

# Long-term Effect of *Helicobacter pylori* Eradication on Risk Factors for Cardiovascular Disease – Is there a Connection?

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## ABSTRACT

**Background & Aims:** Cardiovascular disease (CVD) remains the leading cause of mortality worldwide. Beyond traditional risk factors, chronic inflammation is increasingly recognized in its pathogenesis. *Helicobacter pylori* (*H. pylori*) infection has been proposed as a potential contributor. This study aimed to investigate the long-term effects of successful *H. pylori* eradication on selected CVD risk factors.

**Methods:** Seventy-two patients were enrolled between July 2020 and November 2022 and randomized to two 14-day regimens (group 1: esomeprazole, amoxicillin, clarithromycin; group 2: esomeprazole, amoxicillin, metronidazole, colloidal bismuth subcitrate). Outcomes included homeostatic model assessment of insulin resistance (HOMA-IR) index, lipid profiles and subfractions, and urinary trimethylamine N-oxide (TMAO), assessed by nuclear magnetic resonance spectroscopy. Assessments were performed at baseline, two months, and one year after confirmed *H. pylori* eradication.

**Results:** Of the 72 enrolled patients, 13.9% (10/72) were lost to follow-up. Baseline CVD risk factors did not differ significantly between groups. After successful eradication, both groups demonstrated significant reductions in total cholesterol ( $p=0.003$ ), low-density lipoprotein cholesterol ( $p=0.010$ ), small dense lipoprotein particles ( $p=0.037$ ), and marginal decrease in TMAO concentrations ( $p=0.048$ ). No significant changes were observed in body mass index ( $p=0.799$ ), waist circumference ( $p=0.305$ ), or HOMA-IR index ( $p=0.275$ ).

**Conclusions:** Successful eradication of *H. pylori* infection was associated with favorable changes in lipid metabolism and marginal decrease in TMAO levels. These findings suggest that *H. pylori* may contribute to CVD risk by modulating lipoprotein profiles and systemic inflammation.

**Key words:** *Helicobacter pylori* – cardiovascular disease risk factors – dyslipidaemia – trimethylamine N-oxide.

**Abbreviations:** BMI: body mass index; CHD: coronary heart disease; CVD: cardiovascular disease; *H. pylori*: *Helicobacter pylori*; HDL: high density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; LDL: low density lipoprotein; OR: odds ratio; RR: relative risk; RUT: rapid urease test; SLDL-p: small dense lipoprotein particles; TMAO: trimethylamine N-oxide.

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a spiral-shaped, gram-negative microaerophilic bacterium that infects approximately 43% of the global population [1]. Infection invariably induces chronic active gastritis, and up to 15% of affected individuals develop clinically significant complications such as gastric or duodenal ulcer disease, dyspepsia, idiopathic thrombocytopenic purpura, or iron-deficiency anemia.

Furthermore, approximately 3% of infected patients are at risk of gastric malignancies, including adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Beyond its well-established gastrointestinal sequelae, accumulating evidence suggests that *H. pylori* infection may also contribute to a spectrum of extra-digestive disorders, underscoring its potential role as a systemic pathogen that warrants further investigation [1–3].

Cardiovascular disease (CVD) remains the leading cause of mortality worldwide. Established risk factors for CVD include hypertension, dyslipidaemia, diabetes mellitus, obesity, advanced age, smoking, and physical inactivity [4]. In addition to these traditional risk factors, merging evidence suggests that insulin resistance, oxidative stress, and chronic inflammatory processes significantly contribute to atherosclerosis [5].

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Chronic microbial infections, including *H. pylori*, have been implicated in the pathogenesis of CVD through mechanisms involving low-grade, persistent inflammation and immune-mediated endothelial dysfunction [6–14]. Consequently, several observational studies have reported a link between chronic infection with *H. pylori* and an increased risk of CVD [15–18], although the data are not yet conclusive.

Thus, the present prospective, randomized study aims to evaluate the long-term effects of successful *H. pylori* eradication on various CVD risk factors in a way that has not been done before.

