Clinica e Investigacion en Arteriosclerosis 36 (2024) 51–59

Sociedad Española de Arteriosclerosis

CLÍNICA E INVESTIGACIÓN EN ARTERIOSCLEROSIS

www.elsevier.es/arterio

ORIGINAL ARTICLE

Evaluation of the relationship between atherosclerosis and *Helicobacter pylori* infection with measurement of growth differentiation factor 15 and atherosclerosis indicators in adults with no comorbidity

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Received 6 July 2023; accepted 19 September 2023

KEYWORDS
GDF-15;
Helicobacter pylori;
Atherosclerosis;
Carotid intima-media thickness

Abstract
Background: The aim of this study was to investigate presence of subclinical atherosclerosis by measuring carotid intima-media thickness (CIMT) in patients with *Helicobacter pylori* (HP) and to assess effects of HP on atherosclerosis by evaluating markers of atherosclerosis and blood growth differentiation factor (GDF-15) levels.
Materials and methods: This cross-sectional study included 59 patients without comorbid disease who had HP and 30 healthy controls without HP in upper endoscopic biopsy. In order to assess atherosclerosis, the CIMT measurement was performed by sonography. Serum GDF-15 level was measured by ELISA method. In all patients, atherosclerosis markers were recorded. Atherogenic indices were calculated, including Castelli risk index I and II (TG/HDL-c and LDL-c/HDL-c, respectively), plasma atherogenic index (PAI; log TG/HDL-c), non-HDL-c (TH-HDL-c) and atherogenic coefficient (AC; non-HDL-HDL-c).
Results: The GDF-15 level and CIMT were significantly higher in HP-positive group when compared to HP-negative group ($p \leq 0.001$). There was a significant correlation between serum GDF-15 level and CIMT ($r = 0.445$; $p \leq 0.001$). There was no correlation between other atherosclerosis markers and serum GDF-15 level or CIMT. The bacterial intensity on endoscopic specimen was only correlated with CIMT ($p < 0.001$). Vitamin B12 and D levels were comparable among groups.

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Conclusion: This study suggested that there was a correlation between GDF-15 level and subclinical atherosclerosis development in patients with HP. However, GDF-15 level, which was found to be elevated while atherogenic indices were normal, can be an earlier marker for subclinical atherosclerosis.

PALABRAS CLAVE
GDF-15;
Helicobacter pylori;
Aterosclerosis;
Grosor íntima-media carotídeo

Evaluación de la relación entre aterosclerosis e infección por *Helicobacter pylori* con medición del factor de diferenciación de crecimiento 15 e indicadores de aterosclerosis en adultos sin comorbilidad

Resumen
Antecedentes: El objetivo de este estudio fue investigar la presencia de aterosclerosis subclínica mediante la medición del grosor íntima-media de la carótida (GIMC) en pacientes con *Helicobacter pylori* y evaluar los efectos de *H. pylori* sobre la aterosclerosis mediante la evaluación de marcadores de aterosclerosis y de niveles de factor de diferenciación del crecimiento sanguíneo (*growth differentiation factor 15* [GDF-15]).
Materiales y métodos: Este estudio transversal incluyó 59 pacientes sin enfermedad comórbida que tenían *H. pylori* y 30 controles sanos sin *H. pylori* en la biopsia endoscópica superior. Para evaluar la aterosclerosis, la medición de GIMC se realizó mediante ecografía. El nivel de GDF-15 en suero se midió mediante el método ELISA. En todos los pacientes se registraron marcadores de aterosclerosis. Se calcularon los índices aterogénicos, incluyendo el índice de riesgo de Castelli I y II (TG/cHDL y cLDL-cHDL, respectivamente), el índice aterogénico plasmático (PAI; log TG/HDL-c), no-cHDL (TH-cHDL) y el coeficiente aterogénico (no-HDL-cHDL).
Resultados: Los niveles de GDF-15 y de GIMC fueron significativamente más altos en el grupo *H. pylori* positivo en comparación con el grupo *H. pylori* negativo ($p \leq 0{,}001$). Hubo una fuerte correlación entre el nivel sérico de GDF-15 y el GIMC ($r = 0{,}445$; $p \leq 0{,}001$). No hubo correlación entre otros marcadores de aterosclerosis y el nivel sérico de GDF-15 o GIMC. La intensidad bacteriana en la muestra endoscópica solo se correlacionó con GIMC ($p \leq 0{,}001$). Los niveles de vitamina B12 y de vitamina D fueron comparables entre los grupos.
Conclusión: Este estudio sugirió que había una correlación entre el nivel de GDF-15 y el desarrollo de aterosclerosis subclínica en pacientes con *H. pylori*. Sin embargo, el nivel de GDF-15, que se encontró elevado mientras que los índices aterogénicos eran normales, puede ser un marcador temprano de aterosclerosis subclínica.

