., 1999).

In the study by Aukema and Breukink (1974), serum pepsinogen activities of cattle with abomasal ulcers with fatal hemorrhages were abnormally high only in half of the investigated animals. Mesaric et al. (2002) found out an increased serum pepsinogen activities in the blood of cows with abomasal leukosis. There are reports that cows with various abomasal disorders also have increased serum pepsinogen activities (Mesaric et al., 2002; Mesaric, 2005; Vörös et al., 1984). Displaced abomasum shows a group of pathological events arising from smooth muscle atony and gas and fluid accumulation following the displacement of the

abomasum from its normal ventral position on the abdominal floor (Constable et al., 1992; Geishauser, 1995).

Paynter (1994) described that the mean serum pepsinogen activity was above the physiological range of 5.0 IU/l that was labeled as the normal threshold value for cattle. In addition, Mesaric (2005) reported that low or normal serum pepsinogen activity (<5.0 IU/L) may be useful as a predictor for low susceptibility to major changes to the abomasal mucous membrane.

In the present study, the serum pepsinogen activity in different common gastrointestinal diseases in dairy cows was investigated, and we found a significant serum pepsinogen increase in all abomasal displacement groups rather than the control group in comparison with other gastrointestinal diseases group; beside, the serum activity of pepsinogen in the all cases of LDA and RDA with signs of abomasal ulcer (melena and positive fecal occult blood test) was higher than LDA and RDA groups, and this significant increase was above the predicted value for abomasal ulcer (5 IU/L) which earlier studies reported (Mesaric, 2005; Paynter, 1994). Our finding is similar to that of Vörös et al. (1984) which concluded that blood levels of pepsinogen increased especially in cows with left displacement. On the other hand, Abouzeid et al. (2008) reported that serum pepsinogen markedly decreased in all cows with abomasal displacements. Pepsinogen values in cows with LDA, RDA, and abomasal volvulus (AV) at 7-11 days after the operation were still lower than the reference value; likewise, Ohwada et al. (2002) reported that in cows with abomasal diseases, serum pepsinogen concentrations in LDA, RDA, and AV remarkably decreased compared to the healthy cows. The decreased value of serum pepsinogen in cows with abomasal displacements is thought to be attributed to the atrophy of the mucous membrane of the abomasum. And in human beings, a decrease in pepsinogen in sera of patients with chronic atrophic gastritis was reported (Abouzeid et al., 2008; Samloff et al., 1986). In our study the serum activity of pepsinogen in all cases of abomasal displacements was higher than the control group. The results of our study were generally in agreement with the reports in which serum pepsinogen concentration in abomasal displacements increased compared to the healthy controls; this high level of pepsinogen concentration might deal with vascular permeability in displaced abomasum also any injury to the gastric mucosa allows diffusion of hydrogen ions from the lumen into the mucosal tissues and also allows diffusion of pepsin and pepsinogen into the rest of the mucosa causing further damage (Hajimohammadi et al., 2010; Vörös et al., 1984; Zadnik and Mesaric,

1999). The changes in abomasal environment are due to increased permeability of mucosa. Rise in abomasal pH is an important stimulus for gastrin release; which also increase in pepsinogen secretion (Kataria et al., 2008). Furthermore, in our study the serum pepsinogen activity in all dairy cows with signs of abomasal ulcer (melena and positive fecal occult blood test) was higher than that of our control group and the predicted value for abomasal ulcer (5 IU/L), since all of our cases had negative abomasal parasites, and this increase could contribute to abomasal ulcer. The high levels of serum pepsinogen activity (>5.0 IU/L) could be useful as a predictor of high susceptibility for abomasal ulcers in different gastrointestinal diseases with favorable parasitological status, and this agreed with the study by Mesaric (2005). Results of this study reinforce that serum pepsinogen activities were significantly higher in cows with clinical signs of abomasal ulcer than those with other gastrointestinal diseases. Therefore, this finding indicates that serum pepsinogen level could be a simple serum test to diagnose or evaluate cows with subclinical abomasal ulcer.

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