

## THE EFFECT OF *HELICOBACTER PYLORI* ON INTERLEUKIN-6 IN PATIENTS WITH ISCHEMIC HEART DISEASE

Shahrazad H. Muhi<sup>1</sup>Wisal S. Abd<sup>1</sup>Najah S. Abd<sup>2</sup>

Researcher

Assist. Prof.

Consultant Doctor

<sup>1</sup> Dept. of Biotechnology Coll. of Sci. University of Baghdad-Iraq<sup>2</sup> Iraqi Center for Heart Disease, Ghazy AL-hariri Hospital for Surgical Specialities - Iraqshahrazadhazim@gmail.com<sup>1</sup> wisalimmunology@gmail.com<sup>1</sup>njh\_njh@rocketmail.com<sup>2</sup>

### ABSTRACT

Several studies have shown that the presence of *Helicobacter pylori* (*H. pylori*) may be a risk factor for Ischemic Heart Diseases (IHD). Therefore, it is needed to look into the relation between IHD and *H. pylori* infection. One hundred Iraqi patients with IHD and 60 healthy Iraqis as a control group participated in the current study. The ELISA assay was used for the measurement of the sera levels for anti- *H. pylori* IgG, IgM, and Interleukin-6 (IL-6). Also, *H. pylori* DNA in the human venous blood was investigated by the polymerase chain reaction (PCR) using 16S rDNA primers. Between IHD patients and the control group, *H. pylori*-IgG and IL-6 mean  $\pm$  SD were (Patient = 1.814 $\pm$ 0.08, Control = 0.823 $\pm$ 0.09, Patient = 49.73 $\pm$ 6.60, Control = 0.21 $\pm$ 0.02, respectively), and this results showed a highly significant variation ( $p < 0.05$ ) in anti-*H. pylori* IgG and IL-6, while no significant variations ( $p > 0.05$ ) were found in anti- *H. pylori* IgM. It was also found that PCR amplification of *Helicobacter pylori* DNA using 16S rDNA primers was positive in the venous blood of 81 (81%) IHD patients. The current study results proposed that there was a relation between *H. pylori* and Ischemic Heart Disease.

Keyword: IHD, *H. pylori*-IgG, *H. pylori*-IgM, IL-6, PCR, 16S rDNA, ELISA, DNA.

محي وآخرون

مجلة العلوم الزراعية العراقية -2023: 54(5):1243-1251

تأثير الجرثومة الملوية البوابية على الانترلوكين-6 في المرضى العراقيين المصابين بمرض القلب الاقفاقي

نجاح سلمان عبد<sup>2</sup>وصال سلمان عبد<sup>1</sup>شهرزاد حازم محي<sup>1</sup>

طبيب استشاري

استاذ مساعد

باحث

<sup>1</sup> قسم التقنيات الاحيائية - كلية العلوم - جامعة بغداد<sup>2</sup> المركز العراقي لامراض القلب, مستشفى غازي الحريري

المستخلص

أظهرت العديد من الدراسات أن وجود جرثومة الملوية البوابية قد يكون عامل خطر للإصابة بأمراض القلب الإقفارية. لذلك، من الضروري التحقق من العلاقة بين عدوى الملوية البوابية وأمراض القلب الإقفارية. شارك في الدراسة الحالية 100 مريضاً عراقياً يعانون من أمراض القلب الإقفارية و 60 عراقياً أصحاء كمجموعة ضابطة. تم قياس مستويات المصل لمضادات الملوية البوابية (IL-6 & IgG, IgM) باستخدام اختبار الـ ELISA. أيضاً، تم فحص الحمض النووي للملوية البوابية في الدم المحيطي للإنسان باستخدام بادئات (16S rDNA) بواسطة تفاعل البلمرة المتسلسل. أظهرت النتائج أن المتوسط لكل من IgG و IL-6 كانت (P=1.814 $\pm$ 0.08) ، (C=0.823 $\pm$ 0.09 ، P=49.73 $\pm$ 6.60 ، C=0.21 $\pm$ 0.02) ، وهذه النتائج تدل على وجود فروق ذات دلالة إحصائية ( $p < 0.05$ ) في مضاد جرثومة الملوية البوابية (IgG) وكذلك في (IL-6) بين المرضى المصابين بمرض القلب الاقفاقي والمجموعة الضابطة بينما لم تكن هناك فروق ذات دلالة إحصائية ( $p > 0.05$ ) في مضاد جرثومة الملوية البوابية (IgM) بين المرضى المصابين بالقلب الاقفاقي والمجموعة الضابطة. وجد أيضاً أن الحمض النووي للملوية البوابية المتضخم بواسطة تفاعل البلمرة المتسلسل باستخدام بادئات (16S rDNA) كان إيجابياً في الدم المحيطي لـ 81 (81%) مريضاً بأمراض القلب الإقفارية. اقترحت نتائج الدراسة الحالية أن هناك علاقة بين الإصابة بجرثومة الملوية البوابية وأمراض القلب الإقفارية.

