Water-Soluble Vitamin Deficiencies in Complicated Peptic Ulcer Patients Soon after Ulcer Onset in Japan

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Summary We investigated over time whether contemporary Japanese patients with complicated peptic ulcers have any water-soluble vitamin deficiencies soon after the onset of the complicated peptic ulcers. In this prospective cohort study, fasting serum levels of water-soluble vitamins (vitamins B1, B2, B6, B12, C, and folic acid) and homocysteine were measured at 3 time points (at admission, hospital discharge, and 3 mo after hospital discharge). Among the 20 patients who were enrolled in the study, 10 consecutive patients who completed measurements at all 3 time points were analyzed. The proportion of patients in whom any of the serum water-soluble vitamins that we examined were deficient was as high as 80% at admission, and remained at 70% at discharge. The proportion of patients with vitamin B6 deficiency was significantly higher at admission and discharge (50% and 60%, respectively, p<0.05) than at 3 mo after discharge (10%). In conclusion, most patients with complicated peptic ulcers may have a deficiency of one or more water-soluble vitamins in the early phase of the disease after the onset of ulcer complications, even in a contemporary Japanese population.

Key Words peptic ulcer, gastrointestinal bleeding, water-soluble vitamins

Helicobacter pylori infection and non-steroidal anti-inflammatory drugs (NSAIDs) are two major etiologies of peptic ulcers, and both these etiologies are thought to be involved in excessive oxidative stress and decreased antioxidant levels as common pathogenic mechanisms that drive the formation of peptic ulcers (1–3). Water-soluble vitamins include the B-group vitamins (8 vitamins) and vitamin C. These vitamins have antioxidant activities, and B-group vitamins are required as coenzymes of enzymes that are essential for cellular function (4–6). Unlike fat-soluble vitamins, water-soluble vitamins are more easily depleted because they are unstable, excreted readily in urine, and difficult to store in the body. Deficiencies in water-soluble vitamins may be associated with gastric mucosal injuries and have been reported to affect the development or healing of peptic ulcers in animal models (7–9).

Several early studies reported that vitamin C levels were typically lower in patients with bleeding ulcers than in those with non-bleeding ulcers (10–12). Morris (13) suggested that a diet rich in vitamin C could be a key factor in the treatment of hemorrhagic peptic ulcers. These data indicated that vitamin C deficiency was associated with an increased risk of hemorrhage in peptic ulcers. Therefore, for ideal prevention and treatment strategies for peptic ulcers it is essential to recognize the association between water-soluble vitamin deficiencies and complicated peptic ulcers. However, these data were reported more than half a century ago; nevertheless, the clinical relationship between peptic ulcers and water-soluble vitamins has not been completely characterized in contemporary patient cohorts. In particular, the influence of fasting therapy, which is often required in patients with peptic ulcers accompanied with complications such as bleeding and perforation, on water-soluble vitamin levels is a matter of concern. This study aimed to investigate the time course of changes in the serum levels of water-soluble vitamins and to identify whether patients with complicated peptic ulcers have a deficiency in any water-soluble vitamin in the early phase of disease after the onset of complicated peptic ulcers in a contemporary cohort of Japanese patients.

MATERIALS AND METHODS

Patients. This prospective cohort study included patients aged 20 y or older who were diagnosed with peptic ulcers (gastric or duodenal ulcer) using emergent upper endoscopy at the Department of Gastroenterology, Nippon Medical School, from July 2009 to January 2011 and were judged to require hospitalization for treatment. The following patients were excluded from the study: those who used vitamin supplements before hospitalization, those who underwent gastrectomy, those with severe dysfunction of the liver or kidney, those with impaired consciousness, and those undergoing insulin treatment.

The patients were made to fast after hospitalization, and they were administered an intravenous solution.
containing a proton pump inhibitor (PPI). Once the amelioration of ulcer symptoms and complications (bleeding and perforation) were confirmed using upper endoscopy and blood analysis or abdominal X-rays, an oral diet was initiated. Subsequently, the PPI was switched to an oral PPI. The patients were discharged after initiation of an oral diet and confirmation of a decrease in their anemia condition (Hb, ≥9.0 g/dL) and the absence of recurrent ulcer symptoms or complications. A PPI was administered as an antiulcer drug for 8 wk to patients with gastric ulcers and 6 wk to those with duodenal ulcers. Subsequently, the drug was switched to a histamine H2 receptor antagonist (H2RA). No vitamin preparations were administered during the study period and general dietary counseling for peptic ulcers was administered to all patients at the time of hospital discharge. Upper endoscopy was performed within 2–3 mo after hospital discharge to confirm the condition of the ulcers.