Introduction

Helicobacter pylori (HP) is the most frequently found gram-negative bacillus in human stomach, which is one of the most common chronic infections worldwide.[1] It is known that HP accounts for chronic atrophic gastritis, peptic ulcer, gastric cancer, gastric lymphoma, idiopathic thrombocytopenic purpura and iron deficiency anemia.[3] A series of sero-epidemiological study proposed that there may a correlation between HP and atherosclerosis development.[3] Some studies advocate the hypothesis of a relationship between heat shock proteins and endothelial cells[4] while other studies emphasized presence of HP DNA which act like an inflammation foci in atherosclerotic plaque.[4] In some studies, atherosclerosis development has been limited to virulent genotypes while evidence regarding dyslipidemia was demonstrated in another study.[5]

The recognition of atherosclerosis at early phase is important to prevent potential cardiovascular complications. Measurement of carotid intima-media thickness (CIMT) by sonography is one of the most used methods to assess subclinical atherosclerosis which is an early indicator for atherosclerotic burden. It is considered that CIMT represents early morphological changes of arterial wall caused by many risk factors over a certain period.[6] The smooth muscles cells in media layer undergo proliferation during atherosclerotic process, promoting development of atheroma plaques. CIMT measurement allows identification of changes in arterial wall before onset of atherosclerotic plaque.[7] Thus, degree of atherosclerotic involvement in coronary arteries can be predicted.[8] However, the atherogenic indices, namely Castelli risk index I and II, atherogenic coefficient index, can be used to predict risk for atherosclerotic cardiovascular disease.[9] In addition, growth differentiation

factor-15 (GDF-15) has been shown as a novel parameter for atherosclerosis, which is a member of the transforming growth factor-β (TGF-β) super family.[10] The GDF-15 elevation has been linked to several disorders including glioma, colorectal and pancreas cancer, cardiovascular disorders, multiple myeloma, heart failure and renal failure.[11] Many studies support that elevated GDF-15 level plays an important role in the pathogenesis of atherosclerosis by inducing inflammation and angiogenesis.[12]

The mechanisms which HP trigger atherosclerosis remain unclear.[13] The availability of a novel marker such as GDF-15 can be helpful in clarifying these mechanisms. However, to best of our knowledge, there is no study investigating relationship between blood GDF-15 level and atherosclerosis development in patients with HP infection. The aim of this study was to investigate presence of subclinical atherosclerosis by measuring carotid intima-media thickness (CIMT) in patients with *Helicobacter pylori* (HP) and to assess effects of HP on atherosclerosis by evaluating markers of atherosclerosis and blood growth differentiation factor (GDF-15) levels.

Material and methods

Design and subjects

This cross-sectional study was conducted in a tertiary training and research hospital. The study population included adult patients (aged > 18 years) without comorbid disease who presented to outpatient clinic of Internal Medicine and scheduled for upper gastrointestinal endoscopy between June 2019 and August 2019. Of the patients underwent upper endoscopic biopsy, 59 patients with HP were assigned into the patient group while 30 subjects without HP were employed as healthy controls. The control group was comparable with the patient group regarding age, gender, and body mass index (BMI).

