Seroepidemiology Study of *Helicobacter pylori* Infection and Its Effect on Cardiovascular Diseases in Karaj, Iran

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Helicobacter pylori infection occurs throughout the world between countries and races. A seroprevalence study of *H. pylori* infection in the occurrence of atherosclerosis (ASc) has been considered to be the main cause of cardiovascular diseases (CVDs) in part of Iran. From 600 volunteers, 97 serum samples were selected and tested for the presence of *H. pylori* infection. Positive and negative *H. pylori* infection samples were then selected for *Chlamydia pneumonia* and cytomegalovirus infection. Their serum levels for high-sensitive C-reactive protein (hs-CRP) and IL-6 titer were measured using an ELISA test. Prevalence of *H. pylori* infection was ~68.04% in Karaj. Among the 22 samples of cytokine titration, 4 individuals were *HP*⁻ and 18 were *HP*⁺. It was also noticed that 17 samples had normal titration (77.27%) of IL-6 and 3 had a high level of IL-6 (13.63%), 20 people had high hs-CRP titration (90.90%), and 2 were low level hs-CRP (9.1%). Obtained results showed that there is no significant difference between *HP*⁺ and *HP*⁻ infection and IL-6 and hs-CRP titration. Results showed that *H. pylori* could not lead to ASc by increasing the levels of proinflammatory cytokines such as IL-6 and hs-CRP. Further studies are necessary for the detection of mechanism of *H. pylori* on ASc.

Keywords: atherosclerosis, IL-6, hs-CRP, *Helicobacter pylori*

Introduction

ATHEROSCLEROSIS (ASc) IS THE most important cause of cardiovascular diseases (CVDs), which is caused by pro-inflammatory cytokines secreted by immune cells. Chronic inflammatory responses in ASc could increase the different levels of cytokines such as IL-6, IL-7, IL-8, IL-10, high-sensitive C-reactive protein (hs-CRP), and fibrinogen.^(1,2) Different microorganisms (*Helicobacter pylori*, *Chlamydia pneumonia*, and cytomegalovirus) might also lead to chronic infection, which could result in ASc.⁽²⁾ These organisms, particularly *H. pylori*, could elevate serum level of total cholesterol, low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), and reduce the high-density lipoprotein cholesterol (HDL-c). However, there is insufficient evidence to clearly address this issue.⁽¹⁻⁴⁾ There is a large body of evidence indicating that ASc is associated with smoking, diabetes, hypertension, and hypercholesterolemia.

Although the ASc has a key role in the generation of CVDs and infarction, it is not the only cause of CVDs and myocardial infarction, as recent findings show that ASc plaques could not block lone arteries. There are two main causes of thrombosis, including destroying ASc plaques and the erosion and rescinding of endothelial. Destroying ASc plaques are more risky than the other factors, whereas gathering ac-

tivated immune cells around the plaques and their secreted enzymes lead to rupture of the plaques. Ruptured materials are a kind of signal to absorb activated platelets, interferon γ , IL-1, tumor necrotizing factors, and IL-6. IL-6 induces CRP on liver, which results in increasing CRP levels, amyloids A, and fibrinogen. Activated fibrinogen could cause thrombosis in the arteries, which leads to infarction.⁽¹⁾

Connection between chronic infection and inflammatory cytokines in CVDs increase the idea of an association between bacteria [*C. pneumonia*^(5,6) and *H. pylori*^(7,8)] and cytomegalovirus⁽⁹⁾ in ASc.⁽²⁾ A primary study on this issue was completed by Mendal in 1994 for *H. pylori*⁽¹⁰⁾ and now it is known as one of the main causes of coronary heart disease through the effect of the *Cag A* gene.⁽¹¹⁾ In this regard, a study in 2011 by IgG test in Arak, Iran, revealed that the prevalence of *H. pylori* infection was 673 (58.5% *Hp*⁺) out of 1150 patient samples. There was no relationship between gender and age in the obtained results; however, age did have a positive effect on infection.⁽¹²⁾ In 2009 (Rasht, Iran), an investigation with 475 boys and 486 girls showed that 40% of them were *Hp*⁺ and 60% *Hp*⁻. The test was conducted using the *Hp* antigen T kit and infection had arisen because of water contamination.⁽¹³⁾ A seroprevalence study of 1518 people (Nahavand, Iran) using the IgG test showed 71% of them were *Hp*⁺. Infection had increased by rising age and also the

