Effect of electroacupuncture ST 36 Zusanli on serum levels of nitric oxide in patients with dyspepsia

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Abstract. Dyspepsia is a gastroduodenal syndrome that affects the physical and the emotional quality of life. The available pharmacological therapies vary in effectiveness. Acupuncture can address dyspepsia symptoms effectively. Electroacupuncture (EA) stimulation at ST 36 Zusanli improves dyspepsia by modulation of nitric oxide (NO) in the gastric environment. This double-blind randomized controlled trial compared the effectiveness of EA treatment and manual acupuncture (MA) at ST 36 Zusanli to increase the serum levels of NO in patients with dyspepsia. Forty patients with dyspepsia were randomly allocated to EA or MA. The results showed there were significant differences in the mean serum levels of NO in the EA group compared to the MA group (p = 0.026). The results indicate that electroacupuncture at ST 36 Zusanli was more effective than MA in increasing the serum levels of NO in patients with dyspepsia (p < 0.05).

1. Introduction

Dyspepsia with abdominal discomfort, including epigastric pain and accompanied by other gastrointestinal symptoms is a frequently encountered complaint [1,2]. The worldwide prevalence is estimated at 7% to 45%, depending on the definition and geographic region [3]. The 2005 Republic of Indonesia Health Profile ranked dyspepsia tenth among the most frequently outpatient complaints, diagnosed in 1.5% of those patients [4,5].

The successful treatment of dyspepsia is challenging as shown by a recent meta-analysis reporting a mean response of 34% to proton pump inhibitors and a response of 25% to placebo, a response of 54% to histamine H₂ receptor antagonists versus a response of 40% to placebo, and a response of 37% to Helicobacter pylori eradication therapy versus a response of 29% to placebo [2]. Pharmacotherapy is widely used in clinical practice, but most drugs are directed at only one factor, even though the etiology of dyspepsia is often unclear and the pathophysiology is complex [2-6]. The symptoms of patients with dyspepsia are heterogeneous, and, often, the condition is accompanied by multiple physiological mechanisms [2].

Acupuncture therapy is a neuromodulation technique that has been used to treat gastrointestinal symptoms such as acute and chronic gastroenteritis, diarrhea, constipation, and gastroduodenal ulcers.
The effectiveness of acupuncture therapy for the symptoms of dyspepsia, belching, and anorexia has been confirmed in randomized controlled clinical trials [6]. The protection offered by electroacupuncture (EA) at the ST 36 Zusanli point has been evaluated in animal models of damage to the gastric mucosa,[8,9] and a frequency of 100 Hz had better antinociceptive effects than 5 or 30 Hz in another animal model [10]. The antinociceptive effects were associated with the activation of the opioid pathways and possibly mediated by dynorphin and NO formation [10]. This study compared the effectiveness of manual and EA therapy at ST 36 Zusanli for elevating NO serum levels in dyspeptic patients.

2. Material and Methods
This double-blind randomized controlled trial enrolled 40 dyspeptic patients who were treated as outpatients at the Polyclinic of Internal Medicine of Dr. Cipto Mangunkusumo Hospital, Jakarta between November 2013 and February 2014. All the participants voluntarily gave written informed consent and were guaranteed confidentiality. The Health Research Ethics Committee of Faculty of Medicine, Universitas Indonesia-Dr. Cipto Mangunkusumo Hospital, Jakarta (no. 664/H2.F1/ETIK/ 2013).

Men and women from 18 to 60 years of age were diagnosed with dyspepsia diagnosis following the criteria of the 2011 Asian Consensus Report on Functional Dyspepsia. No participants had unexplained weight loss, progressive dysphagia, recurrent or persistent vomiting, evidence of gastrointestinal bleeding, anemia, fever, family history of gastric cancer, dyspepsia symptoms appearing at > 45 years of age), or diabetes mellitus. Excluded were patients with contraindications to acupuncture or EA, including medical emergencies, surgery, acute fever of > 38°C, clotting disorders or moderate anticoagulant medication, pregnancy, pacemaker use, lack of skin sensibility, or extensive skin lesions. Patients contraindicated for puncture because of skin wounds or infections at the ST 36 Zusanli point were not eligible. The participants who did not complete the study procedures were not included in the analysis.

Patients were randomly assigned to EA (Group A) or MA (Group B). Disposable 0.25 × 40 mm acupuncture needles were used with an electrostimulator (HWATO, SDZ-V Nerve and Muscle Stimulator). Following puncture at the ST 36 Zusanli point, the needle was connected to the electrostimulator at 100 Hz for 30 min. In addition, stimulation was performed for 30 minutes in group B, but was not accompanied by electric stimulation. In both groups needle insertion continued until puncture sensation was achieved.

Venous blood (3 ml) was collected before treatment and within 30 minutes of needle removal. Serum was separated by centrifugation. A colorimetric nitrate/nitrite concentration assay based on the Griess reaction (Cayman Chemical, Ann Arbor, MI, USA), which is an indicator of in vivo NO activity was used to estimate the serum NO [11]. In the assay, nitrate is converted to nitrite followed by the addition of Griess reagents that convert nitrite to a purple azo compound. The total nitrite concentration was determined by absorbance at 540–550 nm and was reported in µM.