This study was conducted in compliance with the ethical principles stated in the Declaration of Helsinki and the ethical guidelines on clinical studies after approval by the Institutional Review Board at Nippon Medical School. Written informed consent was obtained from all participants.

**Serum water-soluble vitamin (vitamins B1, B2, B6, B12, C, and folic acid) and homocysteine measurements.** After obtaining written consent, fasting blood samples were collected at 3 time points: at the time of admission, at the time of hospital discharge, and at 3 mo after hospital discharge. The blood serum was immediately centrifuged and stored at −60°C until further measurement.

The serum levels of water-soluble vitamins (vitamins B1, B2, B6, B12, C, and folic acid) and homocysteine were measured. Vitamins B1, B6, and C and homocysteine levels were measured using high performance liquid chromatography (HPLC; for vitamin B1, Hitachi L-7100 HPLC System, Hitachi, Ltd., Tokyo, Japan; for vitamin B6 and homocysteine, Prominence UFLC, Shimadzu Corp., Tokyo, Japan; and for vitamin C, Hitachi L-2130 HPLC System, Hitachi, Ltd.). The aluminilavine fluorescence method (RF-5300PC fluorescence spectrophotometer; Shimadzu Corp.) was used to measure vitamin B2 levels. Vitamin B12 and folic acid levels were measured by chemiluminescence immunoassay (Advia Centaur® XP Immunoassay System, Siemens Healthcare Diagnostics K.K., Tokyo, Japan).

**H. pylori infection status.** H. pylori infection was diagnosed positive when either serological tests for anti-H. pylori immunoglobulin G using an enzyme-linked immunosorbent assay (E-Plate; Eiken, Tokyo, Japan) or histopathological examination showed a positive result. Eradication therapy was administered to H. pylori-positive patients for up to 2 mo from the time of admission. An H. pylori stool antigen test or a breath test was performed to assess H. pylori eradication 1 mo after initiation of eradication therapy.

**Statistical analysis.** Statistical analysis was performed on data from patients who underwent all blood measurements at all 3 measurement time points (at admission, at discharge, and 3 mo after discharge). Continuous variables were expressed as the means±standard deviations (SDs). Time-specific effects were evaluated on the basis of one-way repeated-measures analysis of variance (ANOVA) for continuous variables and the Friedman test for non parametric variables. For items that exhibited time-dependent effects, Fisher’s protected least significant difference test and the Wilcoxon signed-rank test with Bonferroni correction, respectively, were used to compare continuous variables and non parametric variables obtained at the 3 measurements. All p values were two-tailed, and differences with values of p<0.05 were considered to be statistically significant.

A deficient state was confirmed when serum levels of water-soluble vitamins were below the lower limit of the normal range, whereas an excessive state was confirmed when homocysteine levels were beyond the upper limit of the normal range. Stat View version 5.0 (SAS Institute, Cary, NC) or SPSS 11.0 for Windows (SPSS Inc., Chicago, IL) was used to perform statistical analyses.

**RESULTS**

**Patient characteristics**

Ten consecutive patients who met the inclusion criteria and completed measurements of blood samples...
Table 2. Time course of changes in serum levels of water-soluble vitamins and homocysteine.

|        | Units     | Reference value | Admission | Discharge | 3 mo after discharge |
|--------|-----------|-----------------|-----------|-----------|----------------------|
| B1     | ng/mL     | 21.3–81.9       | 33.7±10.7 | 31.5±11.0 | 45.4±12.2            |
| B2     | μg/dL     | 1.7–4.6         | 2.21±0.80 | 2.54±0.73 | 2.66±0.65            |
| B6     | ng/mL     | 6–40            | 6.6±2.9   | 6.3±3.7   | 11.2±5.4             |
| B12    | pg/mL     | 233–914         | 504.8±228.9 | 665.4±232.5 | 450.2±153.6        |
| C      | μg/mL     | 4.7–17.8        | 4.85±2.51 | 6.75±5.30 | 7.06±3.62            |
| Folic acid | ng/mL   | 3.6–12.9       | 8.26±3.42 | 7.87±2.85 | 7.10±2.11            |
| Homocysteine | mmol/mL | 6.3–18.9       | 11.3±2.4  | 12.0±2.6  | 11.7±1.3             |

Fig. 1. Changes in the serum levels of vitamin B1. Plots indicate the means and standard deviations. Statistical analysis: Fisher’s protected least significant difference test. **p<0.01.

