Seroprevalencija infekcije *Helicobacter pylori* u koronarnoj arterijskoj bolesti

Helicobacter pylori seroprevalence in coronary artery disease

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Sažetak

Uvod: Infekcija bakterijom *Helicobacter (H.) pylori* je dobro poznati uzrok kroničnog gastritisa i želučanog vrijeda. Međutim, sve je više dokaza da kronični upalni odgovor na ovaj patogen može isto tako imati važnu ulogu u koronarnoj arterijskoj bolesti (KAB). Cilj ove studije bio je ispitati razliku u seroprevalenciji infekcije *H. pylori* u kontrolnoj skupini u usporedbi sa skupinom bolesnika s elektrokardiografski dokazanom KAB.

Materijal i metode: U studiju je bilo uključeno 98 zdravih osoba kao kontrolna skupina i 102 bolesnika s elektrokardiografskim dokazima srčane bolesti kao skupina bolesnika. Kvantitativno mjerenje IgG protutijela na *H. pylori* provedeno je automatiziranom metodom EIA u serumu ispitanika obiju skupina.

Rezultati: Seroprevalencija *H. pylori* IgG bila je značajno viša u skupini bolesnika (87,3%) u usporedbi s kontrolnom skupinom (53,1%) (P<0,001).

Zaključak: Rezultati su ukazali na moguću pojačanu seroepidemiološku udruženost infekcije *H. pylori* i KAB.

Ključne riječi: *Helicobacter pylori,* koronarna arterijska bolest, upala

Abstract

Background: *Helicobacter (H.) pylori* infection is a well-established cause of chronic gastritis and peptic ulcer disease. However, there is growing evidence that chronic inflammatory response to this pathogen may also play an important role in coronary artery disease (CAD). The aim of this study was to investigate the difference in seroprevalence of *H. pylori* infection in a control group compared to a group of patients with electrocardiographic evidence of CAD.

Materials and Methods: The study included 98 apparently healthy control subjects as the control group and 102 heart disease patients with electrocardiographic evidence of CAD as the patient group. Quantitative measurement of IgG antibodies to *H. pylori* in sera from subjects of both groups was performed with an automated commercial EIA method.

Results: Seroprevalence of *H. pylori* IgG was significantly higher in the patient group (87.3%) compared to the control group (54.1%) (P<0.001).

Conclusion: The results suggest that there may be an increased seroepidemiological association of *H. pylori* infection with CAD.

Key words: *Helicobacter pylori*, coronary artery disease, inflammation

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Uvod

Iako se u početku činila prilično proturječnom (1), infekcija želučane sluznice bakterijom *Helicobacter (H.) pylori* danas je općenito prepoznata kao vodeći uzrok kroničnog gastritisa i želučanog vrijeda (2), kao i čest uzročnik dermatitisa te želučanog limfoma i karcinoma (3). Međutim, sve je više dokaza za to da bi kronična lokalna infekcija želučane sluznice mogla imati sustavni upalni učinak koji je odgovoran za napredovanje ateroskleroze općenito, a naročito koronarne arterijske bolesti (KAB) (4). Uz to, DNA nekoliko infektivnih uzročnika izolirana je iz aterosklerotskih plakova karotida (5), što može značiti da sastav plaka tvori osobito pogodno okružje za ove uzročnike. Nadalje, klasični upalni biljezi poput fibrinogena i C-reaktivnog

Introduction

Although highly controversial when first reported (1), *Helicobacter* (*H.) pylori* infection of gastric mucosa is widely recognized today as the leading cause of chronic gastritis and peptic ulcer disease (2), as well as a common causative agent of dermatitis and gastric lymphoma and carcinoma (3). There is, however, growing evidence that chronic local infection of gastric mucosa may have systemic inflammatory effects that are responsible for atherosclerosis progression in general, and coronary artery disease (CAD) in particular (4). In addition, DNA from several infective agents has been isolated from carotid atherosclerotic plaques (5), which may mean that plaque composition makes for a particularly suitable nesting ground for

proteina (CRP) mogli bi također biti izravno upleteni u dozrijevanje i prsnuće plaka (6), dok bi sustavna upala kao rezultat infekcije *H. pylori* mogla dodatno dovoditi do endotelijske disfunkcije (7). Postoje isto tako izvješća o tome kako infekcija *H. pylori* možda ima izravan učinak na krvni tlak, iako još nije razjašnjeno je li taj učinak značajan (8). U svjetlu svih ovih spoznaja cilj ove studije bio je utvrditi seroprevalenciju infekcije *H. pylori* u kontrolnoj skupini u usporedbi sa skupinom bolesnika s KAB, kako bismo ispitali udruženost infekcije *H. pylori* i KAB.

