

# A Luminol-enhanced Chemiluminescence Study on the Gastric Mucosa with Portal Hypertensive Gastropathy

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**Summary.** This study measured luminol-enhanced chemiluminescence in patients with portal hypertensive gastropathy and investigated whether superficial reddening, a gastric mucosal change observed in patients with portal hypertension, should be included in the category of endoscopic findings of patients with portal hypertensive gastropathy. The subjects consisted of 36 histologically confirmed cirrhotic patients with a snake skin appearance or discrete red spots on the gastric mucosa (Group 1), 42 patients with superficial reddening on the gastric mucosa who had no liver disease (Group 2) and 32 controls. Luminol-enhanced chemiluminescence study of the gastric mucosal biopsy specimens showed that a significantly larger amount of chemiluminescence was produced in Group 1 than in either Group 2 or the controls, and that there was no significant difference between Group 2 and the controls. In histological examination, no significant differences were observed among the three groups in the extent of mucosal infiltration of small round cells and neutrophils. The mean ratio of the total vascular area to total mucosal stromal area was significantly higher in Group 1 and 2 than in the controls. Moreover, the ratio in Group 1 was statistically higher than that in Group 2. In a gastric mucosal hemodynamic study, Group 1 had a higher mean value of the index of mucosal blood volume (IHB ratio) and a lower mean value of the index of mucosal oxygen saturation ( $ISO_2$ ) than Group 2 or the controls, which suggested congestion. In Group 2,  $ISO_2$  was significantly lower than in the control group, but the IHB ratio was not different from the controls, which suggested that the superficial reddening reflected the increased consumption of oxygen in the mucosa but was not associated with congestion. From these findings, it is suggested that the differences in luminol-enhanced chemiluminescence are related to the presence and absence of congestion. In view of the fact that

congestion plays an important role in portal hypertensive gastropathy and that superficial reddening is rarely observed in patients with chronic liver disease as one of endoscopic findings of portal hypertensive gastropathy, it is concluded that superficial reddening on the surface of gastric rugae should not be included in the category of typical endoscopic findings of patients with portal hypertensive gastropathy.

**Key words**—portal hypertensive gastropathy, chemiluminescence, reactive oxygen metabolites, gastric mucosal blood flow, reflectance spectrophotometry, endoscopy.

## INTRODUCTION

Upper gastrointestinal bleeding in patients with liver cirrhosis associated with portal hypertension is commonly caused by not only esophageal and gastric varices but also gastric mucosal lesions.<sup>1-3)</sup> Merigan et al. reported that gastritis was responsible for upper gastrointestinal bleeding in 38 (22%) of 172 episodes in their patients with cirrhosis of the liver.<sup>1)</sup> Sacchetti et al. showed that the occurrence of gastroduodenal erosions was significantly higher in cirrhotic patients than in patients with minor liver diseases.<sup>3)</sup>

It has been demonstrated that gastric mucosal biopsy specimens from patients with gastritis<sup>4)</sup> produce reactive oxygen metabolites as do colon biopsy specimens from patients with inflammatory bowel disease<sup>5,6)</sup>. Chemiluminescence has been developed as a simple and reproducible technique that estimates the levels of reactive oxygen metabolites in biological specimens.<sup>4-8)</sup> However, no chemiluminescence study on the gastric mucosa with portal hypertensive gastropathy has been carried out yet.

On the other hand, these gastric mucosal lesions in

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patients with portal hypertension are classified endoscopically as mild or severe congestive gastropathy.<sup>9)</sup> Mild congestive gastropathy is characterized by fine pink speckling, superficial reddening — particularly on the surface of rugae, and giving a striped appearance, and a snake skin appearance.<sup>9)</sup> Severe congestive gastropathy is characterized by discrete red spots and diffuse hemorrhagic gastritis.<sup>9)</sup> However, superficial reddening is rarely observed in patients with portal hypertension,<sup>10,11)</sup> though this endoscopic change is commonly observed in younger patients without liver diseases.<sup>12,13)</sup> Therefore, it remains to be established whether superficial reddening should be included in the endoscopic classification of portal hypertensive gastropathy.

