Changes in Serum Levels of ADMA, SDMA and L-NMMA with *Helicobacter Pylori* Eradication

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ABSTRACT

Objective: Increased asymmetric dimethylarginine (ADMA) levels are associated with reduced nitric oxide (NO) levels in many systems, particularly the cardiovascular system, and cause adverse effects. The objective of this study is to evaluate the effect of eradication therapy in patients infected with *Helicobacter pylori* (*H. pylori*) on the serum level of ADMA and other metabolic products of methylarginine.

Methods: Patients who were found positive both in urea breath tests and stool antigen tests were considered to have *H. pylori* infection. These patients received eradication therapy for 14 days (twice daily pantoprazole 40 mg, twice daily amoxicillin 1000 mg, and twice daily clarithromycin 500 mg). Blood samples were taken to measure serum ADMA, symmetric dimethylarginine (SDMA), and N-monomethyl-L-arginine (L-NMMA) levels before eradication therapy and 3 months after the therapy for patients for whom eradication was achieved.

Results: A total of 23 of the 45 patients included in the study were female, whereas 22 were male. The mean age of the patients was 32.4±8 years. Significant reductions in the serum ADMA, SDMA, and L-NMMA levels of the patients were observed post-eradication therapy versus pre-eradication therapy.

Conclusion: This study demonstrated significant reductions in serum ADMA, SDMA, and L-NMMA levels with *H. pylori* eradication. Further extensive long-term studies are needed to evaluate the positive effects that reduced serum ADMA, SDMA, and L-NMMA levels after *H. pylori* eradication can have on all systems, particularly the cardiovascular system.

Keywords: *Helicobacter pylori*, asymmetric dimethylarginine, symmetric dimethylarginine, N-monomethyl-L-arginine

INTRODUCTION

Nitric oxide (NO) is a gaseous molecule with a strong vasodilator effect in all systems, particularly in the cardiovascular and gastrointestinal systems (1). NO plays an important contribution in the maintenance of gastric mucosal integrity, such as increased gastric mucosal blood flow, inhibition of acid secretion, and fundus dilatation, and functions as a mucosa-preserving factor (2-4). NO is synthesized by a reaction including oxygen molecule from L-arginine amino acids and catalyzed by NO synthase (NOS) enzyme (5). Asymmetric dimethylarginine (ADMA) has a structure that is highly similar to L-arginine, and it is the competitive inhibitor of NOS enzyme. Increased ADMA levels decrease NO level in many systems, particularly in the cardiovascular system, and cause negative effects (6).

Helicobacter pylori forms a lifelong inflammation source localized in the mucosa of the gastrointestinal system. Clinical and experimental studies have demonstrated that *H. pylori* infection caused increased levels of ADMA (6). Despite several studies on the importance of ADMA in the development of endothelial dysfunction in the literature, there are a few studies evaluating the relationship between *H. pylori* infection and ADMA levels (7-9). The aim of the present study is to evaluate the effect of eradication treatment on the serum levels of ADMA, symmetric dimethylarginine (SDMA), and N-monomethyl-L-arginine (L-NMMA) in individuals infected with *H. pylori*.

METHODS

The study included 45 patients whose ages were between 18 years and 45 years and who were admitted with dyspeptic complaints to the outpatient clinic of gastroenterology in our university. Patients previously receiving eradication treatment and antiulcer drug therapy for *H. pylori* in the last 1 month; having hypertension, diabetes mellitus, cerebrovascular diseases, ischemic heart disease, malignancy, hepatic or renal failure, inflammatory or infectious diseases, and a history of gastric surgery; becoming pregnant; and breastfeeding were excluded from the study. The study was conducted with the ethical approval of the ethics

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committee of Selçuk University School of Medicine (approval no. 2013/287), and informed consent was obtained from all patients included in the study.

For the diagnosis and eradication control of *H. pylori* infection, ^{14}C urea breath test and stool antigen test were used. These tests were performed twice for the diagnosis of *H. pylori* infection at the beginning of the study and for eradication control after 3 months following the end of a 14-day eradication treatment. Patients having positive results for both tests at the beginning of the study were considered as infected and included in the study. In patients where both tests were negative in the 3rd month after eradication treatment, eradication was assumed to be provided. Eradication was observed in 29 out of the 45 patients included in the study. Serum ADMA, SDMA, and L-NMMA levels of 45 patients before eradication and 29 patients with eradication were compared.

All patients in the study were perorally given 40 mg pantoprazole twice a day, 1000 mg amoxicillin twice a day, and 500 mg clarithromycin twice a day for 14 days.

ADMA, SDMA, and L-NMMA serum levels were analyzed at the beginning of the study and in the 3rd month of the eradication treatment. Blood samples were collected after fasting overnight and stored at 80 °C as serum. The analysis of serum ADMA, SDMA, and L-NMMA levels was performed by using a modified method with Luna C18 (Phenomenex, CA, USA) column in the API 3200 LC–MS/MS system mass spectrometer (Applied Biosystems/MDS SCIEX, CA, USA) device matched with high-performance liquid chromatography (Shimadzu LC-20AD; Shimadzu, Kyoto, Japan) (10).

