Hypovolemia-related gastric tissue damage in the setting of upper gastrointestinal bleeding

Üst gastrointestinal kanama sırasında hipovolemi ilişkili gastrik doku hasarı

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Background/aims: *Much is known about the gastric tissue damage that is associated with hypovolemic stress, but gastrointestinal bleeding due to gastric injury and further gastric injury due to hypovolemia have not been evaluated in previous research. The aim of this study was to assess oxidative gastric tissue damage specifically linked to hypovolemia in patients with upper gastrointestinal bleeding.* ***Methods:*** *The study included 30 patients who presented with acute upper gastrointestinal bleeding and 30 controls. Each patient's history and laboratory findings were recorded, and multiple biopsies of the gastric antrum were obtained at diagnostic endoscopy on admission (day 1) and five days later. A set of antral biopsies was also collected from each control subject. Each tissue specimen was analyzed for levels of glutathione peroxidase, superoxide dismutase and catalase activity, and level of malondialdehyde.* ***Results:*** *First day glutathione peroxidase, superoxide dismutase and catalase levels were significantly lower and malondialdehyde levels were higher than on the 5th day, and 1st day and 5th day levels were significantly different from controls (p<0.05). A moderate level of correlation was detected between catalase and hemoglobin (r:-0.59) and hematocrit (r:-0.61) and between malondialdehyde and systolic blood pressure (p:0.58), hematocrit (r:0.45) and hemoglobin (r:0.49).* ***Conclusions:*** *In this study, gastric tissue oxidative markers showed antral oxidative changes to be significantly correlated with patients' hemodynamics. Oxidative stress may not be a clinical condition but it obviously shows gastric tissue damage and may explain many of the patients' additional diagnosis of gastric erosions. Interestingly, the oxidative change does not completely recover even on the 5th day.*

Key words: Gastrointestinal bleeding, stress, oxidative, reactive oxygen species, antioxidant enzyme

Amaç: *Hipovolemik strese bağlı gastrik doku hasarı iyi bilinmesine rağmen, üst gastrointestinal sistem kanamasının oluşturduğu hipovolemik stresin gastrik doku hasarı daha önce değerlendirilmemiştir. Bu çalışmanın amacı üst gastrointestinal sistem kanamasında oluşan gastrik doku hasarını değerlendirmektir.* ***Yöntem:*** *Çalışma için 30 akut üst gastrointestinal kanamalı ve 30 kontrol hastası alındı. Her hastanın hikayesi ve laboratuvar kayıtlarının yanında müracaatları ve 5. günlerinde endoskopileri yapılarak sağlıklı görünen antrumdan biyopsiler alındı. Alınan biyopsi örneklerinde glutatyon peroksidaz, süperoksit dismutaz, katalaz ve malondialdehit düzeyleri çalışıldı.* ***Sonuçlar:*** *Birinci gün 5. güne göre glutatyon peroksidaz, süperoksit dismutaz, katalaz düzeyleri anlamlı düşük ve malondialdehit yüksek bulundu. 1. gün ve 5. gün sonuçları ise kontrol grubuna göre farklıydı (p<0,05). Katalaz ile hemoglobin (r:-0.59) ve hematokrit (r:-0.61) arasında orta derecede korelasyon, malondialdehit ile sistolik kan basıncı (p:0.58), hematokrit (r:0.45), ve hemoglobin (r:0.49) arasında orta derecede korelasyon bulundu.* ***Sonuç:*** *Çalışmamızda gastrik doku oksidatif değişiklikleri hastaların hemodinamisi ile ilişki göstermektedir. Oksidatif stress klinik bir bulgu olmamasına rağmen gastrik doku hasarı göstergesidir ve bazı hastalarda bulunan gastrik erozyonların açıklayıcı bulgusu olabilir.*

Anahtar kelimeler: Gastrointestinal kanama, stres, oksidatif, reaktif oksjien türleri, antioksidan enzim

INTRODUCTION

Animal studies have shown that free radicals form in body tissues during hemorrhagic shock and ischemia-reperfusion, and that these oxygen species cause tissue injury (1). It is well known that upper gastrointestinal (GI) bleeding leads to hypovolemic stress that may damage the gastric mucosa; however, this form of mucosal injury has not been researched in depth. Hemorrhage-associated gastric tissue damage results in further tissue injury, and the process can become a vicious circle. Blee-

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ding from ectatic vessels in the stomach wall may lead to erosive gastritis due to hypovolemic stress, and such cases may initially be misdiagnosed as erosive gastritis.