This study was therefore undertaken to measure luminol-enhanced chemiluminescence in patients with portal hypertensive gastropathy, and also to investigate whether superficial reddening should be included in the category of endoscopic findings of patients with portal hypertensive gastropathy.

## PATIENTS AND METHODS

### Patients

Among patients who underwent diagnostic upper gastrointestinal endoscopy at Niigata University Hospital from April 1992 to December 1993, 110 with the following endoscopic findings were enrolled in this study (Table 1).

Group 1 consisted of histologically confirmed cirrhotic patients with a snake skin appearance or discrete red spots (Fig. 1) on the gastric mucosa, as described by McCormack et al.<sup>9)</sup>

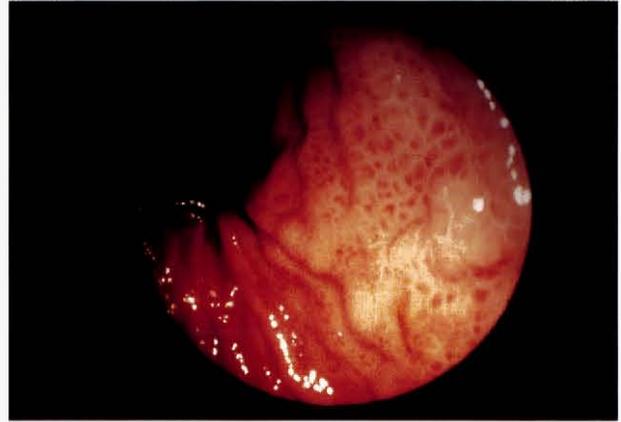
Group 2 consisted of patients with superficial reddening on the gastric mucosa (Fig. 2) as described by McCormack et al.<sup>9)</sup> None had clinical or biochemical evidence of liver disease.

The controls consisted of patients with normal endoscopic gastric mucosa without liver or other

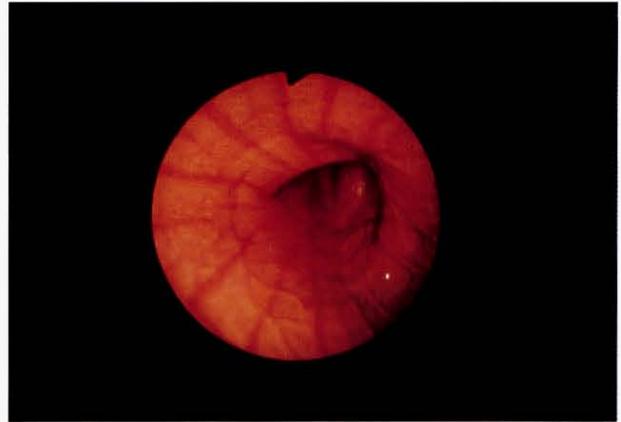
**Table 1.** Clinical data of 36 cirrhotic patients with snake skin or red spots (Group 1), 42 patients with superficial reddening (Group 2), and 32 controls

Characteristics	Group 1	Group 2	Controls
No. of patients	36	42	32
Age (yr) <sup>a)</sup>	59±11	41±14	56±17
Sex (M/F)	15/21	17/25	14/18

<sup>a)</sup> Data expressed as mean±SD.



**Fig. 1.** Endoscopic view of upper gastric body showing red spots.



**Fig. 2.** Endoscopic view of gastric body showing superficial reddening, particularly on the surface of rugae, giving a striped appearance.

organic disease.

Mucosal biopsy specimens of the stomach were obtained endoscopically from the gastric regions in the gastric body that exhibited the above specific endoscopic findings. Endoscopists who were unaware of the patient's profiles and clinical data evaluated the endoscopic photographs on a screen, and the judgment was reached by consensus. All subjects gave oral informed consent to participation in this study. Because not all patients could be included in all of the following investigations for various reasons, the number of patients in each study varied.