Statistical Analysis

Statistical analysis of data was performed by using SPSS version 20.0 (IBM Statistical Package for the Social Sciences, version 20.0 IBM Corp.; Armonk, NY, ABD) software. For parametric data, the comparison of the means in two groups was done with paired Student's t-test. The mean±standard deviation values of parametric data were demonstrated to be convenient. A p-value <0.05 was considered to be statistically significant.

RESULTS

Of 45 patients included in the study, 23 were females, and 22 were males. The mean age of the patients was 32.4±8 years. When serum levels of ADMA, SDMA, and L-NMMA were compared in 45 infected patients before eradication and in 29 patients with eradication, a significant decrease was observed in serum levels in association with eradication treatment (Table 1).

DISCUSSION

NO, which is also considered as a second messenger molecule, is a gaseous molecule with a vasodilator effect in all systems particularly in the cardiovascular system. Its synthesis is directly controlled by NOS expression and activity. Vasodilatation is one of the important inflammatory signals in the body and is widely provided with NO-dependent processes (11).

Methylarginines are formed as a result of the methylation of arginine residues in proteins (12). Protein arginine methylation is a post-translational modification that transfers one or two methyl groups to arginine guanidino nitrogen in proteins. In humans, this process is performed by protein arginine methyltransferase (PRMT) enzymes (13). The products that are formed as a result of type 1 PRMT activity are ADMA and L-NMMA molecules. These molecules can inhibit NOS. Type 2 PRMT plays a role in the formation of SDMA. SDMA cannot inhibit NOS (14). However, in renal failure, the level of SDMA in the circulation is higher than that of ADMA (15).

The reason for the importance of ADMA is that it inhibits NOS activity and causes a significant decrease in NO synthesis. Recently, increasing evidence suggests that ADMA accumulation decreases NO synthesis and bioavailability in many systems and leads to harmful effects and organ dysfunction (16). ADMA-induced endothelial damage can play a role in the development of many diseases such as hypertension, atherosclerosis, coronary artery disease, diabetes mellitus, pulmonary hypertension, and renal failure (16-19). ADMA can directly induce oxidative stress and cell death as well as decreased level of NO (20, 21).

Experimental and clinical studies have demonstrated that *H. pylori* increases ADMA levels. It was reported that in vitro addition of extract obtained with the proteolysis of *H. pylori* in rats caused four times more increase in ADMA level in the duodenal perfusate and five times more increase in ADMA level in the duodenal tissue (22). In the study conducted by Wang et al., increased ADMA and tumor necrosis factor (TNF)-α levels were observed in the gastric mucosal epithelial cell culture incubated with *H. pylori*, and an increased TNF-α level was reported with external ADMA administration (23). Clinical studies demonstrated increased mucosal NOS expression in the gastric antrum tissue and increased ADMA content in patients infected with *H. pylori* (24). In another clinical study, it was reported that elevated plasma ADMA levels were observed in patients with asymptomatic *H. pylori* infection compared with healthy individuals (9). Moreover, in another clinical study, increased ADMA levels were detected in the digestive juice of patients infected with *H. pylori*, but no statistically significant difference was found in plasma ADMA levels of patients with positive and negative *H. pylori* (8).

The only study evaluating *H. pylori* eradication and ADMA levels was conducted by Aydemir et al. (7). In their study, a significant decrease was reported in serum ADMA levels of patients in whom *H. pylori* eradication was provided. Methylarginine metabolism products, except ADMA, were not evaluated in the present study.

Table 1. Comparison of ADMA, SDMA, and L-NMMA serum levels before and after eradication

Parameter	Before eradication (n=45)	After eradication (n=29)	p
ADMA (µmol/l)	0.56±0.09	0.43±0.11	<0.001
SDMA (µmol/l)	0.70±0.19	0.39±0.11	<0.001
L-NMMA (µmol/l)	0.10±0.02	0.009±0.02	<0.001

ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; L-NMMA: N-monomethyl-L-arginine

In our study, a significant decrease was observed with *H. pylori* eradication in ADMA levels, as in the study by Aydemir et al. (7). In addition, a significant decrease in eradication treatment was first revealed in other methylarginine metabolism products (SDMA and L-NMMA) in our study.

Our study has several major limitations. First, it was performed on a small patient group. Second, the patients were not followed up for a long time. Finally, the levels of methylarginine metabolism products were not evaluated in patients in whom eradication could not be provided.

CONCLUSION

In conclusion, it can be suggested that decreased serum ADMA, SDMA, and L-NMMA levels associated with *H. pylori* eradication can lead to beneficial effects in many systems. We believe that these possible useful effects should be evaluated in larger and long-term studies.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Selçuk University School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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