The aim of this study was to assess oxidative gastric tissue damage specifically linked to hypovolemia in patients with upper GI hemorrhage. Levels of gastric tissue stress were evaluated in biopsies of apparently normal antral mucosa, and testing was done to determine levels of the antioxidant enzymes glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT), and concentrations of malondialdehyde (MDA) in this tissue.

MATERIALS AND METHODS

The Ethics Committee of İnönü University Faculty of Medicine approved the study, and all procedures were carried out in accordance with the Helsinki Declaration of Human Rights. Thirty patients with GI hemorrhage and 30 controls were prospectively evaluated. All subjects gave their informed consent to participate.

Patient Group

This group (n = 30) comprised 12 females and 18 males (age range: 19-80 years; mean age: 50 ± 20 years) who presented to our hospital's emergency service with acute upper GI bleeding accompanied by hematemesis or melena. The exclusion criteria were coexisting diseases requiring long-term drug use, hemodynamic conditions in which the patient was not stable lying down, and contraindications for endoscopic biopsy. The details of each patient's medical history, smoking status, physical examination findings (including blood pressure in supine position, complete blood cell count, and blood biochemistry results) were recorded for statistical evaluation. Each individual was evaluated endoscopically within four hours of admission to establish the diagnosis and to secure hemostasis if needed. In each case, eight separate biopsy specimens of the gastric antrum were obtained during initial endoscopy, and another eight were collected five days later. On day 5, eight corpus biopsies were obtained as well, and these were histologically examined for *Helicobacter pylori* infection. To ensure uniformity, all the antral biopsies collected were normal-appearing tissues. The presence of a lesion in or near the antrum was grounds for exclusion from the study, but none of the 30 patients had such lesions. Upon removal, specimens were washed in buffer solution and then frozen for later analysis.

Control Group

The controls (n = 30) included 14 females and 16 males (age range: 21-79 years; mean age: 48 ± 19 years) who were randomly selected from cases of dyspepsia that were diagnosed at our center in the same period. These individuals had no coexisting disease or organic gastric lesions apart from gastritis. Details of each control subject's medical history and smoking status were recorded. Each of these individuals underwent endoscopy, and 24 total biopsy specimens were collected (16 specimens of normal-appearing gastric antrum and 8 corpus specimens). Eight of the antrum samples were washed in buffer solution and frozen for later analysis. The other eight antrum samples and all the corpus specimens were histologically evaluated for *H. pylori* infection.

Biochemical Assays

All agents used in the study were obtained from Sigma Chemical Co. (St. Louis, MO, USA). After defrosting, each antral biopsy specimen was perfused with 50 mM cold phosphate-buffered saline (PBS) solution at pH 7.4, and then homogenized in 500 µl PBS using a PCV Kinematica Status homogenizer. Each homogenate was divided into two portions, and one was immediately used to measure MDA. The second portion was sonicated for four 30-sec periods separated by 20-sec intervals. A VWR Bronson Scientific sonicator was used. The homogenate was then centrifuged at 20,000 g for 15 min in a Beckman L8-70M ultracentrifuge. The supernatant was separated off and stored at -80°C until measurements of protein concentration and enzyme activity levels were carried out. Care was taken to maintain all tissue materials at +4°C when preparing homogenates and supernatants.

Protein Concentration: The concentration of protein in the supernatant from each specimen was determined according to the method of Lowry et al. (2) using bovine serum albumin as the standard. All spectrophotometric assays were done in a Shimadzu 1601 UV/VIS spectrophotometer connected to a Grand LTD 6G thermostability unit adjusted to 37 + 0.1°C.