### Chemiluminescence

The measurement of luminol-enhanced chemiluminescence was performed using Lumat LB9501/9801

(Berthold, Wildbad, Germany), basically in accordance with the method described by Davies et al.<sup>4,7)</sup> Gastric mucosal biopsy specimens obtained endoscopically were immediately suspended in 0.1 ml of CMRL 1066 (Gibco, New York, USA) in scintillation vials, incubated for 10 min at 37°C, and placed in the spectrophotometer. After one minute, luminol (0.5mg) was added to the tissue suspension, and photons were counted for 1 min in the spectrophotometer. The samples were then dried and weighed. Chemiluminescence was expressed as counts per minute per milligram of biopsied specimen after subtraction of the background. Ten, 12 and 10 patients were examined in Group 1, Group 2 and the controls, respectively.

### Histological examination

Biopsied specimens were stained with hematoxylin and eosin and examined by light microscopy. The appearance of the gastric mucosa was evaluated by the degree of infiltration of small round cells and polymorphonuclear cells, and the degree of infiltration of these cells was classified in accordance with the Sydney system<sup>14)</sup> into: none-slight, mild, moderate, and severe. Eleven, 25 and 11 patients were examined in Group 1, Group 2 and the controls, respectively.

### The presence of *Helicobacter pylori*

Biopsied specimens were prepared as mentioned above. The presence of *Helicobacter pylori* (*H pylori*) in the gastric mucosa was examined and ranked by 4 categories: none, mild, moderate, and severe.<sup>14)</sup> Seven, 11 and 10 patients were examined in Group 1, Group 2 and the controls respectively.

### Measurement of vascular area

Since it has been reported earlier that human anti-hemophilic factor (Factor VIII) is present in endothelial cells of arteries, capillaries, and veins,<sup>15,16)</sup> lumens stained with anti-anti-hemophilic factor can be regarded as vessels in the gastric mucosa. Based on this concept, the vascular area was measured and the ratio of total vascular area to total mucosal stromal area was calculated. Using rabbit polyclonal antibody to human anti-hemophilic factor (Dako, Kyowa Medics, Tokyo, Japan) as a primary antibody, sections were prepared by the indirect peroxidase-labeled antibody method,<sup>16)</sup> and measurement was carried out using the PAS-310 system (Olympus Optical Co., Ltd., Tokyo, Japan). Eleven patients in Group 1, 25 in Group 2, and 11 controls were examined.

### Measurement of gastric mucosal blood flow

Gastric mucosal hemodynamics was measured by the reflectance spectrophotometric technique using a spectrophotometer (Tissue Spectrum Analyzer TS-200, Sumitomo Electric Industries, Ltd., Osaka, Japan).<sup>17-22)</sup> This technique permits repeated measurements of an index of mucosal hemoglobin concentration (IHB) and an index of oxygen saturation (ISO<sub>2</sub>). The mucosa was touched gently with the measuring probe inserted through an endoscope, and the measurement of IHB and ISO<sub>2</sub> was repeated five times in each patient. The mean values were then calculated for these indices in each patient. Measurements were taken from the greater curvature in the middle gastric body that exhibited the specific findings. However, in an anemic patient, IHB may not directly reflect the blood volume in the gastric mucosa.<sup>23)</sup> We therefore calculated the index of gastric mucosal blood volume as the IHB ratio by dividing the IHB by blood hemoglobin concentration.<sup>23)</sup> Twenty, 10 and 11 patients were examined in Group 1, Group 2 and the controls, respectively.

### Statistics

The results are shown as mean  $\pm$  SD. The results were statistically analyzed by Scheffe's parametric or non-parametric test or Fisher's exact test.  $P < 0.05$  was defined as statistically significant.

## RESULTS

### Chemiluminescence

Fig. 3 shows the mean values of luminol-enhanced chemiluminescence in the gastric mucosal biopsy specimens from the three groups. The gastric mucosal biopsy specimens from the patients in Group 1 produced significantly more chemiluminescence than did the specimens from either Group 2 or the controls, but there was no significant difference between Group 2 and the control group.

### The presence of *Helicobacter pylori*

The study on *H pylori* confirmed its presence in 2 patients each in Group 2 and the controls, though the degree of infection was mild in all these 4. No patients in Group 1 had *H pylori*. There were no significant differences among the three groups in *H pylori* infection.