الكلمات المفتاحية: مرض القلب الاقفاقي, انترلوكين-6, تفاعل البلمرة المتسلسل, الـ ELISA, الحمض النووي الريبوزي منقوص الأوكسجين.

## INTRODUCTION

Ischemic Heart Disease (IHD) is considered the most popular among cardiovascular disorders (26). Sometime, the term coronary heart disease (CHD) is often used to describe this syndrome, because the reduction of blood flow in IHD is caused by coronary artery atherosclerosis (4). IHD is considered as a pathological case characterized by reduced flow in the cardiac blood that results in a mismatch between myocardial oxygen supply and demand and it is considered a chronic, progressive disease (30). IHD causes 30% of all yearly deaths, which has become the main death reason and a major economic burden worldwide (42). Atherosclerosis is the major pathological process that results in IHD, atherosclerosis is an artery inflammatory disease associated with deposition of lipids and metabolic alterations due to many risk factors (38). Classical risk factors for atherosclerosis and consequently cardiovascular disease (CVD) involve dyslipidemia, smoking, genetic predisposition, diabetes mellitus, arterial hypertension, metabolism disorder, a rich fats diet, and mental or emotional stress (34). Different experimental and scientific researches show that atherosclerosis is significantly affected by inflammatory situations. A significant relationship between coronary ischemia and various infectious agents was shown by many epidemiological types of research. *Helicobacter pylori* (*H. pylori*) is a spiral microaerophilic gram-negative bacterium that causes inflammatory and infectious disease in humans by forming colonies in the stomach (12). *H. pylori* cause many extra digestive disorders, involving functional vascular diseases like Raynaud's (primary) and migraine (primary) and IHD (34). Many studies attested that there is an association between IHD and the stomach *H. pylori* infection (11). Chronic stimulation of inflammatory responses as a result of the gut and gastric organs infection by bacteria causes the stimulation of dyslipidemia, elevates the fibrinogen levels, induces the liberation of CRP, rapidly increases blood leukocyte and homocysteine, stimulates hypercoagulability, induces immune cross-reactivity, elevates pro-inflammatory cytokines (such as Interleukins)

and another cytotoxic agent (27). *H. pylori* might result in a chronic infection that could result in atherosclerosis, chronic inflammatory responses in atherosclerosis can elevate the values of various cytokines such as Interleukin-6 (IL-6), CRP, and fibrinogen. IL-6 stimulates CRP in the liver, which causes elevation in CRP values, fibrinogen, and amyloids A in blood. Arteries thrombosis could result from stimulated fibrinogen, which can cause an infarction (7). IL-6 is an essential cytokine in the inflammatory response, it has two unique and special signaling pathways. The first is named classic signaling of IL-6, in this signaling, IL-6 connects to a membrane-bound IL-6 receptor (IL-6R). The linkage of IL-6 to its receptor stimulates the activation and dimerization of the signal-transducer glycoprotein 130 (gp130). The non receptor tyrosine kinase JAK will be recruited by the active complex, which phosphorylates the gp130's tyrosine residues. Recruitment sites for another protein like SHP2/ERK and STAT1/3 and the activation of many signaling cascades will be initiated as a result of the gp130's phosphorylation. In contrast, the second is named trans-signaling of IL-6, in the IL-6 trans-signaling, soluble IL-6R (sIL-6R) was recognized in several body fluids. In human, sIL-6R was manufactured via dropping of the membrane-bound IL-6R by metalloprotease A disintegrin and metalloproteinase (ADAM10 or ADAM17) or by alternative splice. In circulation, IL-6 can interact with sIL-6R and the IL-6/sIL-6R complex, (also designated Hyper-IL-6), links a membrane bound gp130, triggering a pathway identical to the pathway in the classical signaling that was discussed earlier (42) (21). The first classic signaling pathway is mainly active in hepatocytes and lymphocytes, while the second trans-signaling pathway is active in almost any cell type (32). The soluble gp130 (sgp130) is available and it is manufactured either by shedding using ADAM10 and ADAM17 preferentially or by alternative splicing. Sgp130 binds with the complex of IL-6/sIL-6R but does not bind with IL-6 alone. So, the sgp130 job is to catch the complex of IL-6/sIL-6R selectively, leading to the inhibition of trans-signaling of IL-6 with no disruption to the classical signaling of IL-6. In

animal studies, targeted inhibition of trans-signaling by sgp130 have helpful effects in the inflammatory disorders and in the atherosclerosis. It has been suggested that classical signaling mediates IL-6 anti-inflammatory and regenerative activities, while trans-signaling mediates IL-6 pro-inflammatory actions (42). Researchers stated that there is a correlation between different diseases and many different immune markers (2, 3, 5, 6, 8, 19).