2.1. Statistical analysis
The results were reported as numbers and percentages or means ± standard deviation. The significance of the differences of the mean serum NO concentrations in the EA and MA group after treatment was determined with the one-way Mann–Whitney test. The data were processed statistically using the hypothesis test and the results were considered significant when the p-value of the difference was < 0.05.

3. Results
Forty dyspeptic patients met the selection criteria and were randomly divided into equal numbers to receive EA or MA. All the patients completed the study.
Table 1. Patient characteristics in the two treatment groups

| Characteristic  | Electroacupuncture, n, (%) | Manual Acupuncture, n, (%) |
|----------------|---------------------------|----------------------------|
| Patients       | 20                        | 20                         |
| Age (years)    |                           |                            |
| 18 – 24        | 2 (10)                    | 0 (0)                      |
| 25 – 34        | 6 (30)                    | 2 (10)                     |
| 35 – 44        | 5 (25)                    | 7 (35)                     |
| 45 – 54        | 6 (30)                    | 8 (40)                     |
| 55 – 60        | 1 (5)                     | 3 (15)                     |
| Sex            |                           |                            |
| Male           | 5 (25)                    | 7 (35)                     |
| Female         | 15 (75)                   | 13 (65)                    |
| Education      |                           |                            |
| Low            | 4 (20)                    | 8 (40)                     |
| Moderate       | 9 (45)                    | 9 (45)                     |
| High           | 7 (35)                    | 3 (15)                     |
| Employment     |                           |                            |
| Employed       | 6 (30)                    | 9 (45)                     |
| Unemployed     | 14 (70)                   | 11 (55)                    |

Table 1 shows the patient characteristics. The majority of patients in the EA group was between 25–34 (6/20, 30%) and 45–54 (6/20, 30%) years of age. In the MA group, most were between 45–54 (8/20, 40%) and 35–44 (7/20, 35%) years of age. There were more women than men in both the EA (15/20, 75%) and MA (13/20, 65%) groups. Nearly half of the participants in each group (9/20, 45%) had a moderate level of education. Most patients, 70% in the EA (14/20) and 55% in the AM (11/20) group were unemployed. The differences in age, sex, education level, and employment were not significantly different (p > 0.05). The difference of serum NO concentration before and after treatment is shown in Table 2 below.

Table 2. Serum NO before and after treatment

| Treatment            | Before Treatment | After Treatment |
|----------------------|------------------|-----------------|
|                      | Mean (SD) (μM)   | Mean (SD) (μM)  |
| Electroacupuncture   | 6 (3.17)         | 6.19 (4.01)     |
| Manual Acupuncture   | 8.68 (4.38)      | 8.24 (5.35)     |

Table 3. Mean difference of serum NO concentration before and after treatment

| Variable                  | n   | Mean (SD) (μM) | p*    |
|---------------------------|-----|----------------|-------|
| Δ Electroacupuncture serum NO | 20  | 0.19 (2.44)    | 0.026*|
| Δ Manual acupuncture serum NO  | 20  | −0.44 (2.88)   |       |

*Mann–Whitney Test (one-tailed), Δ Differences ; **p<0.05

The difference in the mean change of serum NO before and after treatment in the EA (0.19 μM ± 2.44 μM) and the MA group (−0.44 μM ± 2.88 μM) was significant (p = 0.026) (Table 3).
4. Discussion

Forty patients who satisfied the Asian Consensus Report on Functional Dyspepsia criteria were randomly allocated to treatment with electro- or mechanical acupuncture. The baseline characteristics of the two study groups, age, sex, education, and employment, were not significantly different, indicating that they were in an equivalent condition at the beginning of the study. The study aim was to determine whether EA was more effective than MA in increasing NO serum levels in dyspeptic patients. Serum NO was assayed before and 30 minutes after completion of a single acupuncture treatment. The treatment outcome was the change in serum NO from before to after treatment, which was significantly greater in the EA than in the MA group.

EA at ST 36 Zusanli is the most commonly investigated acupuncture treatment of gastric problems, and has been shown to protect and to maintain the integrity of the gastric mucosa and improve gastric motility [8,9]. EA in this study was applied at 100 Hz. In a rat microdialysis model, EA at 100 Hz produced analgesia associated with increased release of dynorphin bound to κ opioid receptor in the spinal cord dorsal cauda. EA at 2 Hz seems to induce analgesia by releasing endomorphins, β-endorphins, and enkephalin bound to μ and δ opioid receptors. A study comparing the effects of various EA frequencies at ST 36 Zusanli found that 100 Hz had better antinociceptive effects than 5 and 30 Hz [10].

