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Original article

DIAGNOSTIC SIGNIFICANCE OF PLASMA GASTRIN CONCENTRATION FOR THE DIAGNOSIS OF TYPE 1 ABOMASAL ULCER IN WATER BUFFALO: A PRELIMINARY CASE CONTROL STUDY

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Summary

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The type 1 abomasal ulcer (AU1) does not have specific clinical signs, so there is a need to identify some early biochemical markers for its diagnosis in cattle and buffaloes. The plasma gastrin is reported to reflect the gastric mucosa damage but its utility for the diagnosis of AU1 in buffaloes has not been evaluated. The objective of this study was to investigate the test performance of plasma gastrin to distinguish between healthy buffaloes and the buffaloes with AU1. Twenty-three buffaloes with AU1 and six buffaloes without any abomasal ulcer were used. The blood samples were collected from buffaloes, slaughtered in a buffalo specific slaughterhouse for estimation of the plasma gastrin. After slaughter the abomasa were examined for the presence of AU1 and the blood samples were collected for estimation of plasma gastrin. The mean plasma gastrin concentration of the ulcer-positive buffaloes was significantly ($P<0.05$) higher than the ulcer-negative buffaloes. The receiver operating characteristic curve analysis suggested that the optimal value of plasma gastrin for the diagnosis of AU1 was 106.2 pg/mL. This preliminary work suggests that plasma gastrin could be a valid diagnostic test for the detection of AU1 in the buffaloes. The sensitivity, specificity, positive predictive value and negative predictive value of the plasma gastrin to diagnose AU1 in the buffaloes were 78.3, 100, 100 and 69.9 pg/mL, respectively.

Key words: abomasal ulcer, buffalo, diagnostic performance, gastrin

INTRODUCTION

Abomasal ulcers occur in several forms and produce different clinical signs (Smith *et al.,* 1983). Type 1 abomasal ulcer (AU1) is a non-perforated ulcer of the abomasal mucosa associated with minimal haemorrhage and non-specific clinical signs (Braun *et al.,* 2020). The currently available literature advocates use of faecal occult blood test for the diagnosis of AU1 in both cattle and buffaloes (Hussain *et*

al., 2015). However, the faecal occult blood test has not proved to be suitable for detecting the abomasal ulcers due to its low sensitivity (Hund *et al.,* 2016). Unlike humans and horses, the use of gastroscopy for the visualisation of the gastric mucosa in bovids is difficult and has not been reported so far in this species. Due to high prevalence of AU1 in water buffalo (Tajik *et al.,* 2013; Hussain *et al.,* 2019), there is a need to identify some biomarkers for the diagnosis and management of AU1 in this species.

Gastrin is secreted from the gastrin cells of the pyloric region of the abomasum into the blood circulation, reaches the parietal cells and is an important stimulator of gastric acid and pepsinogen secretion (Argenzio, 2005). The plasma gastrin is reported to reflect the damage to abomasal mucosa especially due to parasites like *Haemonchus* and *Ostertagia* (Fox *et al.,* 1993; Kataria *et al.,* 2008) but its utility for diagnosis of AU1 in buffaloes has not been evaluated. This case control study was designed to investigate the plasma gastrin in ulcer-negative and ulcer-positive buffaloes and to evaluate the possible role of plasma gastrin in identification of the abomasal mucosa damage due to AU1 in the buffaloes. Two null hypotheses for this study were formulated; first, the variances of the plasma gastrin will be equal for the ulcer-negative and ulcer-positive buffaloes and second, the plasma gastrin levels will not differ significantly ($P<0.05$) between the ulcer-negative and ulcer-positive buffaloes.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University. This study was conducted on the blood samples collected from a mixed population of buffaloes, slaughtered in a buffalo specific abattoir. The buffaloes were selected randomly as has been discussed in our published findings (Hussain *et al.,* 2019). Blood samples were collected by jugular venepuncture into commercially available sodium citrate coated tubes. The plasma was separated with centrifugation (2000–3000 g for at least 15 minutes) and frozen at –20 °C until analysis. The abomasa were examined for the presence or absence of ulcers, and the findings are discussed in detail (Hussain *et al.,* 2019). For this study, 31 plasma samples were used, representing 24 samples of AU1-positive buffaloes and seven ulcer-negative buffaloes. The plasma samples from each group were selected randomly using lottery method of randomisation. The plasma gastrin was analysed by IMMULITE 1000 Immunoassay System (Siemens Healthineers, Germany) by using Immulite® Gastrin Kit. The procedure for gastrin estimation was standardised during the preliminary study of the PhD dissertation of the first author. The procedure is fully automated except for the sample loading in the sample cups. In brief, IMMUITE/IMMULITE 1000 gastrin is a chemiluminescent, enzyme labelled immunometric assay based on ligand labeled murine monoclonal capture antibody specific for gastrin and separation by anti-ligand coated solid phase. The sample along with the ligand labelled anti-gastrin monoclonal antibody, an alkaline phosphatase-conjugated rabbit polyclonal antigastrin antibody and an alkaline phosphatase-conjugated murine antibody are simultaneously incubated in presence of the immobilised anti-ligand bead within an IMMULITE Test Unit. During the 60 minute incubation, gastrin molecule in the sample forms antibody sandwich com-