Epidemiological studies have recorded a direct relation between the values of circulating IL-6 and the danger or severity of CHD (31).

#### **MATERIALS AND METHODS**

**Subject:** This study was done in Baghdad, Iraq between the period Oct. 2020 to Mar. 2021. Subject included 100 patients with clinically defined IHD who were admitted to the Iraqi Center for Heart Disease (male:55 and female:45 aged 40-75 years) and 60 healthy volunteers (male:34 and female:26 aged 39-60 years) as the control group.

#### **Blood sample**

The sample collection and laboratory investigation was done as the following, peripheral blood was drawn with a 5 ml sterilized syringe and was discharged in two aliquots. Three milliliters of blood were discharged in a gel tube and the tubes were centrifuged (4000 rpm\10 min.) after clot formation at room temperature. Eppendorf tubes were used for the serum storage until the ELISA measurements were done and the other 2 ml of blood was stored in EDTA tubes until the nucleic acid measurement was done. All tubes were stored at 20° C (22).

#### **Measurements of IL-6, IgG, and IgM**

Human Interleukin-6 (IL-6) ELISA kit (Elabscience), human *H. pylori* IgG (Hp-IgG) ELISA kit (Shanghai Yehua), and human *H. pylori* IgM (Hp-IgM) ELISA kit (Shanghai Yehua) were used to identify the IL-6 and *H. pylori* IgG and IgM values depending on the protocol of the manufacturer company.

#### **DNA extraction**

Extraction of the genomic DNA from peripheral blood mononuclear cells (PBMCs) was done by ReliaPrep Blood gDNA Miniprep System (Promega) (40) (7) to examine DNA of *H. pylori* in the human venous blood by PCR. Nano-Drop device was used to measure

the purity and the concentration of the extracted DNA (12).

#### **Molecular diagnosis of *H. pylori* by PCR**

For the detection of *H. pylori* DNA presence in the blood, DNA of blood samples were amplified by *H. pylori* specific 16s rDNA (or 16s rRNA) gene, sequence of primers was: C-97, 5'- GCT ATG ACG GGT ATC C-3' (276–291 forward), C-98, 5'- GAT TTT ACC CCT ACA CCA -3' (681–698 reverse), and the product size was ~ 400 base-pair (45) (24) (22) (13). Singleplex PCR program was as followed: initial-denaturation 94 °C and final extension 72 °C were for five minutes and 1 cycle for each, while denaturation, annealing, and extension were for only one minute but 35 cycles and the temperature was (94, 50, 72) °C respectively. The product was examined by 1 % agarose gel stained by ethidium bromide (1) and visualized using UV transilluminator (9).

#### **Statistical analysis**

The SAS 2012 {Statistical Analysis System} application was employed to detect the impact of different groups in the parameters of the study. The T-test was performed to significance and compare between the means. A Chi-square test was employed to significance compare the percentage (0.05 and 0.01) probability in the current study.

#### **RESULTS AND DISCUSSION**

In this case-control study, many parameters were examined, including the serum level of IL-6, Hp-IgG, Hp-IgM, and the presence of *H. pylori* DNA in the blood among IHD and healthy controls. This study was recruited 100 IHD patients and 60 healthy subjects.

#### **Serum levels of IL-6, Hp-IgG, Hp-IgM**

The results showed highly significant differences in the *H. pylori* IgG antibodies and IL-6 concentration in the serum of the patients infected with IHD compared to the healthy control group ( $p < 0.0001$ , for IgG and IL-6). However, there were no significant differences ( $p = 0.279$ ) in the *H. pylori* IgM antibodies concentration in the serum of IHD patients compared to the healthy control group, (Table 1).