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TABLE 1. PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN DIFFERENT AGES IN NAHAVAND-IRAN, 2009^a

Age (years)	No. of women	No. of men	Total
6–10	17/31 (54.8%)	11/30 (36.7%)	28/61 (45.9%)
11–20	79/172 (45.9%)	96/179 (53.6%)	175/351 (49.9%)
21–30	124/169 (73.4%)	55/100 (55.0%)	179/269 (66.5%)
31–40	134/165 (81.2%)	80/102 (78.4%)	214/267 (80.1%)
41–50	104/122 (85.2%)	63/75 (84.0%)	167/197 (84.8%)
51–60	70/81 (86.4%)	47/58 (81.0%)	117/139 (84.2%)
61–70	60/71 (84.5%)	39/51 (76.5%)	99/122 (81.1%)
>70	48/54 (88.9%)	45/58 (77.6%)	93/112 (83.0%)

^aReference⁽¹⁴⁾

number of infected women were greater than the number of infected men⁽¹⁴⁾ (Table 1). Similar research in China presented 3435 (63.41%) out of 5415 people who were between 30 and 39 years, were *Hp*⁺, and also indicated that men had a higher risk of infection than women.⁽¹⁵⁾ Work in Sanandaj, Iran in 2013 showed that 64.2% of the studied population were *Hp*⁺ (ages 4 months–15 years)⁽¹⁶⁾ (Table 2).

In contrast, the first report of cytomegalovirus infection was in 1891 in which the writer had worked on a relationship between virus and ASC.⁽¹⁷⁾ In 2012, Fazel and colleagues, using a PCR, IgG, and IgM test, presented a report where 26.7% of people were DNA positive, 85.7% of them were IgG positive, and 3.8% IgM positive.⁽⁹⁾

Subsequently, this study was conducted to understand the effect of *H. pylori*, *C. pneumonia*, and cytomegalovirus on CVDs and their effect on the expression of proinflammatory cytokine (IL-6) and hs-CRP.

Materials and Methods

Study subjects

Five milliliter blood samples of 600 patients were collected, kept in a water bath at 37°C for 1 hour, centrifuged (3000 rpm/5 minutes) to separate their serum, and stored at –20°C. At first, biochemical tests of fasting blood sugar (FBS), TG, cholesterol, HDL, and LDL were measured. Then the samples of 13 men and 84 women who had a normal range of FBS, TG, cholesterol, HDL, and LDL were selected for use in other chemical tests and were kept at –20°C.

Data collection

Demographic data, including information about diseases and medical history, medication, diabetes, and smoking, were gathered using a standardized questionnaire.

Detection of bacteria and virus

Anti-*H. pylori* IgG kit (Euroimmun), anti-*C. pneumonia* IgG kit (Euroimmun), and anti-CMV IgM kit (Euroimmun) were applied to detect the IgG level of *H. pylori* and *C. pneumonia* and the IgM level of cytomegalovirus according to the manufacturer's protocol.

Detection of proinflammatory cytokines

An ELISA test was applied to detect cytokines of IL-6 (eBioscience) and hs-CRP (IBL, Germany) according to the manufacturer's protocol.

Statistics

Data were analyzed using descriptive statistical methods using SPSS software (version 16.0, SPSS, Inc., Chicago, IL). *p* Value of ≤0.05 was considered statistically significant.

Results

H. pylori infection

From 97 selected people (age between 20 and 61 years) samples from 84 women and 13 men were isolated. The average rate of the anti-*H. pylori* IgG results was 67.35% and the highest IgG titer was 28% among the population samples with ages between 26 and 30 years.