The patients with ischemic cardiac disorder, cerebrovascular disease, hypertension, diabetes mellitus or peptic ulcer; the patients consuming alcohol; smokers, those with hepatic or renal failure; those with history of cancer; those with hyperlipidemia; and those with active infection were excluded. The patients receiving treatment for HP and those with gastric surgery were also excluded.

The study was conducted in accordance with tenets of Helsinki Declaration. The study was approved by Ethics Committee of Kayseri City Training and Research Hospital (Approval Number: 423 Date: 2019)

Diagnosis of HP

The diagnosis of HP was made via endoscopic biopsy performed by experienced gastroenterologists using Fujinon video endoscopy (EG-L580NW, Tokyo, Japan). The biopsy materials were stained by hematoxylin–eosin and modified Giemsa solution and evaluated under light microscope. The preparations were classified as mild (+), intermediate (++) or severe (+++) positivity according to bacterial intensity. Based on biopsy results, intestinal metaplasia, atrophy, chronicity and activity were also classified as mild (+), intermediate (++) or severe (+++) positivity together with biopsy site (corpus, antrum).

Laboratory evaluation and atherosclerotic indicators

Routine blood parameters and lipid profile were studied using standard assays in venous blood samples obtained on the same day with endoscopy after overnight fasting.

Clinical data, demographic characteristics (age, gender), laboratory findings (white blood cell count, neutrophil count, lymphocyte count, monocyte count, hemoglobin, platelet count, mean corpuscular volume, procalcitonin, platelet distribution width, creatinine, aspartate transaminase, alanine transaminase, thyroid stimulating hormone, vitamin B12, vitamin D, C-reactive protein, fasting blood glucose) were recorded. In all patients, blood lipid profile (total cholesterol [TC], triglyceride, high-density lipoprotein cholesterol [HDL-c], low-density lipoprotein cholesterol [LDL-c]) was assessed. The atherogenic indices including Castelli risk index I and II (TC/HDL-c and LDL-c/HDL-c, respectively), plasma atherogenic index (PAI; log TG/HDL-c), non-HDL-c (TC-HDL-c) and atherogenic coefficient (AC; non-HDL-c/HDL-c) were calculated.

In all patients, body weight and height were measured, and body mass index was calculated using following formula: weight (kg)/height (meter).[2]

GDF-15 measurement

From 59 patients and 30 healthy controls, blood samples were drawn on the same day with endoscopy after overnight fasting to study GDF-15 level. The blood samples (approximately 5 mL) were drawn into EDTA tubes. The blood samples were centrifuged as soon as possible, and sera obtained were transferred to Eppendorf tubes and stored at 80 °C. The serum GDF-15 level was measured using Enzyme-Linked Immunosorbent Assay (ELISA), (DY957, R&D Systems, Minneapolis, MN, USA) in accordance with manufacturer's instructions. The test range was 0–5000 pg/mL.

CIMT measurement

The CIMT measurement was performed by an experienced radiologist on the same day as post-endoscopy and radiologist was blinded to the results of the upper gastrointestinal endoscopy. The CIMT was measured at supine position with slight cervical extension using high-resolution 7.5 MHz linear probe (Hitachi EUB 6500, Osaka, Japan). The measurements were performed at a point 10-mm away from internal carotid artery and carotid artery bifurcation using 2-dimensional sonography. To minimize effects of arterial compliance on results, cardiac monitorization and peak-R wave coupling (for correlation with each phase of cardiac cycle) were used. Measurements were made at 3 different sites in each session on both side. Mean CIMT was defined average of 12 measurements obtained in 2 different sessions. The CIMT $\geq$ 0.9 mm was defined as abnormal.