Fig. 2. Changes in the serum levels of vitamin B6. Plots indicate the means and standard deviations. Statistical analysis: Fisher’s protected least significant difference test, *p<0.05.

at all 3 time points (at admission, discharge, and 3 mo after discharge) were enrolled in the study. The patient characteristics are shown in Table 1. All patients were males, with an average age of 58.4±15.1 y. Three patients had gastric ulcers and 7 had duodenal ulcers. The fasting period after hospitalization was 4.0±1.6 d, and the duration of hospitalization was 12.2±4.6 d. Peptic ulcers in all 10 patients were confirmed to be in the scarring stage, and none of the biopsy specimens obtained at 3 mo after hospital discharge showed malignant findings. *H. pylori* infection was confirmed in 8 patients. These patients underwent eradication therapy for up to 2 mo after admission, and successful eradication was confirmed in all patients. The remaining 2 patients without *H. pylori* infection were NSAID users (loxoprofen for neuralgia and meloxicam for lumbago). One of the patients with *H. pylori* infection was a low-dose aspirin user.

*Serum levels of water-soluble vitamins and homocysteine*

Serum levels of water-soluble vitamins at each measurement time point are shown in Table 2. The mean levels of vitamins B1, B2, B6, and C, but not those of vitamin B12 and folic acid, were below normal at the time of admission. As shown in Table 2, time-dependent effects during the time course of changes in serum levels of the water-soluble vitamins were significantly different for vitamin B1 (p<0.01) and vitamin B6 (p<0.05) using one-way repeated-measure ANOVA; however, significant differences were not observed for vitamins B2, B12, C, or folic acid. Subsequently, the mean values of vitamin B1 and B6 levels were compared between the 3 measurement time points. Vitamin B1 and B6 levels were significantly higher at 3 mo after discharge than at admission (vitamin B1, 45.4±12.2 ng/mL vs. 33.7±10.7 ng/mL, p<0.01, Fig. 1; vitamin B6, 11.2±5.4 ng/mL vs. 6.6±2.9 ng/mL, p<0.05, Fig. 2). Furthermore, vitamin B1 and B6 levels were significantly higher at 3 mo after discharge than at discharge (vitamin B1, 45.4±12.2 ng/mL vs. 31.5±11.0 ng/mL, p<0.01, Fig. 1; vitamin B6, 11.2±5.4 ng/mL vs. 6.3±3.7 ng/mL, p<0.05, Fig. 2). The mean levels at discharge and at admission were not significantly different. In contrast, serum homocysteine levels showed neither an excessive state nor time-dependent effects (Table 2).

Although the mean levels of water-soluble vitamins were not below the lower limit of the normal range, as shown in Table 3, the proportion of patients with a deficiency of serum water-soluble vitamins were as high as 50% for vitamin B6 and vitamin C (5 patients each) at admission and remained present in 60% (6 patients) for vitamin B6 and 40% (4 patients) for vitamin C at discharge. In addition, the proportion of patients in whom any of the serum water-soluble vitamins were deficient was as high as 80% at admission, and remained at 70% of patients at discharge. Time-dependent changes in the proportion of patients with a deficiency of serum water-
soluble vitamins were found to be significantly different by the Friedman test for vitamin B₆ (p<0.05), but not for vitamins B₁, B₂, B₁₂, or C, or folic acid. Therefore, the proportion of patients with vitamin B₆ deficiency was compared between the measurement time points, and was found to be significantly higher at admission and discharge (50% and 60%, respectively, p<0.05, Wilcoxon signed-rank test with Bonferroni correction) than that at 3 mo after discharge (10%).

**DISCUSSION**

In this study, the proportion of patients with a deficiency in any water-soluble vitamin, a vitamin B₆ deficiency, or a vitamin C deficiency was as high as 80%, 50%, and 50%, respectively, at admission and 70%, 60%, and 40%, respectively, at discharge. These results indicated that water-soluble vitamins, particularly vitamins B₆ and C, were deficient in patients with complicated peptic ulcers, at admission and discharge. Because the patients underwent nutrition management on the basis of daily life guidance for peptic ulcers during hospitalization, the serum levels of water-soluble vitamins at 3 mo after discharge were assumed to be at favorable nutritional levels in each patient. In fact, the proportion of serum levels that was below the lower limit of the normal range for any of the water-soluble vitamins levels tested decreased from 25% (15/60) at admission to 5% (3/60) at 3 mo after discharge. Our findings suggest that, clinically, most patients with complicated peptic ulcers have a deficiency in one or more water-soluble vitamins soon after the onset of complicated peptic ulcers, even in the contemporary Japanese population.