## METHODS

A total of 72 patients aged 20 to 70 years with chronic *H. pylori* gastritis who met the inclusion criteria were recruited at the Diagnostic Centre Rogaška. Inclusion criteria included confirmed *H. pylori* infection with positive rapid urease test (RUT) and histological analysis or *H. pylori* cultivation. Exclusion criteria included (i) previous *H. pylori* eradication therapy, (ii) history of gastric or gastrointestinal surgery, (iii) use of biological or immunosuppressive therapy, (iv) psychiatric disorders precluding participation, (v) pregnancy or breastfeeding, (vi) known allergy to the drugs used in the study, (vii) use of proton pump inhibitors 14 days prior to study enrolment, (viii) recent (within 4 weeks) treatment with broad-spectrum antibiotics, (ix) ongoing treatment for malignant diseases, (x) impaired coagulation, and (xi) continuous use of anticoagulant medication preventing safe biopsy collection.

The study was approved by Medical Ethics Committee of the Republic of Slovenia (application number 0120-574/2019/4), and all participants provided written informed consent prior to study inclusion. The study was conducted in accordance with the ethical standards set forth in the 1975 Declaration of Helsinki.

Each patient underwent upper gastrointestinal (UGI) endoscopy at the Diagnostic Centre Rogaška, Slovenia. Biopsies were obtained from corpus and antrum for RUT, histological examination and *H. pylori* cultivation (for additional confirmation of *H. pylori* infection). Demographic and clinical data were collected, including sex, age, comorbidities, chronic therapy, and smoking. Body weight (BW), height (BH), waist circumference, blood pressure (BP), heart rate (HR) and body mass index (BMI) were also recorded. Baseline laboratory measurements included serum concentrations of glucose, insulin, lipid profiles and subfractions, creatinine, and urea, as well as urinary trimethylamine N-oxide (TMAO) metabolites. Analysis of TMAO concentrations and lipid profiles was performed at the INFAI GmbH laboratories in Cologne, Germany.

Patients were randomly allocated into two treatment groups using computerized randomization software. The aim of randomization was not to compare therapeutic efficacy of two commonly used therapeutic protocols. The randomization was originally implemented as part of a larger, ongoing study in which we are also evaluating long term changes in gut microbiota, as well as alterations in the resistome following different treatment regimens and to minimize potential treatment-related biases. According to the recommendations

of the Slovenian Society of Gastroenterology and Hepatology [19], patients received either esomeprazole 40 mg BID, amoxicillin 1000 mg BID and clarithromycin 500 mg BID for 14 days (group 1) or colloidal bismuth subcitrate 120 mg QID, esomeprazole 40 mg BID, amoxicillin 500 mg QID and metronidazole 400 mg QID for 14 days (group 2).

Two months post-treatment, a <sup>13</sup>C-urea breath test (UBT) was performed to confirm *H. pylori* eradication. Patients with successful *H. pylori* eradication were included in the follow-up analysis. Urine and blood samples as well as clinical data were collected at baseline, two months, and one-year post-eradication to evaluate the long-term changes in the investigated parameters.

Venous blood samples for lipid profiling, collected from fasting patients in the morning, were stored at -20°C and transported on dry ice for subsequent analysis. Nuclear magnetic resonance (NMR) spectroscopy using the Bruker Avance III spectrometer was used for determining metabolic profiles. The AXINON® lipoFIT®-S100 test system was used to determine the lipoprotein fractions and subfractions.

Urine samples were collected in the morning from fasting patients, stored at -20°C and transported for analysis on dry ice. The samples were analysed by 1D-<sup>1</sup>H NMR spectroscopy. Bruker ADVANCE III 500 MHz and 600 MHz high throughput NMR spectroscopy was used.

Statistical analysis was performed using the SPSS software (SPSS Inc., Chicago, USA). All data were presented as mean ± standard deviation for both normally and abnormally distributed variables for aligned presentation of the data. The normality of distribution was confirmed using the Shapiro-Wilk test. Patients were divided into two treatment groups, which represented independent variables. The dependent variable was measured 3-times, namely at the baseline, 2 months after and 1 year after *H. pylori* eradication. Times of measurements were also evaluated as independent variables. Repeated-measure ANOVA was used in case of normally distributed variables with treatment group as independent variable and over time. For variables that were abnormally distributed, comparisons over time and between test groups were performed using the Generalized Estimating Equations (GEE) test. The significance level for all statistical analyses was set at p<0.05. The degree of correlation between the values examined were checked using the Pearson's correlation coefficient.