Table 1 Laboratory measurements and atherosclerosis indicators of patients who were *Helicobacter pylori* positive and negative.

	Helicobacter pylori positive $n = 59$	*Helicobacter pylori* negative $n = 30$	p
Body mass index (kg/m^2)	25.6 (23–28)	24.9 (23–27)	0.284
GDF-15 (pg/mL)	5435 (2429–7825)	945 (714–1258)	**<0.001**
White blood cell count ($\times 10^9$/L)	7.1 (6.2–8.5)	7.2 (6.4–8.6)	0.818
Hemoglobin (g/dl)	13.2 (12.7–15.5)	13.6 (12.0–15.2)	0.965
Platelet ($\times 10^3/\mu$L)	253 (218–297)	284 (224–343)	0.115
LDL-C (mg/dL)	95.0 (75.0–118)	91.5 (80.7–114)	0.983
Triglycerides (mg/dL)	112 (73.0–161)	116 (79.2–180)	0.512
HDL-C (mg/dL)	46.0 (38.0–55.2)	44.5 (37.7–53.0)	0.598
Total cholesterol (mg/dL)	167 (142–193)	162 (143–193)	0.948
Fasting plasma glucose (mg/dL)	88.0 (83.0–93.0)	86.0 (80.0–95.5)	0.611
Creatinine (mg/dL)	0.70 (0.63–0.81)	0.74 (0.61–0.82)	0.485
Aspartate aminotransferase (IU/L)	17.0 (14.0–19.0)	17.0 (13.7–22.0)	0.917
Alanine aminotransferase (IU/L)	15.0 (13.0–21.0)	15.5 (12.5–23.2)	0.951
Thyroid stimulating hormone	1.6 (1.2–2.1)	1.7 (1.3–2.4)	0.617
Vitamin B12 (μg/d)	282 (226–339)	296 (211–353)	0.709
Vitamin D (μg/d)	14.0 (10.0–19.6)	20.6 (13.5–21.0)	0.125
C-reactive Protein (mg/L)	1.5 (0.6–3.0)	1.2 (0.5–2.8)	0.524
CIMT (mm)	0.66 (0.48–0.75)	0.44 (0.40–0.47)	**<0.001**
Castelli index 1	3.66 (3.03–4.30)	3.84 (3.27–4.35)	0.516
Castelli index 2	2.18 (1.53–2.60)	2.12 (1.77–2.65)	0.661
Non-HDL-C	126.5 (91–148)	111 (103–156)	0.926
AC	2.66 (2.03–3.30)	2.84 (2.27–3.35)	0.516
PAI	0.38 (0.20–0.57)	0.43 (0.15–0.67)	0.588

Data are expressed as median (including the lower and upper quartiles). GDF-15: growth differentiation factor 15, LDL-C: low-density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, CIMT: carotid intima-media thickness, PAI: atherogenic index of plasma, AC: atherogenic coefficient.
The significant values were provided as bold.

Statistical analysis

For estimating the sample size, a preliminary study including 5 patients per group was performed. According to intermediate evaluation with power analysis, a sample size of 30 patients per group was obtained. Considering the possible dropouts in HP-positive group, the final sample size was determined to be at least 50 patients. Data are expressed as the mean $\pm$ standard deviation (SD) or the median (including the lower and upper quartiles). The normality and the homogeneity of the data were evaluated by Shapiro–Wilk test and Levene test, respectively. Comparisons among the groups for continuous variables were performed by using the Student *t*-test or one-way ANOVA for normal distribution and the Mann–Whitney *U* test or Kruskal–Wallis test for non normal distribution. Fisher test or the χ^2 test was used for all categorical data. Spearman's correlation was used to evaluate the association of continuous variables. All calculations used the SPSS statistical package (version 15.0; SPSS, Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

In the study, 59 patients positive for HP and 30 healthy subjects negative for HP were assessed. Twenty-two (37.3%) of patients with HP were male while 11 (36.7%) of subjects without HP were male. Mean age was 34.8 ± 8.4 years in patients with HP whereas 36.1 ± 7.2 years in the subjects without HP. There was no significant difference in age and gender between groups ($p = 0.501$ and $p = 0.954$).