plexes which in turn, bind to anti-ligand on the solid phase. Unbound conjugate is then removed by a centrifugal wash, after which luminogenic substrate is added and the Test unit is incubated for a further ten minutes. The chemiluminescent substrate, a phosphate ester of adamantly dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of these intermediates results in the sustained emission of light. The bound output, as measured by the luminometer is proportional to concentration of gastrin in the sample.

Statistical analysis

The data were analysed by using SPSS for Windows (Version19.0, SPSS Inc. 83 Munich, Germany) and MedCalc (Version 19.6.1, MedCalc Software). Normal distribution was tested by Shapiro-Wilk's test and visual examination of the histograms, normal Q-Q plots and box plots. Two gastrin values (one ulcer-negative and one ulcer-positive) were outliers, as indicated by box plots, so these two values were not used in the subsequent statistical analysis. Levene's test was used to assess the equality of variances for the two groups. Welch's t-test and Brown-Forsythe test were used for the comparison of the plasma gastrin concentration between the ulcer negative and the ulcer positive buffaloes.

The sensitivity, specificity, positive predictive value and negative predictive value were calculated with a receiver operating characteristic (ROC) curve analysis. The continuous variable was the plasma gastrin and the classification variable – presence or absence of AU1. The 95% confidence interval was calculated for all the test characteristics. The predictive values calculated were based on the apparent prevalence (89/134=66.42%) of AU1 in the present study population. The optimal test criterion was determined using the Youden index giving equal weight to the sensitivity and specificity.

RESULTS

The Shapiro-Wilk's test ($P>0.05$), visual examination histograms, normal Q-Q plots and box plots showed that the plasma gastrin concentrations were approximately normally distributed for both the ulcer-negative and ulcer-positive buffaloes. Although our data were a little skewed and kurtotic, did not differ significantly from the normality. As the P-value of Levene's test was 0.04, the null hypothesis of equal

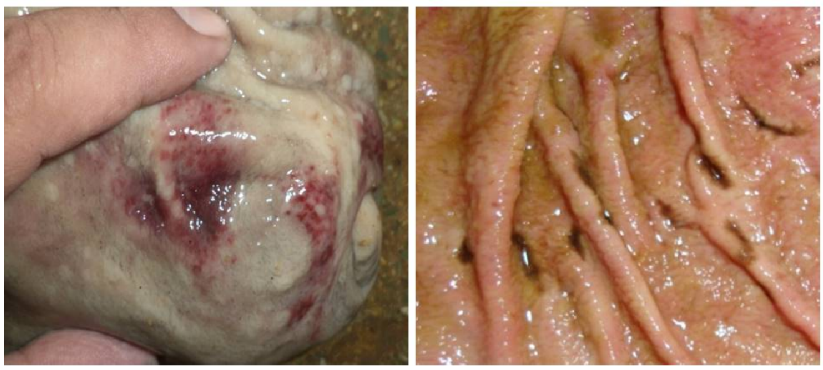

Fig. 1. Gross appearance of different degrees of type 1 abomasal ulcers.