Enzyme Activity Levels

GPX Activity: GPX activity was measured according to the method of Lawrence and Burk (3). One milliliter of 50 mM PBS solution (pH 7.4) contai-

ning 5 mM ethylenediaminetetraacetic acid (EDTA), 2 µM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 20 µM GPX, 10 µM sodium azide, and 23 mU glutathione reductase was incubated at 37°C for 5 min. Then 20 µL of 0.25 mM H2O2 solution and 110 µl of supernatant from the specimen were added to the assay mixture. The change in absorbance at 340 nm was monitored for 1 min. A blank containing all ingredients except supernatant was also monitored for the same period. Specific activity was calculated as U/mg protein.

SOD Activity: The activity of this enzyme was measured using the technique of McCord and Fridovich (4). Solution A was prepared by mixing 100 ml of 50 mM PBS (pH 7.4) containing 0.1 mM EDTA and 2 µmol cytochrome-C with 10 ml of 0.001 N NaOH solution containing 5 µmol xanthine. A second solution (Solution B) of 0.2 U/ml xanthine oxidase and 0.1 mM EDTA was prepared. Fifty microliters of supernatant from each specimen were mixed with 2.9 ml of Solution A, and the reaction was started by adding 50 µL of Solution B. Change in absorbance at 550 nm was monitored for 1 min. A blank was prepared in which 50 µL of ultrapure water was substituted for the supernatant, and absorbance was monitored for the same period. The SOD activity in each sample was expressed as U/mg protein.

CAT Activity: CAT activity was measured using the method described by Luck (5). Decomposition of the substrate H2O2 was monitored spectrophotometrically at 240 nm. Specific activity was expressed as U/mg protein.

MDA Levels: The level of MDA in the homogenate from each specimen was measured using the method of Mihara and Uchiyama (6). Briefly, 250 µl of homogenate was mixed with 3 ml of 1% H3PO4. One milliliter of 0.67% thiobarbituric acid was added, and then the mixture was heated in boiling water for 45 min. The colored phase was extracted into n-butanol, and absorption at 532 nm was measured using tetramethoxypropane as the standard. MDA concentrations were expressed as nmol/mg protein.

Statistics

Statistical testing was done using the software SPSS for Windows, version 10 (SPSS, Chicago, IL, USA). The independent-samples *t*-test was used to compare patient group versus control group results; the paired-samples t-test was used to compare findings in the patients' antral biopsies collected on day 1 and day 5; and the Mann-Whitney U test was used to compare differences between patients and controls categorized according to *H. pylori* infection status. Pearson's correlation analysis was used to assess relationships between tissue enzyme levels and patient data, and between MDA levels and patient data. P values <0.05 were considered statistically significant.

RESULTS

Patient Features and Endoscopy Findings

At admission, 27 (90%) of the 30 patients had melena and 22 (73%) had hematemesis. Three patients (10%) had a history of syncope, 9 (30%) had a history of nonsteroidal anti-inflammatory drug (NSAID) use, and 8 (27%) of the patients were smokers. The mean findings on initial physical examination were systolic blood pressure 113 ± 18 mmHg (range: 113-80 mmHg), diastolic blood pressure 68 ± 11 mmHg (range: 40-90 mmHg), and heart rate 94 ± 11 beats/min (range: 70-110 beats/min). The mean hemoglobin (Hb) and hematocrit (Ht) values on admission were 10.2 ± 2.6 g/dl (range: 6-14 g/dl) and 29 ± 7.7% (range: 17-43%), respectively. Sixteen patients (53%) needed blood transfusions, and the average number of units required in this group was 2 ± 2.7. As noted, all patients were evaluated endoscopically within 4 hours of admission. These exams revealed that the cause of bleeding was gastric ulcer in 6 cases (20%), duodenal ulcer in 10 cases (33%), erosive gastritis and bulbitis in 13 cases (43%), and was of vascular origin (Dieulafoy's lesion) in 1 case (3%).