In all patients, biopsy materials were obtained from gastric antrum. In the biopsy material from patients with HP, mild positivity (+) was detected in 20 patients whereas intermediate positivity (++) in 20 patients and severe positivity (+++) in 19 patients. Intestinal metaplasia was detected in only one patient whereas mild (+) in 7 patients and intermediate atrophy (++) in one patient. The chronicity was mild (+) in 19 patients, intermediate (++) in 26 patients and severe (+++) in 11 patients. In addition, there was mild activity (+) in 15 patients, intermediate activity (++) in 22 patients and severe activity (+++) in 8 patients.

Table 1 presents laboratory measurements and atherosclerosis markers in patients with and without HP. Median GDF-15 level was significantly higher in HP positive group when compared to HP negative group (5435 vs. 945; $p < 0.001$). There was no significant difference in body mass index and lipid profile (LDL-C, triglycerides, HDL-C, total cholesterol) between groups ($p > 0.05$). Again, atherogenic indices (Castelli index 1, Castelli index 2, Non-HDL-C, AC, PAI) were comparable between groups. The median CIMT measurements were significantly higher in HP

Table 2 Correlation of GDF-15 and CIMT with age, laboratory measurements and atherosclerosis indicators.

	GDF-15		CIMT	
	r	*p*	*r*	*p*
GDF-15	–	–	0.427	**<0.001**
Body mass index	0.346	**0.001**	0.226	**0.033**
Age	−0.179	0.093	0.292	**0.006**
White blood cell count ($\times 10^9$/L)	−0.032	0.763	0.034	0.749
Hemoglobin (g/dl)	−0.012	0.909	0.055	0.611
Platelet ($\times 10^3/\mu$L)	−0.134	0.210	−0.128	0.231
LDL-C (mg/dL)	−0.011	0.921	0.102	0.342
Triglycerides (mg/dL)	−0.113	0.294	−0.050	0.640
HDL-C (mg/dL)	−0.002	0.983	0.124	0.263
Total cholesterol (mg/dL)	−0.047	0.659	0.104	0.331
Fasting plasma glucose (mg/dL)	−0.031	0.774	0.050	0.645
Creatinine (mg/dL)	0.023	0.832	−0.011	0.916
Aspartate aminotransferase (IU/L)	0.072	0.505	0.069	0.519
Alanine aminotransferase (IU/L)	0.060	0.577	0.081	0.451
Thyroid stimulating hormone	0.001	0.994	−0.028	0.802
Vitamin B12 (μg/d)	−0.033	0.771	0.112	0.324
Vitamin D (μg/d)	−0.030	0.870	−0.219	0.228
C-reactive protein (mg/L)	0.071	0.582	0.146	0.253
Castelli index 1	−0.048	0.667	−0.023	0.837
Castelli index 2	−0.020	0.859	−0.030	0.785
Non-HDL-C	−0.043	0.695	−0.097	0.382
AC	−0.048	0.667	−0.023	0.837
PAI	−0.060	0.587	−0.077	0.487

GDF-15: growth differentiation factor 15, LDL-C: low-density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, CIMT: carotid intima-media thickness, PAI: atherogenic index of plasma, AC: atherogenic coefficient.
The significant values were provided as bold.

Table 3 Evaluation of GDF-15 and atherosclerosis indicators according to bacterial density in endoscopic biopsy material.