MA, which is reported to improve dyspepsia symptoms, has autonomic effects by blocking the sympathetic nerves and by increasing the parasympathetic activity. Zhou et al. found that both MA and low 2 Hz EA had stronger inhibitory effects on sympathetic nerves than EA at 40 or 100 Hz. In traditional acupuncture theory, ST 36 Zusanli is on the Foot–Yangming meridian. Yan et al. reported a significant decrease of the gastric mucosal injury index in rabbits with acupuncture therapy at Foot–Yangming meridian points. NO, NO synthase, prostaglandin E2, and epidermal growth factor all increased significantly in the treated animals, confirming the gastric effects of the Foot–Yangming meridian [12]. The ST 36 Zusanli point was selected on the basis in this study was based on evidence that acupuncture at that point cures and reduces gastric mucosal damage induced by ethanol [9,12] and acetylsalicylic acid [8], protects the gastric mucosa by regulating blood flow [9], regulates NO levels [13,14], decreases gastric acid secretion, and regulates other gastroprotective functions [15]. NO is an endothelial-derived relaxing factor that relaxes the smooth muscle of blood vessels. Pei et al. reported that acupuncture and moxibustion at the ST 36 Zusanli point increased NO and gastric mucosal blood flow and protected against mucosal damage as the decrease in the lesion index values shows. The response to acupuncture and moxibustion was also associated with activation of endogenous NOS [9]. NO or NO donors have been shown to stimulate prostaglandin production and regulate the secretion of acetylsalicylic acid (AAS)-induced HCO3. Hwang et al. found that EA pretreatment significantly increased NO levels in a rat model of acetylsalicylic acid-induced acute gastritis compared control animals without EA pretreatment. The increase in total NO may have protected the gastric mucosa from AAS-induced toxicity and increased the production of prostaglandins and HCO3 [8].

To the best of our knowledge, the effects of acupuncture on serum NO in dyspeptic patients have not been reported in Indonesia. Serum NO serum was elevated in patients treated with EA. The mean serum NO concentration in the EA group was 6 ± 3.17 μM before treatment and increased to 6.19 μM ± 4.01 μM at 30 min after the EA treatment was completed. The increase in serum NO in response to EA is consistent with previous reports by Hwang et al. [8] Pei et al. [9] and Yan et al. [12]. The mean serum NO before MA treatment was 8.68 μM ± 4.38 μM, and it decreased to 8.24 μM ± 5.35 μM 30 min after treatment. The mean change in serum NO in the EA group was an increase of 0.19 ± 2.44 μM. The mean change in in the MA group was a decrease of 0.44 μM ± 2.88 μM. The between-group difference was 0.63 μM, which was significant (one-way Mann–Whitney test, p = 0.026). The difference in treatment-related change in serum NO (p < 0.05) shows that EA was more effective than MA in increasing serum NO serum in dyspeptic patients. The decrease of serum NO in the MA group was not consistent with the results of previous studies in experimental animal models of gastrointestinal disorders. However, a study comparing the effects of ear and body acupuncture on blood pressure and NO in patients with essential hypertension found a decrease in serum NO shortly after completion of body acupuncture [16].

A study of the effect of catgut suture imbedding on NO and blood pressure in patients with essential hypertension reported that continuing damage of the vascular endothelium increased the levels of...
superoxide anions. NO produced by blood vessels was bound to superoxide anions to form peroxinitrite that in turn oxidized tetrahydrobiopterin (BH4), which is a cofactor of NOS. Thus, NOS produced more superoxide anions than NO did [17].

Acupuncture may act by activating the opioid pathway and involve NO formation [10]. EA inhibits sympathetic effects by modulating NOS expression in the central nervous system. Acupuncture appears to decrease the activation of the sympathetic nervous system and the activation of the cholinergic system or opioid receptors in the rostral ventrolateral medulla. Some parasympathetic motor nerves release NO as a neurotransmitter. The needle puncture at ST 36 Zusanli induces NO release near the puncture area. In a study of the distribution of NO in rat skin near acupuncture points, Chen et al. found that the L-arginine-derived NO mediated noradrenergic activity in sympathetic nerves, contributing to the skin’s electrical resistance around the acupuncture points and meridians. Pei et al. proposed that stimulation at ST 36 Zusanli reduces dyspeptic symptoms by mediating NO production and involving vagal innervation of the stomach and the dorsal nucleus of the vagus nerve (the posterior motor nucleus of vagus). Acupuncture needles with or without electrostimulation stimulate somatic afferent nerves in the skin and muscles. The stimulus is transmitted to the dorsal vagus nucleus by somatic sensory nerves in the spinal cord. In addition, the vagus dorsal nucleus receives afferent signals from the gastrointestinal tract and integrates other information from the central nervous system, and passes it on to the stomach through efferent vagal nerves, resulting in increased NO synthesis and release [18]. The increase in gastric motility that ultimately occurs after the excitation of vagal afferents helps to reduce the symptoms of dyspepsia [9]. No patients in this study experienced adverse effects associated with the treatment. Some EA patients commented on the loudness of the electrostimulator vibration; however, none complained of pain caused by the treatment. Both EA and MA were safe and well tolerated in clinical practice.

5. Conclusion
EA at ST 36 Zusanli was more effective than MA in increasing NO serum levels in dyspeptic patients, with a difference in the mean increase of 0.63 μM.

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