### Histological examination and measurement of vascular area

Table 2 summarizes the histological findings of the gastric mucosal specimens from the three groups. No significant differences were observed among the three groups in the extent of infiltration of small round cells and neutrophils. The mean value of the ratio of total vascular area to total mucosal stromal area was significantly larger in Group 1 and 2 than in the controls. Moreover, the mean ratio in Group 1 was statistically higher than that in Group 2.

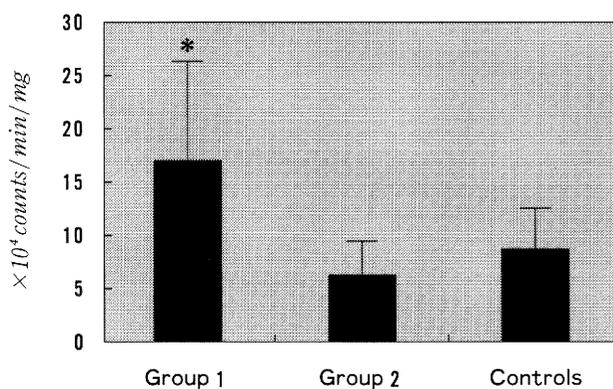
### Gastric mucosal blood flow

Table 3 shows the results of the gastric hemodynamic study in the three groups. The mean value of the IHB ratio in Group 1 significantly exceeded that in the controls. The difference between Group 2 and the controls was not statistically significant, however, the mean values of ISO<sub>2</sub> in Group 1 and 2 were significantly lower than that in the control group.

## DISCUSSION

The present study is the first to report luminol-enhanced chemiluminescence in gastric biopsy specimens obtained from patients with portal hypertensive gastropathy.

We demonstrated that the gastric mucosa exhibiting a snake skin appearance or red spots emitted a significantly larger amount of luminol-enhanced chemiluminescence than did the controls, but the



**Fig. 3.** Comparison of the mean values of chemiluminescence in the gastric mucosa among Group 1 (n=10), Group 2 (n=12) and controls (n=10). Data are expressed as mean  $\pm$  SD. \* $p < 0.05$  compared with Group 2 and controls.

**Table 2.** Histological findings on gastric mucosa of the three groups

	Group 1 (n=11)	Group 2 (n=25)	Controls (n=11)
Small round cell infiltration			
None-slight	11	19	11
Mild	0	5	0
Moderate	0	1	0
Severe	0	0	0
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Polymorphonuclear cell infiltration			
None-slight	7	24	6
Mild	4	1	4
Moderate	0	0	1
Severe	0	0	0
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The ratio of total vascular area to total mucosal stromal area (%) <sup>a)</sup>			
	9.0 $\pm$ 3.5 <sup>b,c)</sup>	4.7 $\pm$ 2.1 <sup>b)</sup>	2.1 $\pm$ 0.5

<sup>a)</sup>Data are mean  $\pm$  SD; <sup>b)</sup> $p < 0.05$  compared with controls; <sup>c)</sup> $p < 0.05$  compared with Group 2.

**Table 3.** The mean values of IHB, IHB ratio, and ISO<sub>2</sub> of the gastric mucosa in the three groups

	Group 1 (n=20)	Group 2 (n=10)	Controls (n=11)
IHB <sup>a)</sup>	136.1 $\pm$ 30.2	144.6 $\pm$ 17.3	146.2 $\pm$ 17.2
IHB ratio	12.8 $\pm$ 3.3 <sup>c)</sup>	10.5 $\pm$ 1.5	10.5 $\pm$ 1.3
ISO <sub>2</sub> <sup>b)</sup>	39.2 $\pm$ 5.1 <sup>c)</sup>	42.5 $\pm$ 4.5 <sup>c)</sup>	48.3 $\pm$ 4.4

All data are mean  $\pm$  SD. <sup>a)</sup>Index of mucosal hemoglobin concentration; <sup>b)</sup>Index of mucosal oxygen saturation; <sup>c)</sup> $p < 0.05$  compared with controls.