In the initial endoscopic examinations, Forrest 1b bleeding was detected in 4 cases, and Forrest 2a lesion (visible vessel) was detected in 1 patient. All 5 of these patients were treated with a heater probe during the scoping session. The patient with Dieulafoy's lesion was treated with an injection of polidocanol 0.5% during the session. In 3 of the 6 treated cases, endoscopy had to be repeated within 24 hours due to bleeding recurrence. One of these 3 repeat cases was the Dieulafoy's case, and the recurrent hemorrhage was from this lesion. In this case, it was not possible to stop the bleeding with endoscopic intervention and the patient had to be treated surgically. However, the other 2 cases of bleeding recurrence were successfully managed endoscopically with injections of polidocanol 0.5%, and there was no further recurrence.

The other 24 patients were not treated in the session. None of these individuals experienced bleeding recurrence, and repeat endoscopy was done on day 5 (as described above).

There was no other morbidity in any of the 30 patients, and none of the patients died.

The histological assessments of the corpus specimens collected during the day-5 endoscopic evaluation revealed that 18 patients (60%) had H. pylori infection.

Control Findings

Analysis of age and sex distribution in the control group revealed no significant differences from the patient group. Six of the control subjects were smokers, and 16 (53%) were *H. pylori*-positive.

Biochemical Results

The findings for antioxidant enzyme activity and MDA levels in the patient group biopsies from days 1 and 5 and in the control biopsies (3 sets total) are shown in the Figures. The means (ranges) for the GPX activity levels in these three sets of biopsies were 0.349±0.169 (0.188-0.916), 0.455±0.146 (0.218-0.860), and 0.689±0.151 U/mg protein (Figure 1a). The corresponding results for SOD were 22.56±9.06 (9.99-43.41), 30.68±8.46 (17-03-49.82), and 45.67±17.09 (14.33-87.34) U/mg protein (Figure 1b). The corresponding findings for CAT were 46.38±26.07 (20.24-136.06), 75.99±23.55 (35.08-135.92), and 111.64±59.93 (51.06-316.00) U/mg protein (Figure 1c). The MDA levels in the three sets of biopsies were 8.03±1.66 (4.80-11.82), 4.75±0.87 (3.00-6.20), and 3.82±0.55 (2.98-4.90) nmol/mg protein (Figure 1d).

For all three enzymes investigated, the patient specimens obtained at initial endoscopy on admission (day 1) exhibited the lowest levels of activity. On day 5, the patient specimens showed higher