Materijali i metode

U studiju je bilo uključeno 102 bolesnika, 59 muškaraca i 43 žene, u dobi od 45 do 70 godina, s bolovima u prsištu i elektrokardiografski dokazanom KAB (povišenje ST-segmenta). Kontrolnu skupinu činilo je 98 zdravih osoba (davatelji krvi), 61 muškarac i 27 žena, u dobi od 40 do 60 godina, bez nalaza koji bi ukazivali na KAB na kliničkom pregledu i sa vrijednostima rutinskih biokemijskih pretraga unutar referentnih granica. Kriteriji za uključivanje u studiju u skupini bolesnika bili su: dob od 45 do 75 godina; bolovi u prsištu i povišenje ST-segmenta kod prijma; uzorak krvi uzet na kontrolnom pregledu za rutinske pretrage. Kriteriji za uključivanje u kontrolnu skupinu bili su: dob od 40 do 60 godina; zdravi davatelji krvi bez nepravilnosti u nalazima kliničkog pregleda i rutinskih biokemijskih pretraga; odsutnost dokaza za KAB u vrijeme uključivanja u studiju/uzimanja krvi. Kriteriji za isključenje bolesnika bili su: dob ispod 45 i iznad 75 godina; dokazi za postojanje drugih srčanih bolesti osim KAB; novija anamneza infektivnih bolesti. Kriteriji za isključenje kontrolnih ispitanika bili su: dob ispod 40 i iznad 60 godina; dokazi postojanja neke aktualne bolesti; uporaba lijekova poput inhibitora protonske crpke, H2-blokatora ili antibiotika. Podaci o socioekonomskom statusu prikupljeni su za sve ispitanike pri ulasku u studiju.

Uzorci krvi uzimali su se u epruvete *vacutainer* bez antikoagulansa, a potom odijeljeni serum je pohranjen na -20 °C do analize. Titar IgG protutijela na *H. pylori* određen je automatiziranom metodom EIA Roche Diagnostics (F. Hoffmann-La Roche Ltd., Basel, Švicarska) na imunoanalizatoru Cobas Core II. Svi bolesnici s titrom anti-*H. pylori* protutijela iznad 6,6 U/mL klasificirani su kao seropozitivni.

Statistička obrada rezultata provedena je pomoću programa SAS Institute StatView (verzija 5.0) uz primjenu Fisherova egzaktnog testa za izračunavanje χ^2 i vrijednosti P. Vrijednosti P niže od 0,05 smatrane su statistički značajnima.

Rezultati

Od 98 osoba u kontrolnoj skupini, 53 (54,1%) ih je imalo pozitivan rezultat testa na anti-*H. pylori* IgG (titar >6,6

these agents. Furthermore, classic inflammatory markers, such as fibrinogen and C-reactive protein (CRP), may also be directly involved in plaque maturation and rupture (6), and systemic inflammation as a result of H. pylori infection may additionally lead to endothelial dysfunction (7). There have also been reports that *H. pylori* infection may have a direct effect on blood pressure, although the significance of this effect remains controversial (8). In the light of all the above mentioned findings, the aim of this study was to determine the seroprevalence of *H. pylori* infection in a control group compared to a group of patients with coronary artery disease in order to investigate the association of *H. pylori* infection and CAD.