## RESULTS

A total of 72 patients with a positive RUT were enrolled in the study. In the final analysis, 13.9% (10/72) of patients were excluded due to unsuccessful *H. pylori* eradication (3/72), lack of compliance (5/72), or discrepancies in *H. pylori* testing (2/72, e.g., positive RUT with negative histology and culture for *H. pylori*), as shown in Fig. 1. Baseline characteristics were comparable between the two treatment groups, as detailed in Table I. The study cohort comprised 46.8% male and 53.2% female participants with a mean of age 47.9 years [standard deviation (SD)] ±13.1. Smoking was present in 27.4% of patients, 22.6% of patients were receiving antihypertensive drugs and 8.1% therapy with statins for dyslipidaemia, with no statistically significant differences between the treatment

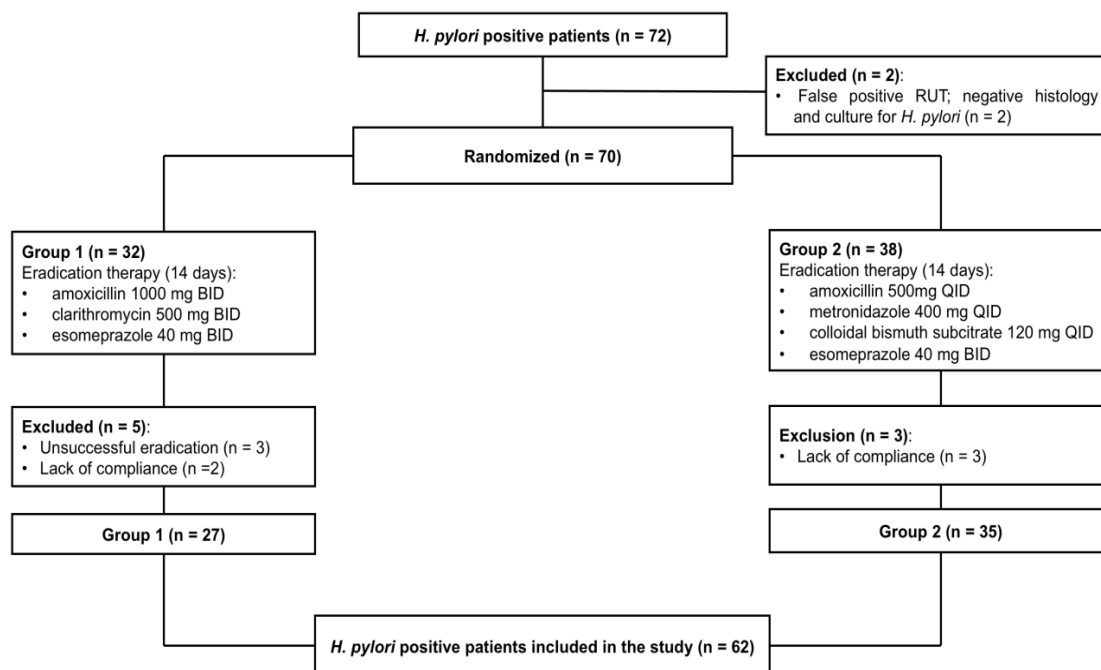


Fig. 1. Flowchart of the study.

groups. BMI was 26.9±4.5 and 27.0±4.4 in group 1 and 2, respectively. Other comorbidities not related to metabolic syndrome and CVD were rare.

The Framingham risk score for coronary heart disease (CHD) was comparable between groups. Specifically, more than half of the patients (56.5%; 35/62) were classified as low risk, 14.5% (9/62) as intermediate risk, and 27.4% (17/62) as high risk.

Most patients were slightly overweight, with a BMI of 27.0±4.4 (26.9±4.5 in group 1 and 27.0±4.4 in group 2). No statistically significant change in BMI was observed at follow-

up after 2 months (27.0±4.3) or 1 year (26.8±5.2) after *H. pylori* eradication (p=0.910; Table II). There was no statistically significant change in BMI between the two treatment groups (p=0.821), as shown in Table III.

Waist circumference at baseline was 92.8±11.8 cm (group 1: 91.6±12 cm; group 2: 93.7±11.8 cm). As shown in Table II, there were no significant changes at 2 months (92.6±12 cm) or 1 year (93.7±11.8 cm) after *H. pylori* eradication (p=0.325). During follow-up, there were no significant changes in the treatment groups (p=0.737), as shown in Table III.