Positive epidemiological prevalence of *H. pylori* infection in this study was around 68.04% and the negative rate was 31.96%. The maximum rate of infection was observed in the population with ages 26–30 years (33 people). The average rate of gender infection was obtained. In the men's group it was 92.3% (12 out of 13 people) and 64.28% (54 out of 84) in women's group (Table 3).

TABLE 2. PREVALENCE OF *H. PYLORI* INFECTION AMONG CHILDREN IN SANANDAJ, IRAN, 2013^a

Age (years)	Total	Total positive	Female number	Positive female	Male number	Positive male
4 months	78	34 (43.6%)	41	18 (44%)	37	16 (43%)
1	73	30 (41.1%)	34	17 (50%)	39	13 (33%)
2	78	52 (66.7%)	43	26 (60%)	35	26 (74%)
5	76	59 (77.6%)	40	29 (72%)	36	30 (83%)
10	73	47 (64.4%)	29	18 (62%)	44	29 (66%)
15	80	70 (90%)	40	35 (87.5%)	40	37 (92.5%)
Total	458	294 (64.2%)	227	143 (63%)	231	151 (65%)

^aReference⁽¹⁶⁾

TABLE 3. PREVALENCE OF *H. PYLORI* INFECTION IN KARAJ, IRAN, THIS STUDY

Age (years)	Total number	Total positive	Total negative	Female number	Positive female	Male number	Positive male
≤20	6	2	4	6	4	0	0
21–25	16	10	6	13	8	0	0
26–30	33	28	5	28	21	5	4
31–35	17	13	4	15	10	2	2
36–40	7	2	5	6	3	1	1
41–45	4	2	2	3	2	1	1
46–50	4	3	1	3	2	1	1
51–55	2	2	0	1	0	1	1
56–60	4	3	1	3	2	1	1
≥61	4	1	3	3	2	1	1
Total	97 (100%)	66 (68.04%)	31 (31.96%)	84 (86.6%)	54 (64.28%)	13 (13.4%)	12 (92.3%)

C. pneumonia infection

Results of the test showed that prevalence of infection was around 48% and the average titer of infection was about 35.15%. The proportion of infection in men and women were 53.85% and 47.62%, respectively, which indicates 50% of infections (Table 4).

Cytomegalovirus infection

Results showed that prevalence of virus infection was equal to 56% of individuals and the average IgM titer was 45.71%. In this part of study, 61.37% of women had a high titer of antibody and close to 38.63% had a normal titer, whereas in 16.67% of men the titer was high and in 83.33% of them it was negative (Table 5).

IL-6 assessment

From 97 selected people, 22 who were without cytomegalovirus and *C. pneumonia* infection were isolated to measure their IL-6 level. Four people were *Hp*⁻ and 18 people were *Hp*⁺ in which 17 people (77.27%) had a normal level of IL-6 and 3 people (13.63%) had a high level of IL-6. Two people (9.1%) were nondetectable. Frequency and mean of IL-6 were 5.52 using SPSS 16.0 (Table 6).

hs-CRP assessment

Results of this part of the study on 22 people showed that 20 (90.90%) out of 22 people had a high concentration of hs-CRP. This indicates that they had a high risk of CVD and one

person (4.55%) had a low risk, whereas one person was in the middle rate of CVD. Frequency of hs-CRP cytokine was reported as 23.33% (Table 7).

Discussion

Recent studies have indicated that since the mean age of incidence of CVD has dropped to <65 years, hypertension, diabetes, and high cholesterol could not be the only risk factors of ASc and CVDs. Subsequently, chronic infection through different microorganisms (*H. pylori*, *C. pneumonia*, *Perfiromonas gengivalis*, and cytomegalovirus), and their effects on the expression of proinflammatory cytokines could play an important role in the generation and development of ASc and CVDs.⁽¹⁴⁾ Current immunological studies on *H. pylori* have also pointed this out.⁽¹⁸⁾ Findings of this study showed that the prevalence of infection with *H. pylori* was 68.04%, and the mean of the IgG test was 67.35%. The age group of 25–30 years had the highest prevalence rate of infection. The rate of *H. pylori* infection was reported (2014) between 31% and 35% with the highest prevalence in 44 years.⁽¹⁹⁾ The results of this study revealed a close relationship between age and morbidity rate. Moreover, the study showed that although the number of men was less than the number of women, the percentages of positive antibody in men were more than in women indicating a high gender morbidity. This analysis showed that the population under study had a high number of *H. pylori* infection that increases the possibility of the incidence of ASc and CVD disorders.