Materials and methods

The study included 102 patients (59 male and 43 female) aged 45 to 70, with chest pain and electrocardiographic evidence of CAD (ST-segment elevation). The control group consisted of 98 apparently healthy subjects, blood donors (61 male and 37 female) aged 40 to 60, with no findings indicative of CAD on clinical examination and routine biochemical tests in the reference ranges. Inclusion criteria for the patient group were: age 45 to 75 years; chest pain and ST-segment elevation upon admission; and blood sample obtained at clinic visit for routine tests. For the control group, inclusion criteria were: age 40 to 60 years; apparently healthy blood donors, with no irregular findings on clinical examination and routine biochemistry; and no evidence of CAD at the time of recruitment/blood collection. Exclusion criteria for patients were: age under 45 or over 75 years; evidence of cardiac disease other than CAD; and recent history of infectious diseases. Exclusion criteria for controls were: age under 40 or over 60 years; any evidence of overt disease; and history of using drugs such as proton pump inhibitors, H2-blockers or antibiotics. Data on socioeconomic status were collected from all study participants at recruitment.

Blood samples were taken from all subjects into vacutainers without anticoagulant, and separated serum was stored frozen at -20 °C until analysis. The titer of IgG antibodies against *H. pylori* was determined with the automated EIA method from Roche Diagnostics (F. Hoffmann-La Roche Ltd., Basel, Switzerland) on a Cobas Core II immunoanalyzer. Patients with a titer of anti-*H. pylori* IgG antibodies above 6.6 U/mL were considered seropositive.

Statistical processing of the results was performed with the SAS Institute StatView software (version 5.0), using Fisher's exact test to calculate χ^2 and *p* values. A P value lower than 0.05 was considered significant.

Results

Out of 98 control subjects, 53 (54.1%) tested positive for anti-*H. pylori* IgG (titer >6.6 U/mL), i.e. 34 of 61 (55.7%) male subjects and 19 of 37 (51.3%) female subjects; there was

U/mL), tj. 34 od 61 (55,7%) muških i 19 od 37 (51,3%) ženskih osoba; razlika prema spolu nije bila statistički značajna. Od 102 bolesnika s KAB, 89 (87,2%) ih je bilo pozitivno na anti-*H. pylori* IgG, tj. 52 od 59 (88,1%) muških i 37 od 43 (86,0%) ženskih bolesnika. Broj seropozitivnih ispitanika značajno se razlikovao između kontrolne i bolesničke skupine (P<0,001). Nadalje smo skupinu bolesnika s KAB podijelili prema socioekonomskom statusu, pričem su oni s niskim (N=49) socioekonomskim statusom svi bili seropozitivni. Broj seropozitivnih ispitanika značajno se razlikovao između ovih podskupina s niskim i visokim socioekonomskim statusom (100% *vs.* 75,5%; P<0,001). Rezultati su prikazani u tablicama 1. i 2.

no significant difference between the two subgroups. Out of 102 patients with CAD, 89 (87.2%) were positive for anti-*H. pylori* IgG, i.e. 52 of 59 (88.1%) male patients and 37 of 43 (86.0%) female patients. The number of seropositive individuals differed significantly between the control group and patient group (P<0.001). In addition, seropositivity was detected in the entire subgroup of patients with low socioeconomic status (N=49; 100%). The number of seropositive patients in this group differed significantly from the respective figure in patients with higher socioeconomic status (75.5% of seropositive subjects) (P<0.001). These results are summarized in Tables 1 and 2.

Tablica 1. Seropozitivnost na *Helicobacter pylori* u skupini bolesnika s KAB i kontrolnih ispitanika

Table 1. *Helicobacter pylori* seropositivity in CAD patients and controls

	Controls (N=98)	CAD (N=102)	P
H. pylori positive (%)	53 (54.1)	89 (87.2)	<0.001
Seropositive male/female ratio	34/19	52/37	0.500

Tablica 2. Seropozitivnost na *Helicobacter pylori* u skupini bolesnika s KAB prema socioekonomskom statusu

Table 2. *Helicobacter pylori* seropositivity in CAD patients according to socioeconomic status

	CAD patients with low socioeconomic status (N=49)	CAD patients with high socioeconomic status (N=53)	p
H. pylori positive (%)	49 (100)	40 (75.5)	<0.001
Seropositive male/female ratio	34/15	18/22	0.020