Table I. Patient characteristics

	All patients (N=62)	Group 1 (N=27)	Group 2 (N=35)	p
Gender (male/female)	29/33	14/13	15/20	0.482
Age (years)	47.9 ± 13.1	46.6 ± 15.8	49.0 ± 10.7	0.476
Smoking, n (%)	17 (27.4)	10 (37.0)	7 (20.0)	0.136
Body mass index	27.0 ± 4.4	26.9 ± 4.5	27.0 ± 4.4	0.894
Dyslipidaemia therapy, n (%)	6 (9.7)	3 (11.1)	3 (8.6)	0.737
Antihypertensive therapy, n (%)	14 (22.6)	5 (18.5)	9 (25.7)	0.502
Framingham score, n (%)				0.313
Low	35 (56.5)	16 (59.3)	19 (54.3)	
Median	9 (14.5)	2 (7.4)	7 (20.0)	
High	17 (27.4)	9 (33.3)	8 (22.9)	
Comorbidities, n (%)	22 (35.5)	10 (37.0)	12 (34.3)	0.822
Arterial hypertension	14 (22.6)	5 (18.5)	9 (25.7)	0.502
Dyslipidaemia	6 (9.7)	3 (11.1)	3 (8.6)	0.737
Depression	1 (1.6)	0	1 (2.9)	0.376
Arrhythmia (palpitations)	2 (3.2)	1 (3.7)	1 (2.9)	0.852
Hypothyroidism	2 (3.2)	2 (7.4)	0	0.102
Benign prostatic hyperplasia	1 (1.6)	0	1 (2.9%)	0.376

**Table II.** Changes in various parameters over time in the entire cohort

	T1 (n=62)	T2 (n=62)	T3 (n=62)	p-value between times
Body mass index	27.0 ± 4.4	27.0 ± 4.3	26.8 ± 5.2	0.910
Waist circumference (cm)	92.8 ± 11.8	92.6 ± 12.0	93.7 ± 11.8	0.325
Antihypertensive therapy, n (%)	14 (22.6)	14 (22.6)	14 (22.6)	1.000
Dyslipidaemia therapy, n (%)	6 (9.7)	7 (11.3)	7 (11.3)	0.321
HOMA-IR	2.3 ± 2.1	2.2 ± 1.4	2.0 ± 1.1	0.342
Cholesterol (mg/dl)	212.1 ± 39.5	200.3 ± 40.4	197.3 ± 41.9	0.004
LDL (mg/dl)	131.9 ± 34.0	124.1 ± 37.0	120.2 ± 35.4	0.011
HDL (mg/dl)	56.1 ± 15.5	54.0 ± 14.7	55.2 ± 15.3	0.159
Triglycerides (mg/dl)	141.3 ± 74.7	136.8 ± 63.1	135.8 ± 76.6	0.686
LDL-p (nmol/l)	1588.1 ± 412.9	1532.3 ± 465.4	1483.0 ± 441.4	0.066
SLDL-p (nmol/l)	604.7 ± 334.0	552.0 ± 351.4	542.7 ± 351.9	0.029
LDL.C-c (mg/dl)	5.5 ± 2.9	5.4 ± 2.6	5.4 ± 3.0	0.876
LDL-s (nm)	21.1 ± 0.6	21.1 ± 0.6	21.1 ± 0.6	0.985
HDL-s (nm)	8.9 ± 0.6	8.9 ± 0.6	8.8 ± 1.3	0.488
HDL-p (nmol/l)	37657.4 ± 4943.8	36347.5 ± 5415.8	36719.7 ± 4761.0	0.047
TMAO (mg/l)	38.3 ± 29.9	29.2 ± 18.8	31.5 ± 30.4	0.050
TMAO (mmol/mol creatinine)	47.3 ± 42.9	47.7 ± 26.3	45.5 ± 31.9	0.915

T1 – at baseline; T2 – 2 months post-eradication; T3 – 1-year post-eradication; LDL: low-density lipoproteins; HDL: high-density lipoproteins; LDL-p: low-density lipoprotein particles; SLDL-p: small low-density lipoprotein particles; LDL.C-c: cholesterol in small dense lipoprotein particles; LDL-s: diameter of low-density lipoprotein particles; HDL-s: diameter of high-density lipoprotein particles; HDL-p: high-density lipoprotein particles; TMAO: trimethylamine N-oxide (measured in urine).

Arterial hypertension, requiring treatment with antihypertensive drugs, was present in 22.6% (14/62) of patients at baseline and remained unchanged during follow-up ( $p=1.000$ ).