TABLE 6. PREVALENCE OF PROINFLAMMATORY CYTOKINE IL-6 IN KARAJ, IRAN, THIS STUDY

	Total number	Normal titer	High titer	Nondetectable
IL-6	22	17 (77.27%)	3 (13.63%)	2 (9.1%)

TABLE 7. PREVALENCE OF HIGH-SENSITIVE C-REACTIVE PROTEIN IN KARAJ, IRAN, THIS STUDY

Titer	Total number	High-risk CVDs	Low-risk CVDs	Intermediate CVDs
hs-CRP	22	20 (90.90%)	1 (4.55%)	1 (4.55%)

CVDs, cardiovascular diseases; hs-CRP, high-sensitive C-reactive protein.

TABLE 4. PREVALENCE OF *CHLAMYDIA PNEUMONIA* INFECTION IN KARAJ, IRAN, THIS STUDY

Gender	Total number	Total positive	Total negative
Women	84	40 (47.62%)	44 (52.38%)
Men	13	7 (53.85%)	6 (46.15%)

TABLE 5. PREVALENCE OF CYTOMEGALOVIRUS INFECTION IN KARAJ, IRAN, THIS STUDY

Gender	Total number	Total positive	Total negative
Women	44	27 (61.37%)	17 (38.63%)
Men	6	1 (16.67%)	5 (83.33%)

Seroepidemiological evidence on *C. pneumonia* expresses 69% of population infection,⁽⁵⁾ whereas this study showed that the prevalence of infection with *H. pylori* is 48.45% with the mean of 35.15%. There was no significant association between gender and prevalence of infection.

A study (2009) on 400 people showed that the frequency of anti-CMV IgM and IgG tests were 6.3% and 96.9%, respectively.⁽²⁰⁾ However, the result of this study was not in accordance with these results and found 56% for IgM with a significant association between gender and infection.

Studies on the expression of IL-6 through the effect of *H. pylori* have shown that the microbe had no significant effect on its expression, and there were no major differences between the titer of *Hp*⁺ and *Hp*⁻ people and IL-6. This indicates that there is no relationship between infected people and CVDs,⁽²¹⁾ which was similar to the results of this study.

Regarding the effect of *H. pylori* on the expression of hs-CRP, most studies indicate that its expression has been observed in people with peptic ulcer rather than the control group.⁽²²⁾ The titer of hs-CRP in *H. pylori*-infected patients was reported higher than in healthy people in a study,⁽²³⁾ which was not in accordance with the results of this study indicating *Hp*⁺ and *Hp*⁻ patients had an equal level of hs-CRP titer, and there were no significant differences between them.

This study showed that prevalence of *H. pylori* infection in Karaj, Iran, was around 68.04% and the highest prevalence rate was between the ages of 25–30 years. There was a significant association between age and gender in infection by microbe, whereas men were infected more than women. The study also determined that there is no significant difference between *Hp*⁺ and *Hp*⁻ patients and level of IL-6 and hs-CRP. This indicates that *H. pylori* could play any role in the expression of these cytokines that involve ASc and CVDs. As a result, the mechanism of ASc and CVDs could not only be through the changing of IL-6 and hs-CRP and there is still room for further investigation.

Authors' Contributions

T.M. contributed to this article with conception and design of the study, literature review and analysis, drafting, and final approval of the final version. S.A. contributed to this article with revision and editing, and final approval of the final version.

Author Disclosure Statement

No competing financial interests exist.

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