	+	++	+++	*p*
	n = 20	*n* = 20	*n* = 19	
GDF-15 (pg/mL)	3981 (2422–6118)	6501 (3732–8357)	5623 (1973–8011)	0.068
CIMT (mm)	0.49 (0.44–0.64)	0.60 (0.51–0.71)	0.77 (0.67–0.86)	**<0.001**
Castelli index 1	3.54 (3.02–4.04)	3.50 (2.91–3.88)	4.14 (3.25–4.55)	0.163
Castelli index 2	2 (1.31–2.46)	2.09 (1.37–2.37)	2.43 (1.90–2.93)	0.145
Non-HDL-C	128 (79–136)	113 (87.5–142.5)	134 (104–148)	0.300
AC	2.54 (2.02–3.04)	2.50 (1.91–2.88)	3.14 (2.25–3.55)	0.163
PAI	0.33 (0.12–0.55)	0.40 (0.23–0.58)	0.41 (0.23–0.57)	0.752

Data are expressed as median (including the lower and upper quartiles). GDF-15: growth differentiation factor 15, HDL-C: high density lipoprotein cholesterol, CIMT: carotid intima-media thickness, PAI: atherogenic index of plasma, AC: atherogenic coefficient.
The significant values were provided as bold.

positive patients than those in HP negative patients (0.66 vs. 0.44; $p \leq 0.001$). No significant difference was detected in remaining blood parameters between groups ($p > 0.05$).

Table 2 presents correlation of CIMT, and GDF-15 level with age, laboratory measurements and atherosclerosis markers. Neither CIMT nor GDF-15 levels showed correlation with atherogenic indices (Castelli index 1, Castelli index 2, Non-HDL-C, AC, PAI) and lipid profile (LDL-C, triglycerides, HDL-C, total cholesterol) ($p > 0.05$); however, there was a marked correlation between GDF-15 and CIMT ($r = 0.44$; $p \leq 0.001$). Furthermore, there was a significant positive weak correlation between body mass index and GDF-15 and CIMT. Age also had a positive weak correlation with CIMT. We found no significant correlation between remaining blood parameters and GDF-15 or CIMT ($p > 0.05$).

Table 3 presents comparison of atherosclerosis markers and GDF-15 levels according to bacterial intensity in endoscopic biopsy material. A significant increase was observed in median CIMT value by increasing bacterial intensity in groups with mild, intermediate and severe positivity (0.49 mm, 0.60 mm and 0.77 mm, respectively; $p < 0.001$). Remaining parameters were comparable among groups ($p > 0.05$). However, albeit not significant, an

incremental trend was present in GDF-15 by increasing bacterial intensity.

Discussion

In this study, we evaluated the relationship between HP infection and atherosclerosis development by serum GDF-15 level and atherosclerosis markers. Our results showed that HP-positive patients had higher serum GDF-15 and CIMT. In addition, there was a significant correlation between GDF-15 and CIMT; however, no correlation was found between remaining atherosclerosis markers and GDF-15 or CIMT level. We observed that CIMT level was significantly increased by increasing bacterial intensity in biopsy material. Similarly, there was an incremental trend in GDF-15 levels by increasing bacterial intensity at near-significance level, suggesting that the trend would be significant in a larger sample.

In epidemiological studies, it was shown that there is a relationship between HP infection and atherosclerosis.[14] Although many hypotheses have been proposed about role HP in the development of atherosclerosis, it is controversial which mechanisms are involved in the atherosclerosis process.[3–5] HP causes release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-16, IL-8 and γ-interferon by infiltrating gastric epithelium. It has been proposed that these cytokines can play role in the pathogenesis of atherosclerosis by injuring vessel wall via chronic inflammation.[15] A series of in vitro study reported HP-related endothelial injury via reduced angiogenesis, decreased proliferation and high apoptosis rate.[16]