Dyslipidaemia requiring statin therapy was present in 9.7% (6/62) of patients at baseline. During follow-up, one additional patient received long-term statin therapy (7/62; 11.3%,  $p=0.321$ ), as shown in Table III.

The Framingham risk score for CHD decreased following successful eradication of *H. pylori* in both groups over 1 year (from 14.7±11% at baseline to: 13.4±10.8% after 1 year in group 1 and from 14.7±11% to 12.1±9.5% after 1 year in group 2), although these changes were not statistically significant ( $p=0.076$ ; Table III).

Total cholesterol levels decreased significantly following successful *H. pylori* eradication ( $p=0.004$ ). In group 1, baseline total cholesterol was 206.4±45.5 mg/dl (optimal value < 200 mg/dl), decreasing to 192.7±44.3 mg/dl at 2 months and 187.5±44.0 mg/dl at 1 year. Similar trends were observed in group 2 (baseline: 216.5±34.1 mg/dl, 2 months: 206.4±36.7 mg/dl, 1 year: 204.9±39.2 mg/dl).

Low dense lipoprotein (LDL) levels also decreased statistically significantly post-eradication ( $p=0.011$ ). In group 1, baseline LDL values decreased from 129.6±37.7 mg/dl (optimal value < 130 mg/dl) to 122.0±39.3 mg/dl at 2 months and 115.8±36.9 mg/dl at 1 year. Similar trends were observed in group 2 (baseline: 133.8±31.3 mg/dl, 2 months: 125.7±35.6 mg/dl, 1 year: 123.7±34.3 mg/dl).

Further analysis revealed a statistically significant reduction in small dense lipoprotein particles (SLDL-p), which are generally associated with CVD ( $p=0.029$ ). Baseline values

decreased from 604.7±334.0 nmol/l (optimal value < 500 nmol/l) to 552.9±351.4 nmol/l at 2 months and 542.7±351.9 nmol/l at 1 year. This trend persisted when analysing both treatment groups ( $p=0.037$ ) and the entire cohort over time ( $p=0.029$ ; Tables II and III, Fig. 2A–C).

Insulin resistance, assessed using the homeostatic model assessment of insulin resistance (HOMA-IR) score, showed a non-significant reduction over time (baseline: 2.3± 2.1, 2 months: 2.2±1.4, 1 year: 2.0±1.1;  $p=0.342$ ; Fig. 2D; Table II).

Trimethylamine N-oxide concentrations decreased after successful eradication of *H. pylori* ( $p=0.048$ , Table III). Baseline TMAO levels were 40.1±22.1 mg/l in group 1 and 36.9±35.0 mg/l in group 2, decreasing to 36.0±30.2 mg/l and 28.0±30.5 mg/l, respectively, after 1 year (Table II). However, although TMAO levels decreased after eradication therapy ( $p=0.048$ ), this association was marginal and lost significance after adjusting for creatinine ( $p=0.915$ ), reflecting the substantial baseline variability in TMAO concentrations.

## DISCUSSION

This study analysed the long-term impact of *H. pylori* eradication on various CVD risk factors. Up till now some studies and meta-analyses were performed to evaluate the association between chronic *H. pylori* infection and CVD. Specifically, Fang et al. [20] conducted a meta-analysis of 48 studies, encompassing 7,522 cases and 8,311 controls, and reported that patients with chronic *H. pylori* infection had a higher risk of acute coronary syndrome [odds ratio (OR)=2.03, 95%CI: 1.66–2.47] than the controls.