gastric mucosa exhibiting superficial reddening had no difference from controls (Fig. 3). Earlier studies have shown that mucosal biopsy specimens obtained from patients with inflammatory bowel disease<sup>5,6)</sup>, duodenitis or duodenal ulcer<sup>7)</sup> and H pylori-positive gastritis<sup>4)</sup> produced a large amount of luminol-enhanced chemiluminescence. Taking into consideration that chemiluminescence is a technique for estimating the levels of reactive oxygen metabolites,<sup>4-8)</sup> it is thought that chemiluminescence reflects the amount of reactive oxygen metabolites in biological specimens in these disorders.<sup>4-7)</sup> It is presumed that excessive reactive oxygen metabolites are generally produced by neutrophils infiltrating into mucosa. It has actually been ascertained that the value of chemiluminescence correlates to the macroscopically or histologically assessed degree of activity and severity

of diseases.<sup>4,6,7)</sup> Since no *H pylori* were detected in the gastric mucosa exhibiting a snake skin appearance or red spots in this study, high levels of luminol-enhanced chemiluminescence in these patients may be due to portal hypertensive gastropathy. Therefore, even in portal hypertensive gastropathy, excessive reactive oxygen metabolites may also play an important role in the mucosal injury.

On the other hand, histological examination disclosed that the gastric mucosa exhibiting a snake skin appearance or red spots and that exhibiting superficial reddening had both a higher ratio of vascular area, and infrequent infiltration of inflammatory cells into the mucosa (Table 2). These results are consistent with the previously reported histologic findings of gastric mucosa with snake skin and red spots,<sup>9,24,25)</sup> and are not inconsistent with the histology of portal hypertensive gastropathy. Therefore, it can not be predicated that the high levels of chemiluminescence in Group 1 are attributable solely to the infiltration of neutrophils.

We used the reflectance spectrophotometric technique to evaluate mucosal blood flow. We demonstrated a larger mean value of IHB ratio and a lower mean value of ISO<sub>2</sub> in gastric mucosa with snake skin or red spots than in that with superficial reddening or the control group, which suggests that gastric mucosa exhibiting snake skin or red spots is associated with congestion<sup>20)</sup> (Table 3). This result was consistent with the previous findings obtained by laser Doppler flowmetry<sup>11)</sup> and reflectance spectrophotometry<sup>25)</sup>. On the other hand, in gastric mucosa with superficial reddening, ISO<sub>2</sub> was significantly lower compared with the controls, while the IHB ratio did not differ from the controls, suggesting that superficial reddening is associated with a condition in which the amount of oxygen supply into mucosa per hemoglobin is increased but is not associated with congestion (Table 3). If it is supposed that the differences in luminol-enhanced chemiluminescence reflect the amount of oxygen supply in gastric mucosa, it may be considered that the gastric mucosa exhibiting snake skin or red spots has a larger amount of oxygen supply than that exhibiting superficial reddening, and that the difference is due to the presence of congestion. In congestive condition, since neutrophils remain in the regional gastric mucosa for long time, a small number of neutrophils might interact with vascular endothelial cells and produce excessive reactive oxygen metabolites. In view of the fact that congestion plays an important role in portal hypertensive gastropathy,<sup>9,11,25)</sup> a different pathophysiological background is involved between the gastric mucosa exhibiting snake skin or red spots and

that exhibiting superficial reddening, and it would seem difficult to include them in the same category. This concept is not inconsistent with clinical studies reporting that snake skin is the most common type of gastric mucosal lesion in patients with portal hypertension, red spots are clinically important as a cause of bleeding,<sup>9-11,24,26,27)</sup> that the incidence of superficial reddening observed in patients with portal hypertension is low,<sup>10,11)</sup> and that this endoscopic change is rather common in younger patients without liver diseases.<sup>12,13)</sup>

However, superficial reddening may be interpreted as a change leading to snake skin or red spots. We intend to study this point further.

In conclusion, gastric mucosa exhibiting a snake skin appearance or red spots in patients with portal hypertensive gastropathy produced significantly more luminol-enhanced chemiluminescence than gastric mucosa exhibiting superficial reddening. This difference is thought to be due to the presence of congestion. In view of the fact that congestion plays an important role in portal hypertensive gastropathy,<sup>9,11,25)</sup> we conclude that superficial reddening on the surface of gastric rugae should not be included in the category of typical endoscopic findings of patients with portal hypertensive gastropathy.

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