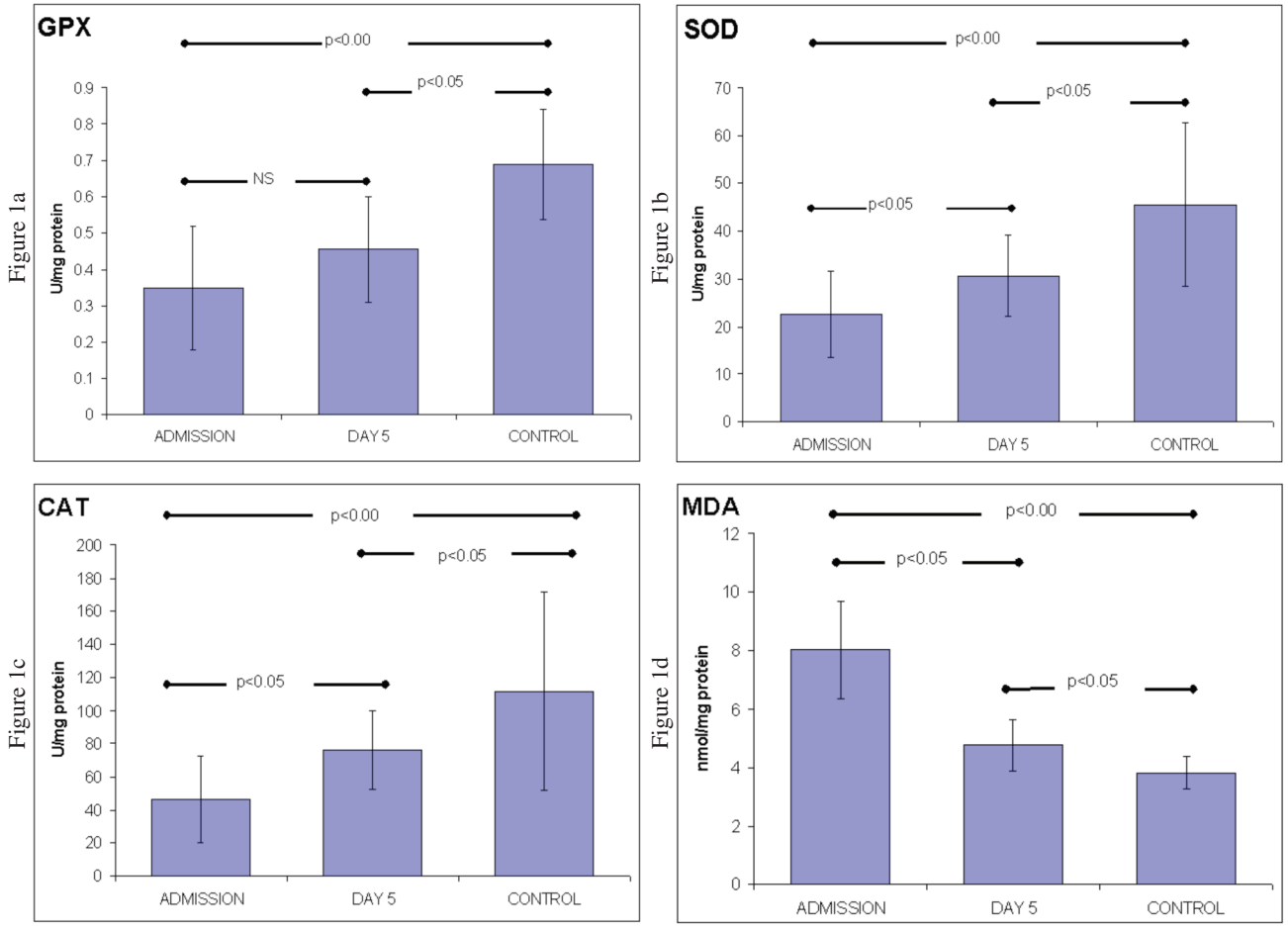

Figure 1. 1 a, b, c, d: Mean enzyme activity levels and MDA levels in the antral biopsies from the patient and control groups on admission (day 1) and day 5: a) GPX: Glutathione peroxidase (U/mg protein, multiplied by 10); b) SOD: Superoxide dismutase (U/mg protein), c) CAT: Catalase (U/mg protein), d) MDA: Malondialdehyde (nmol/mg protein).

enzyme activity levels than on day 1, but the findings for all three enzymes were still lower than the control group findings. Specifically, the mean activity levels of GPX, SOD and CAT in the patient specimens from days 1 and 5 were all significantly lower than the corresponding control group levels ($p<0.001$ for all on day 1, and $p<0.05$ for all on day 5). The changes in SOD, CAT and MDA levels from day 1 to day 5 were significant ($p<0.001$ for all), but the change in GPX over this period was not significant ($p>0.05$). The patient specimens from day 1 contained the highest levels of MDA. By day 5, the levels in the patient tissues had decreased to some degree, but were still higher than the control group MDA level. The mean MDA levels in the patient specimens from days 1 and 5 were both significantly higher than the mean MDA level in the control tissues ($p<0.001$ and $p<0.05$ for days 1 and 5, respectively).

When the control subjects were categorized according to sex, smoking status, and *H. pylori* infection status, there were no statistically significant differences with respect to enzyme activity levels or MDA levels. When the patients were categorized according to smoking status, *H. pylori* infection status, and use/non-use of NSAIDs, there were no statistical differences with respect to levels of enzyme activity or MDA levels.