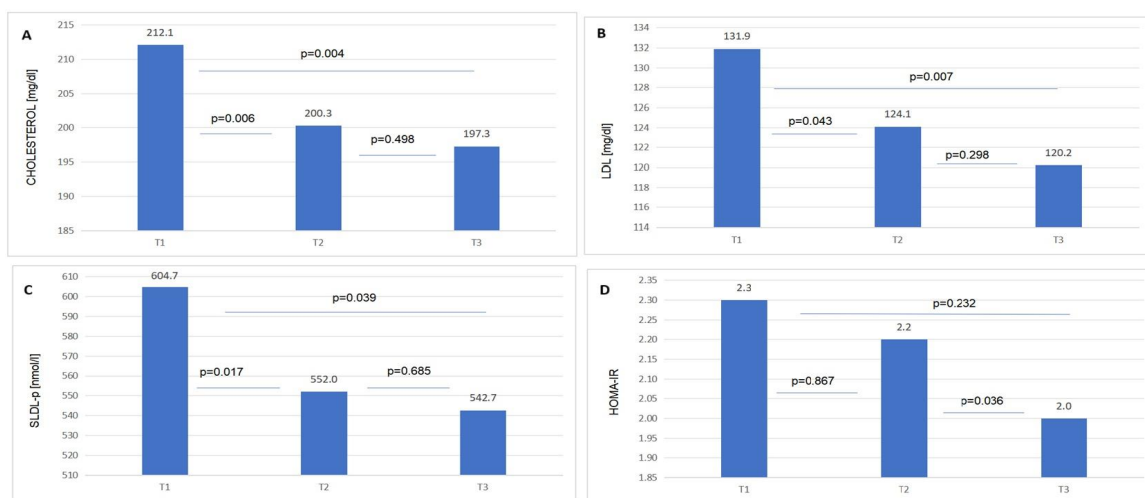
**Table III.** Changes in various parameters over time between both groups

	T1		T2		T3		p-value between times	p-value between factor subjects x time
	Group 1 (n=27)	Group 2 (n=35)	Group 1 (n=27)	Group 2 (n=35)	Group 1 (n=27)	Group 2 (n=35)		
Body mass index	26.9 ± 4.5	27.3 ± 4.3	26.7 ± 4.1	27.4 ± 4.0	27.0 ± 3.8	27.4 ± 4.2	0.799	0.821
Waist circumference (cm)	91.6 ± 12.0	93.7 ± 11.8	91.9 ± 12.6	93.2 ± 11.7	93.2 ± 11.4	94.1 ± 12.2	0.305	0.737
Antihypertensive therapy	5 (18.5%)	9 (25.7%)	5 (18.5%)	9 (25.7%)	5 (18.5%)	9 (25.7%)	1.000	1.000
Dyslipidaemia therapy	3 (11.1%)	3 (8.6%)	3 (11.1%)	4 (11.4%)	3 (11.1%)	4 (11.4%)	0.769	0.769
Systolic blood pressure	140.4 ± 17.3	144.0 ± 16.1	143.3 ± 19.4	142.5 ± 17.7	141.6 ± 17.5	139.0 ± 13.6	0.302	0.097
HOMA-IR	2.6 ± 3.0	2.1 ± 1.0	2.4 ± 1.9	2.1 ± 0.9	1.9 ± 1.2	2.0 ± 1.0	0.275	0.484
Creatinine (qmol/l)	75.6 ± 20.6	72.6 ± 11.8	76.9 ± 21.6	70.6 ± 11.9	77.5 ± 18.2	70.6 ± 11.9	0.912	0.070
Urea (mmol/l)	4.9 ± 1.9	4.9 ± 1.3	5.2 ± 2.1	5.0 ± 1.4	5.1 ± 2.0	4.9 ± 1.6	0.477	0.655
Cholesterol (mg/dl)	206.4 ± 45.5	216.5 ± 34.1	192.7 ± 44.3	206.4 ± 36.7	187.5 ± 44.0	204.9 ± 39.2	0.003	0.730
LDL (mg/dl)	129.6 ± 37.7	133.8 ± 31.3	122.0 ± 39.3	125.7 ± 35.6	115.8 ± 36.9	123.7 ± 34.3	0.010	0.003
HDL (mg/dl)	53.2 ± 16.3	58.4 ± 14.6	49.4 ± 13.8	57.5 ± 14.8	51.0 ± 14.8	58.5 ± 15.1	0.124	0.439
Triglycerides (mg/dl)	142.3 ± 64.3	140.5 ± 83.0	132.7 ± 53.4	140.1 ± 70.5	128.8 ± 56.5	141.3 ± 89.7	0.622	0.572
LDL-p (nmol/l)	1569.5 ± 441.1	1602.8 ± 395.6	1525.8 ± 462.8	1537.5 ± 474.5	1429.9 ± 424.2	1524.8 ± 456.6	0.055	0.626
SLDL-p (nmol/l)	605.7 ± 335.6	603.9 ± 338.4	572.8 ± 312.8	534.7 ± 385.1	560.3 ± 337.3	527.8 ± 368.5	0.037	0.737
LDL.C-c (mg/dl)	5.3 ± 2.2	5.7 ± 3.3	5.1 ± 2.3	5.5 ± 2.9	5.0 ± 2.2	5.7 ± 3.6	0.869	0.873
LDL-s (nm)	21.1 ± 0.5	21.1 ± 0.6	21.0 ± 0.4	21.2 ± 0.7	21.1 ± 0.5	21.2 ± 0.6	0.990	0.692
HDL-s (nm)	8.9 ± 0.6	9.0 ± 0.6	8.9 ± 0.6	9.0 ± 0.6	8.9 ± 0.5	8.8 ± 1.7	0.582	0.447
HDL-p (nmol/l)	36387.9 ± 5101.5	38657.5 ± 4650.5	34212.1 ± 3995.8	38029.8 ± 5836.5	34793.5 ± 4125.0	38237.3 ± 4733.0	0.031	0.331
TMAO (mg/l)	40.1 ± 22.1	36.9 ± 35.0	28.8 ± 19.2	29.5 ± 18.7	36.0 ± 30.2	28.0 ± 30.5	0.048	0.520
TMAO mmol/mol creatinine	44.8 ± 32.5	49.3 ± 49.8	39.9 ± 15.3	53.6 ± 31.3	43.9 ± 17.5	46.7 ± 39.8	0.946	0.589
Framingham score [%] <sup>‡</sup>	14.7 ± 11.0	14.7 ± 11.0	14.0 ± 11.3	11.3 ± 10.0	13.4 ± 10.8	12.1 ± 9.5	0.076	0.353