Table 1. Plasma gastrin concentration (mean±SEM) in buffaloes with or without type 1 abomasal ulcer

	Plasma gastrin concentration (pg/mL)		
	Number		Minimum, maximum
Ulcer negative	6	91.43±4.79	76.6, 106.2
Ulcer positive	23	148.59±8.25*	98.6, 248.9

* P<0.05 vs the ulcer-negative group

Table 2. Test performance of the plasma gastrin concentration to diagnose type 1 abomasal ulcer in buffaloes

Plasma gastrin concentration (pg/mL)	Test performance (95% confidence interval)				
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	AUC
>106.2	78.26 (56.3–92.5)	100 (54.1–100)	100	69.9 (51.7–83.5)	0.942 (0.79–0.995)*

*P<0.001.

variances was rejected and it was concluded that there was a difference between the variance of ulcer-negative and ulcer-positive buffaloes. The plasma gastrin concentrations of the ulcer negative and ulcer positive buffaloes (Fig. 1) are presented in Table 1. The mean plasma gastrin concentration of the ulcer negative buffaloes was significantly (P<0.05) lower than the ulcer-positive buffaloes.

Fig. 2 illustrates the ROC curve of plasma gastrin for the diagnosis of AU1 in buffaloes. The area under the ROC curve (AUC) was 0.942 with standard error of 0.045 (P<0.001). Table 2 presents the test performance of plasma gastrin to diagnose AU1 in buffaloes, for the optimal threshold determined with Youden index. As suggested by the AUC and the corresponding P-value, the plasma gastrin had the ability to distinguish between the healthy buffaloes and the buffaloes with AU1 and was highly accurate (AUC > 0.9).

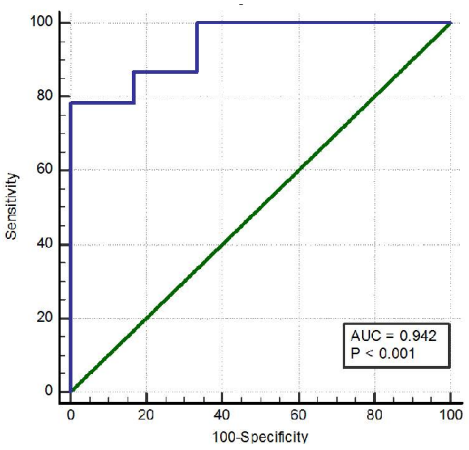

Fig. 2. Receiver-operating characteristic (ROC) curve of plasma gastrin concentration in the diagnosis of abomasal ulcer in buffaloes.

DISCUSSION

The present case control study was conducted on buffaloes with confirmed diagnosis of AU1 along with a control group. The changes in the plasma gastrin concentration are reported to be associated with the neoplastic and ulcerogenic gastric diseases in cattle and have been studied in cattle and sheep with the parasitic diseases, bleeding abomasal ulcers and abomasal dysfunction (Fox *et al.,* 1993; Ok *et al.,* 2001; Kataria *et al.,* 2008). However, the plasma gastrin levels in cattle or buffaloes with AU1 have not been evaluated so far. In fact, the plasma gastrin concentration has not been previously evaluated in water buffalo. This is the first study to document the relationship between plasma gastrin concentration and the abomasal diseases in buffaloes. The rejection of null hypothesis about the equal variances of the two groups meant that we had to use a more robust test than the conventional *t*-test. So, the Welch's t test and Brown-Forsythe test were used and a significant difference in plasma gastrin between the healthy buffaloes and the buffaloes with AU1 was detected. The results of the present study could not be compared to previous studies due to lack of available literature in buffaloes. However, the values could be compared to the studies conducted on cattle and sheep. The plasma gastrin has been evaluated in *Haemonchus*-affected sheep and reported to be 489.61±12.23 pg/mL (Kataria *et al.,* 2008). The mean plasma gastrin concentration in healthy cattle (103.2 pg/mL) and sheep (103.45±10.41 pg/mL) reported earlier is similar to our suggested cutoff value in buffaloes (Ok *et al.,* 2001; Kataria *et al.,* 2008). In humans, hypergastrinaemia is defined by gastrin levels >100–150 pg/mL (Berna *et al.,* 2006). In this study, the mean gastrin value of the AU1 positive group was also within this range, indicating hypergastrinaemia.