In clinical trials, it was shown that GDF-15 level was increased in patients with vascular dysfunction and atherosclerosis and in those developing atherosclerosis complications later.[17] The GDF-15 appears to be a stress-derived cytokine that reflects damage in several tissues including heart and vasculature.[18] The GDF-15 has pro-atherogenic effects on vascular system through LDL oxidation. The alterations in lipoproteins induced by cytokines predispose patients to atherosclerosis in an indirect manner.[19] In a study by Deepa M. Gopal et al. elevated GDF-15 levels found to be associated with subclinical atherosclerosis.[20] In the context of metabolic effects, the GDF-15 can act as an adipokine; thus, it may contribute inflammation via transcription factor p53.[21]

In our study, elevated serum GDF-15 levels were detected in patients with HP. However, this is the first study assessing atherosclerosis development by GDF-15 in patients with HP. It is known that GDF-15 expression is regulated by several pro-inflammatory cytokines including IL-1β, TNF-α, IL-2 and macrophage colony stimulating factor (M-CSF).[22] The fact that cytokines responsible for endothelial dysfunction also play role in GDF-15 expression[23] may explain elevated blood GDF-15 levels in case of endothelial injury. Given that HP induces release of pro-inflammatory cytokine, it may contribute to GDF-15 expression. Our results demonstrated that HP can be associated with endothelial dysfunction through cytokine release by the finding of elevated GDF-15 level in these patients. In addition, they also support the previous evidence indicating that HP may play role in the development of atherosclerosis by inducing vascular injury.[15] There is limited data regarding pathophysiological function of GDF-15 at molecular level; thus, GDF-15 can be a novel parameter that may shed light on uncertainties regarding atherosclerosis development in HP infected patients.

CIMT is a good and inexpensive marker to assess subclinical atherosclerosis by measuring thickness of intima and media layers.[24] In our study, CIMT was higher in HP-positive patients when compared to HP-negative patients, indicating presence of subclinical atherosclerosis in HP-positive patients. Our findings were consistent with previous findings[25]; however, our findings better demonstrate relationship between HP and atherosclerosis due to younger age and lack of atherosclerotic comorbidities such as diabetes mellitus in our study population. On the other hand, there was a strong correlation between GDF-15 level and CIMT. Our data are consistent with previous findings indicating a relationship between CIMT and GDF-15.[21] However, this is a novel finding in patients with HP infection and GDF-15 can play an important role in the assessment of subclinical atherosclerosis in HP-positive patients. According to our results, CIMT and GDF 15 are two complementary parameters in evaluating atherosclerosis and GDF-15 has additional utility to elucidate the mechanism of atherosclerosis in HP-positive patients. Since the CIMT of all patients in our study was within the normal limits, the results we obtained should be evaluated carefully. Although these normal CIMT values are due to the fact that all of our patients were young and did not have any comorbiditiy, we think that higher CIMT values are valuable in this young patient population.

Atherogenic dyslipidemia is defined as elevated TG and normal and/or elevated LDL-C levels with decreased HDL-C levels. The individual levels of TG, HDL-C and LDL-C not always reflect general cardiovascular risk.[26] In our study, LDL-C, triglyceride, HDL-C and total cholesterol levels were comparable among HP-positive and -negative patients. In some studies, a positive correlation has been proposed between lipid levels and degree of mucosal inflammation.[2] Although it was shown that HP infection is associated with dyslipidemia in some studies on different populations, it remains to be unclear.[14] Our study may contribute to literature.

When compared to individual lipid parameters, comprehensive atherogenic indices such as PAI, non-HDL-C, Castelli index 1, Castelli index 2 and AC are considered to be better predictors of cardiovascular disease (CVD). In our study, there was no significant difference between patient and controls groups regarding atherogenic indices without significant association between atherogenic indices and CIMT in the correlation analysis. These findings are inconsistent with previous studies on association between atherogenic indices and subclinical atherosclerosis or CIMT.[27] In our study, CIMT values were within normal range for all patients, which may indicate earlier stage of atherosclerosis in our patients. This implies that atherogenic indices may be helpful in later stages of atherosclerosis. Cheah WL et al. suggested these parameters can be reliable for CVD risk only with elevated triglyceride concentration.[28] The predictive value of these parameters showed variability across studies representing different populations.[29] There are published studies demonstrating that race/ethnicity and adipose distribution largely affect lipid profile.[30] In a study including patients with CVD and healthy controls, it was shown that elevated PAI was present in multiple vessel involvement but not in those with