<sup>‡</sup>Framingham score (1 – low risk, 2 – moderate risk, 3 – high risk). For abbreviations see Table II.

The proposed mechanism linking chronic *H. pylori* infection to an increased risk of CVD is chronic inflammation

mediated by oxidative stress and immune responses that may potentially lead to endothelial dysfunction [21, 22].



**Fig. 2.** Total cholesterol, low density lipoprotein (LDL), small low density lipoprotein particles (SLDL-p) and insulin resistance (HOMA-IR) levels following *H. pylori* eradication. T1: at baseline; T2: 2 months post-eradication; T3: 1-year post-eradication.

In addition, direct effects of the microorganism on atherosclerotic plaque have also been proposed, along with its influence on lipid metabolism, including reduction in high density lipoprotein (HDL) levels and oxidation of LDL [15, 23–25]. Sun et al. [18] conducted an analysis of cohort studies that accounted for potential confounding factors and found a significant association between *H. pylori* and CVD risk, although the relative risk (RR) was low (1.10, 95%CI: 1.03–1.18). One possible explanation for this relatively low risk estimate could be the use of serology as a diagnostic test for determining the *H. pylori* status, as it is known that the presence of IgG cannot differentiate between past and active *H. pylori* infections. However, a stronger association was observed in patients infected with CagA-positive *H. pylori* strains (RR=1.58, 95%CI: 1.03–2.41).

Dyslipidaemia, a well-established risk factor for CVD, is influenced by variations in serum lipoprotein composition, including both small, dense lipoprotein particles and larger, less dense lipoproteins particles, which have differing atherogenic potential [26, 27]. Numerous studies have demonstrated that it is mainly the SLDL-p within the LDL fraction that exhibit a strong association with CVD risk [28–30]. A recent meta-analysis reported an increase in HDL levels following *H. pylori* eradication, while LDL levels remained largely unchanged [31]. Conversely, another study found that *H. pylori*-infected patients exhibited higher LDL and lower HDL levels [32].

In the present study, CVD risk factors were analysed following successful *H. pylori* eradication with a one-year follow-up. No significant changes in BMI and waist circumference were found, which differs from some previous findings [33–35]. On the other hand, a significant decrease in total cholesterol, LDL and SLDL-p was noted in both groups after eradication, with a sustained decline over the follow-up period. Since only one additional patient received statin therapy during the study, this factor is unlikely to explain the observed lipid profile changes. The divergence between our findings and previous meta-analysis likely reflects methodological differences. In the meta-analysis by Watanabe et al. [31], most included studies had heterogeneous diagnostic approaches, limited adjustment for comorbidities affecting lipid metabolism, short follow-up intervals (18 out of 24 included studies had follow-up <1 month), and inconsistent reporting or low rates of eradication success. Such factors constrain the interpretation of lipid changes after therapy. In contrast, our study employed uniform diagnostic criteria, using NMR spectroscopy, confirmed eradication, excluded conditions known to alter lipid profiles, and assessed outcomes at one year. These differences in study design may explain the reductions in total and LDL cholesterol observed in our cohort.