Rasprava

Uloga klasičnih čimbenika rizika za aterosklerozu, tj. hipertenzije, hiperlipidemije, šećerne bolesti i pušenja, dobro je utvrđena. Danas se istraživanja sve više usmjeravaju ka razjašnjavanju uloge alternativnih čimbenika rizika poput citokina, infekcije i imunosti. Postoji mnoštvo podataka o mogućim patogenetskim mehanizmima, koji su većinom usredotočeni na ulogu posrednika upalnog odgovora, kao što su fibrinogen i CRP, te odnedavno interleukini (IL) poput IL-1 i IL-10, te osteopontin (9). U tom smjeru još se uvijek ispituje udruženost kronične infekcije *H. pylori* i KAB. Prema nekim izvješćima, protein toplinskog šoka 60 iz *H. pylori* (Hsp60) dovoljno je sličan endotelijskom Hsp, pa bi tako kronična infekcija *H. pylori* mogla dovoditi do odgovora autoimunih protutijela na endotelijske stanice (10). Nekoliko je studija isto tako našlo povišen broj leuko-

Discussion

The role of classic risk factors for atherosclerosis, i.e. hypertension, hyperlipidemia, diabetes and smoking, has been well established. Today, there are increased research efforts in elucidating the role of alternative risk factors, such as cytokines, infection and immunity. There is abundant body of data concerning the possible pathogenetic mechanisms involved, most of which have been concentrated on the role of the inflammatory response mediators such as fibrinogen and CRP, and more recently interleukins (IL) such as IL-1 and IL-10, and osteopontin (9). In this regard, the association of chronic *H. pylori* infection and CAD is still under investigation. There have been reports that *H. pylori* heat shock protein 60 (Hsp60) is sufficiently similar to endothelial Hsp, so that chronic infection with *H. pylori* may lead to an autoimmune antibody response to endot-

cita i niži HDL-kolesterol u bolesnika s infekcijom *H. pylori* (11). Bez obzira na mehanizam koji je tu uključen, naši podaci pružaju dodatne dokaze za povezanost infekcije *H. pylori* i KAB, poglavito u bolesnika s elektrofiziološkim znacima srčane ishemije (12). Još uvijek su prisutna neka metodologijska ograničenja koja bi mogla pomutiti istraživanja u ovom području (13).

Naše je ispitivanje bilo skromno zamišljeno, što je rezultiralo nekim ograničenjima koja se nadamo prevladati u budućnosti. Jedno od ograničenja bio je mali broj ispitanika uključenih u studiju te neujednačenost kontrolne i bolesničke prema spolu, dobi i rizičnim čimbenicima za KAB. Nadalje, nije provedena procjena hipertenzije, hiperlipidemije, šećerne bolesti i pušenja te korelacija sa seropozitivnošću na *H. pylori*. Daljnje ograničenje ove studije bilo je to što nije pružila dokaze za ulogu virulentnih (tzv. CagA+) sojeva *H. pylori* (14). Ipak, smatramo kako će naša studija potaknuti daljnja istraživanja povezanosti između seropozitivnosti na *H. pylori* i KAB, jer naši rezultati ukazuju na moguću pojačanu seroepidemiološku udruženost infekcije *H. pylori* i KAB.

helial cells (10). Several studies have also found increased leukocyte counts and lower HDL-cholesterol in patients infected with *H. pylori* (11). Irrespective of the mechanisms involved, our data provide further evidence on the link between *H. pylori* infection and CAD, especially in patients with electrophysiological signs of cardiac ischemia (12). There still are several methodological limitations that may confound research in the field (13).

The present study was quite modest in design and therefore suffering from some limitations that we hope to overcome in the future. One limitation was the small number of study participants, whereby the control group was not matched for sex, age and CAD risk factors. Furthermore, hypertension, hyperlipidemia, diabetes and smoking were not evaluated and correlated to *H. pylori* seropositivity. Another limitation of the study was that it did not provide any evidence on the role of virulent (so called CagA+) *H. pylori* strains (14). Despite all this, we believe that this study may stimulate further research into the link between *H. pylori* seropositivity and CAD, as our data suggest that there may be an increased seroepidemiological association of *H. pylori* infection with CAD.

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