In this study, all patients either had *H. pylori* infection or were NSAID users. Water-soluble vitamins have antioxidant activity. Among these, vitamin C is a particularly important dietary antioxidant that significantly decreases the adverse effects of molecules such as reactive oxygen and nitrogen species that can cause oxidative damage. These species have been implicated as reactive oxygen and nitrogen species that can cause oxidative damage. These species have been implicated as reactive oxygen and nitrogen species that can cause oxidative damage. Vitamin C is known to have antioxidant activity. Although vitamin C is locally secreted into gastric juices under physiological conditions, hypochlorhydria, a pH exceeding 4, is associated with lower vitamin C levels in gastric juices. Vitamin C may play a key role in healing and protecting the gastric mucosa from injurious insults, and vitamin C deficiency may repeatedly have been linked with development and healing of peptic ulcer disease and its complications.

In this study, the proportion of patients with vitamin B₆ deficiency was significantly higher at admission and discharge (50% and 60%, respectively) than at 3 mo after discharge (10%). Serum levels of water-soluble vitamins are thought to be affected by various factors, such as consumption through bleeding or inflammation, or inadequate intake. It is well established that water-soluble vitamins have antioxidant effects. Although vitamin C is locally secreted into gastric juices under physiological conditions, hypochlorhydria, a pH exceeding 4, is associated with lower vitamin C levels in gastric juices. Vitamin C-induced inflammatory response is known to decrease vitamin C levels in gastric juices. Thus, a decrease in vitamin C levels in gastric juices may be one of causes of peptic ulcer development. However, little is known about the potential for preventing and healing peptic ulcers by increasing water-soluble vitamin levels in gastric juices, or the relationship between serum and gastric juice levels of water-soluble vitamins. Further investigation should be carried out to estimate water-soluble vitamin levels in gastric juices and to investigate the local antioxidant effects of water-soluble vitamins in patients with peptic ulcers.

In this study, the proportion of patients with vitamin B₆ deficiency was significantly higher at admission and discharge (50% and 60%, respectively) than at 3 mo after discharge (10%). Deficiency of vitamin B₆, which is involved in the metabolism of homocysteine, can cause hyperhomocysteinemia. Homocysteine is one of the causes of arteriosclerosis and phlebothrombosis. Rimm et al. (29) suggested that the intake of vitamin B₆ at levels above the current recommended dietary allowance might be important for the primary prevention of coronary heart disease among women. Robinson et al. (30) reported that low pyridoxal 50-phosphate (the active form of vitamin B₆) was an independent risk factor for ischemic heart disease. Maintenance of adequate vitamin B₆ levels is thought to be an important factor for the primary prevention of not only ischemic heart disease but also peptic ulcers caused as a consequence.
of arteriosclerosis.

PPIs and H2RAs, which are acid-suppressant drugs, are commonly prescribed as chronic preventive and therapeutic drugs for the treatment of peptic ulcers and gastroesophageal reflux disease. However, studies have reported that chronic administration of PPIs decreases vitamin C levels in gastric juices through hyposecretion and the instability of ascorbic acid, which is related to hypochlorhydria induced by potent acid suppression (31–33). Furthermore, chronic H2RA or PPI use has been shown to be associated with the development of serum vitamin B12 deficiency through PPI-associated malabsorption due to decreased release of vitamin B12 from ingested food (34–36). Discontinuation of prophylactic long-term PPI treatment may easily lead to peptic ulcer recurrence through the malabsorption of water-soluble vitamins. Therefore, supplementation of water-soluble vitamins seems to be of particular importance in high-risk patients with complicated peptic ulcers that require prophylactic long-term PPI treatment (37).

In this study, most patients with complicated peptic ulcers showed a deficiency in one or more water-soluble vitamins at admission. It is thought that deficiencies in water-soluble vitamins can be a trigger for the development of peptic ulcers in patients with H. pylori infection or in NSAID users. The clinical significance of the water-soluble vitamin deficiencies determined in our study is unclear. The deficiencies could be associated with poorer nutrition during the period before the diagnosis of peptic ulcers, which could have been a consequence rather than a cause of the clinical manifestations. However, because patients with complicated peptic ulcers were under dietary restriction during their initial management, any deficiencies in water-soluble vitamins were sustained during hospitalization. Therefore, although the Japanese health care system allows for the supplementation of multivitamins only in patients on total parenteral nutrition, supplementation of water-soluble vitamins may be indicated for the prevention and healing of complicated peptic ulcers and to compensate for H. pylori-induced gastritis and NSAID-associated gastric mucosal damage. Further investigations will be required to determine whether water-soluble vitamin deficiencies promote the development of peptic ulcers and their complications or interfere with ulcer healing.

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Declaration of Interest
The authors declare no potential conflicts of interest.

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