Correlations

As described above, statistical testing was done to assess links between enzyme activity levels in patients' gastric antrum specimens and patient characteristics, and similar analysis was done for MDA levels. The CAT activity level in the specimens from day 1 was negatively correlated with Hb at admission ($p<0.05$, r = -0.59) and with Ht at admission ($p<0.05$, r = -0.61). The MDA level in the day-1 specimens was positively correlated with systolic blood pressure at admission ($p<0.05$, r = 0.58), Ht at admission ($p<0.05$, r = 0.45) and Hb at admission ($p<0.05$, r = 0.49).

DISCUSSION

Numerous studies have revealed that risk factors for gastric epithelial damage and ulceration, including stress, alcohol use, *H. pylori* infection, NSAID use, and others, are associated with formation of free radicals. Regardless of the type of irritant that damages the gastric mucosa, production of these oxygen radicals plays a role in the tissue damage. It is well established that damaged tissue contains decreased levels of antioxidant enzymes and increased levels of lipid peroxidation products (1, 9-14).

Ischemia-reperfusion injury has been thoroughly investigated in many studies; however, the specific tissue damage that takes place in hypovolemic stress (shock), prior to actual ischemia or reperfusion, has not. Hypovolemia-related injury has not been studied adequately in animals. Currently, there are also no related human data available on this phase of tissue damage (9-14).

This study focused on a series of events that are well known but difficult to interpret: the causes of gastric injury and the further gastric injury due to resultant hypovolemia. We measured activity levels of antioxidant enzymes and levels of a lipid peroxidation product in patients with upper GI bleeding. Tissue biopsies from normal-appearing gastric antrum were assessed, and the results confirmed oxidative tissue damage at these sites. Compared to findings in antral biopsies from the control group, the patient specimens collected at admission (day 1) and on day 5 showed significantly lower activity of all three enzymes tested (GPX, SOD, CAT) and significantly higher levels of MDA ($p<0.01$ for all on day 1, and $p<0.05$ for all on day 5). The patient biopsies collected 5 days after admission exhibited less oxidative damage than the specimens collected on day 1; however, as mentioned, the status of patient tissues on day 5 was still significantly worse than that of the control group tissues. Even in the cases where GI bleeding was the result of erosion only, the antioxidant enzyme activity levels on day 5 were significantly lower than the levels in control tissues.

As explained, in cases of GI bleeding, there is oxidative damage in normal-looking gastric tissue, and it may be related to factors that cause the bleeding, but these oxidative damage findings were shown to be directly related to the resultant hypovolemia. The strongest pieces of evidence for hypovolemia-related tissue damage were the statistical correlations between CAT activity and Hb and Ht at admission, and between MDA and blood pressure, Hb, and Ht at admission. The fact that the levels of antioxidant enzymes in the patient group tissues had not completely normalized even by day 5 is also important evidence of hypovolemia-related damage. In addition, one of the patients had endoscopic and surgically diagnosed Dieulafoy's lesion, which is not erosive or ulcerative in nature. The biopsies in this case showed the same

enzyme changes as were noted in the other patients. The only risk factor for mucosal damage in a Dieulafoy's patient is hypovolemia.

This study showed that GI bleeding exhibits oxidative tissue damage directly linked to hypovolemia. Normal-appearing antrum tissue is actually injured by hypovolemia and this finding may explain the erosive gastritis that accompanies most bleeding lesions in the stomach or duodenum, and that often occurs in bleeding of vascular origin. One of our most interesting findings was that antioxidant enzyme activity in the patients' antral specimens had not returned to normal even 5 days after the start of treatment. This hindered healing ability in this period may promote recurrent bleeding or lead to further problems with other gastric stress factors. It is not possible to prevent the gastric mucosa damage that is associated with hypovolemic shock in cases of GI bleeding, but the findings of this study suggest that treatment with antioxidant substances may prevent recurrent bleeding episodes and help the mucosa heal more rapidly and effectively. This theory requires further investigation.

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