single-vessel involvement.[31] In the literature, there is no study which definitively showed HP positivity and atherogenic indices. However, the finding that GDF-15 level was increased and showed correlation with CIMT while remaining atherogenic indices were normal suggests that GDF-15 can be an earlier marker for subclinical atherosclerosis when compared to other parameters.

There are controversial results in the studies investigating effects of vitamin on atherosclerosis development resulting from HP. Recent studies have proposed that B12 deficiency can play role in the pathogenesis of atherosclerosis in association with hyper-homocysteinemia.[32] The role of vitamin D in atherogenesis is explained with favorable effects on functions of circulating lipoproteins.[33] Although there are studies proposing that supplementation with vitamin B12 and/or D can slow down subclinical atherosclerosis, there is no definitive evidence that these vitamins can lead regression of available lesions.[34] In our study, no significant difference was found in vitamin B12 and D levels between HP-positive and HP-negative groups.

CRP plays role in atherogenesis by increasing production of intercellular adhesion molecule 1 (ICAM-1) and monocyte chemoattractan protein 1 (MCP-1).[34] In a study investigating HP-related atherosclerosis, the major increase in hsCRP was linked to infection itself rather than atherosclerosis and no significant increase was detected in hsCRP in atherosclerotic patients when compared to controls.[35] In our study, CRP levels were comparable in HP-positive and HP-negative patients in agreement with literature.

In the literature, there is limited number of studies assessing atherosclerosis according to bacterial intensity in patients with HP. In a study on monkeys infected with HP, a strong correlation was found between gastric bacterial intensity and bacterial intensity in the coronary artery plaques.[36] We observed that CIMT value was increased by increasing bacterial intensity in biopsy material in the histological examination of gastric mucosa. Additionally, an incremental trend was present in GDF-15 by increasing bacterial intensity. This finding supports the accelerated atherosclerosis by increased bacterial intensity.

This study has some limitation. It has relatively small sample size. Furthermore, the cross-sectional, single-center design limits generalizability of the results and it is not possible to attribute a causal relationship between GDF-15 and atherosclerosis due to the characteristics of the study. We did not assess status of nutrition and physical activity level which can be additional cardiovascular risk factors for these patients. The younger age and lack of atherosclerotic disorders are strengths of our study.

Conclusion

This study reveals that HP can be associated with endothelial dysfunction through cytokine release by the finding of elevated GDF-15 level in these patients. Thus, presence of correlation between GDF-15 level and CIMT suggest that cytokine-related endothelial injury may have role in atherosclerotic process. In addition, GDF-15 level can be a potential early marker for subclinical atherosclerosis since it was elevated while remaining atherosclerotic indices were normal. However, our results should be interpreted with caution, since CIMT was normal in all patients and atherosclerotic indices did not differ significantly in HP-positive patients. Our study makes important contribution in topic of relationship between atherosclerosis and vitamin B12 and D where there are controversial publications in the literature. It was seen that levels of these vitamins were not associated to any atherosclerotic parameter in our study population including young adults without comorbidity. Further prospective-controlled studies are needed to assess atherosclerosis development in HP-positive patients.

Authors' contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Funding

During this study, any pharmaceutical company that has a direct connection with the subject of the research, a company that provides and/or produces medical tools, equipment, and materials, or any commercial company, during the evaluation process of the study, financial and/or no moral support was received.

Conflict of interest

Regarding this study, the authors and/or their family members do not have a scientific and medical committee membership or relationship with their members, consultancy, expertise, working status in any company, shareholding, or similar situations with a potential conflict of interest.

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