A plausible explanation for the decrease in LDL levels and SLDL-p levels after eradication is a reduction in chronic inflammatory burden. Another contributing factor may be the impact of antibiotics on the diversity of the gut microbiome and its metabolic function. For example, bacteria from the *Bacteroidetes* phylum have previously been associated with higher LDL and lower HDL levels, whereas bacteria from the *Firmicutes* phylum have been associated with the opposite effect [36]. Further investigations are needed to evaluate

gut microbiome alterations and potential correlations with lipoprotein profiles.

The pathogenesis of atherosclerosis is multifactorial, with gut microbiome-derived metabolites, including TMAO, playing a significant role [37, 38]. Experimental studies in mice have shown that TMAO promotes atherosclerosis by impairing reverse cholesterol transport, macrophages activation, and increasing platelet activation [39, 40]. Conversely, a reduction in TMAO production has been shown to mitigate atherosclerotic plaque formation [41]. Clinical studies have reported significantly elevated serum TMAO levels in patients with coronary atherosclerosis compared to those with normal coronary arteries [42]. A meta-analysis of three studies involving 923 high-risk CVD patients showed that elevated TMAO levels were associated with an increased incidence of major cardiovascular events (RR=2.05; 95%CI: 1.61–2.61) [43]. Consistent with these findings, our study demonstrated a decrease in urinary TMAO levels following *H. pylori* eradication. However, interpretation of TMAO findings requires caution. Baseline TMAO values in our cohort exhibited considerable variability (mainly due to different patients diets), and the observed post-eradication reduction was statistically marginal and not maintained after adjustment for creatinine. These factors suggest that the association between *H. pylori* eradication and TMAO levels may be unstable, and larger studies are needed to clarify this relationship.

This study was designed as a prospective, randomised trial without dietary or drug interventions during the follow-up period. However, the main limitation of our study is the relatively small sample size, so it may have been underpowered to detect small metabolic effects, raising the possibility of type II error for negative findings. Given that participants were symptomatic, withholding *H. pylori* eradication therapy for the purpose of a control group was deemed ethically inappropriate. On the other hand, we analysed different atherosclerosis risk factors as has not been done before, and we have a one-year follow-up period for all patients. Furthermore, as several metabolic parameters were evaluated, the possibility of type I error for marginally significant associations (as for TMAO) cannot be excluded. These results should therefore be interpreted with appropriate caution and confirmed in larger studies.

In our study, reductions in several cardiometabolic markers, including total cholesterol, LDL, and SLDL-p levels, were observed following eradication therapy. Although these findings are consistent with some previous reports, the study design does not allow us to determine whether these changes were driven specifically by *H. pylori* eradication, exposure to antibiotics, or other unmeasured factors, as all participants received eradication therapy and no untreated control group was included. Similarly, a small decrease in TMAO, a metabolite increasingly recognized for its role in the development of atherosclerosis, was observed after therapy, but causality cannot be inferred. While no direct correlation between chronic *H. pylori* infection and insulin resistance or body-weight changes has been demonstrated in our cohort, these results contribute to the growing body of evidence suggesting that *H. pylori* infection and its treatment may have systemic effects beyond gastric pathology.

As our study suggested positive impact of *H. pylori* eradication on improved lipid profile, confirmation in a larger randomised study is needed. Considering the global prevalence of *H. pylori* infection and its potential impact on CVD risk factors, routine screening and eradication in high-risk individuals could have significant public health implications beside gastric cancer prevention [44].

## CONCLUSIONS

In our study, successful eradication of *H. pylori* infection was associated with favorable changes in lipid metabolism and marginal decrease in TMAO levels. These findings suggest that *H. pylori* may contribute to CVD risk by modulating lipoprotein profiles and systemic inflammation.

**Conflicts of interest:** None to declare.

**Authors' contributions:** K.T, G.A, R.O. and B.T, conceived the study. K.T, S.A and B.T. designed the methodology of the study. K.T, S.A and B.T. investigated the patients. D.Š. analyzed the data. K.T. drafted the manuscript. K.T, G.A, R.O and B.T. revised the manuscript. B.T. supervised the study. All authors have read and agreed to the published version of the manuscript.

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