Although the causes of abomasal ulcer have not been elucidated, the peptic ulcer syndrome in humans resembles so closely to that of the abomasal ulcer disease, that a similar etiology and pathogenesis is suspected (Smith *et al.,* 1983). The main mechanism for increased gastrin level appears to be an impairment of the acid-mediated inhibitory control of gastrin release (McColl *et al.,* 2000). The mechanism by which gastritis or gastric ulcers impair the acid-mediated inhibitory control of gastrin release is not fully understood. However, the increased gastrin is responsible for the increased gastric acidity leading to ulcers in the stomach and duodenum and even refluxes oesophagitis (Roy *et al.,* 2001; Orlando *et al.,* 2007). Another satisfactory explanation for the increased gastrin in AU1 may be disturbances in the food intake and resultant increase in gastric acidity and hence increased secretion of gastrin (Dacha *et al*., 2015).

Hypergastrinaemia has become an important topic of research and clinical concern after the development of proton pump inhibitors (Peghini *et al.,* 2002; Orlando *et al.,* 2007). These powerful acid inhibitory drugs elevate the intragastric pH and thus remove the acid-mediated inhibition of gastrin release. In humans, proton pump inhibitors (PPIs) are used indiscriminately for treating dyspepsia, acid reflux, gastritis and peptic ulcers without appropriate indication. Due to over the counter availability of PPIs and the resulting hypergastrinaemia, there has been immense interest in the pathophysiology of gastrin during the last two decades (Dacha *et al.,* 2015). However, the use of PPIs in bovine gastroenterology is still

in infancy stage, so the indiscriminate use of PPIs as a cause of hypergastrinaemia in bovines is ruled out.

This is the first study where a ROC analysis was performed to determine the optimal thresholds of plasma gastrin for the diagnosis of AU1 in buffaloes. The ROC analysis helped to determine the optimal threshold of plasma gastrin with Youden index. As this study aimed to suggest a diagnostic test for AU1 in buffaloes, it was decided to include the buffaloes with the best available definition of AU1 (Braun *et al.,* 1991; Hussain *et al.,* 2019). As both the ulcer positive and ulcer negative animals were established to be so on the histopathological examination, we suggest that this threshhold value of the plasma gastrin could be a valid test for the detection of AU1 in buffaloes.

With regard to test the performance it is evident that plasma gastrin concentration >106.2 pg/mL was the most acceptable combination of sensitivity and specificity. The AUC value of 0.942 indicated that plasma gastrin was a highly accurate test to distinguish between the AU1 positive and negative buffaloes (Greiner *et al.,* 2000) and justifies its use for the diagnosis of AU1 in clinical setting.

In this preliminary study, the affected abomasa were not grouped on the basis of age or parity of the buffaloes, as was done in the previous prevalence study (Hussain *et al.,* 2019). Also, the correlation between the plasma gastrin concentration and the number of ulcers in the abomasum was not evaluated in this study. Further, the plasma gastrin dynamics was not evaluated with respect to AU1 severity.

CONCLUSIONS

This is the first study that determined the test characteristics of plasma gastrin to diagnose AU1 in buffaloes. The overall test performance of plasma gastrin was highly accurate. So, we suggest that plasma gastrin could be used as a diagnostic marker to reflect the abomasal mucosa damage due to AU1 in buffaloes. However, information on the effects of age, sex, and the number and severity of abomasal ulcers on plasma gastrin concentrations is not available and requires further studies. Further, there is need to determine the reference range of the plasma gastrin levels in the buffaloes.

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