**Table 1. Relation of some immune *H. pylori* parameters in IHD patients compared with healthy individuals**

Variables	Patients	Controls
Mean of <i>H. pylori</i> IgM conc. (ug/ml)	5.97±0.37	5.34±0.43
Mean of <i>H. pylori</i> IgG conc. (ug/ml)	1.814±0.08	0.823±0.09
Mean of IL-6 conc.(pg/ml)	49.73±6.60	0.21±0.02

*H. pylori* infection stimulates the manufacturing of immune antibodies. Serodiagnostic tests give the sensitivity, rapidity, and specificity for the identification of the bacterium. The anti-*H. pylori* IgM seroprevalence in the current study showed no significant differences between IHD patients and healthy individuals. The current study findings were in accordance with a few studies which estimated the relation of *H. pylori* with acute myocardial infarction and CHD. The results were in agreement with the study showed that there was no significant difference in *H. pylori* IgM levels between cases and controls ( $p = 0.29$ ) (31). Also, Viswanath *et al.*, reported that *H. pylori* IgM antibodies were analyzed and it was noticed that all of the subjects were negative for IgM antibodies (43). While a study was done in 2008 disagreed with the current study, Jha *et al.*, showed that heavy smokers were significantly associated with seropositivity ( $p = 0.044$  for IgM of *H. pylori*) (28). The results were in agreement with Jafarzadeh *et al.*, study who showed that the infection with *H. pylori* bacteria might participate in IHD pathogenesis directly or indirectly, and the study also reported that the anti-*H. pylori* IgG seroprevalence was 58.3 % among healthy individuals and 89.2 % among patients with IHD. Anti-*H. pylori* IgG was shown to be much more prevalent in patients with IHD than in healthy individuals ( $p < 0.0001$ ). Additionally, the association of *H. pylori* with IHD may vary by countries or even within a country. The variances are largely explained via variations in age, race, socio-economic condition, and ethnic origin. Additionally, variation in the inclusion criteria for patients and controls, genetic heterogeneity of *Helicobacter pylori*, and variations in the distribution of the IHD risk factors may

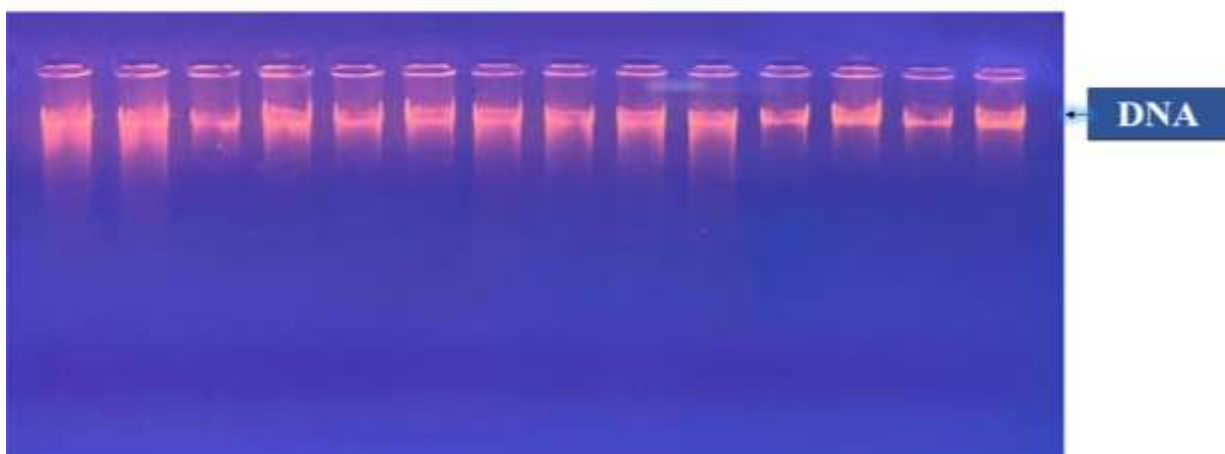
account for some of the differences. As a result, the findings of one study may not be applicable to other populations, even within the same area or the same country (26). Ismael *et al.*, suggested that there was a relationship between coronary artery disease and *H. pylori* infection, and reported that 78.57 % (55/70) of CAD patients had a positive result for anti-*H. pylori* IgG and these results showed great significant differences between the studied groups ( $p < 0.01$ ). This study and others support the hypothesis that *H. pylori* can be correlated with CAD or even be considered a risk factor for atherosclerosis plaque formation. *H. pylori* infection has been implicated in the development of atherosclerotic changes in coronary arteries, implying that this pathogen or its products (cytokines, cytotoxins, endotoxins, and several other virulence factors) have a detrimental effect on the coronary endothelium (23). An Egyptian study that targeted the relation between acute coronary syndrome (ACS) and *H. pylori* chronic infection. Ghaleb *et al.*, reported that ACS was assured in 94 % of the entire study sample with *H. pylori* IgG antibodies (mean 2.36 U/mL). The danger of ACS development in patients with greater *H. pylori* IgG was 1.6 times (95% CI: 0.662-1.704) (18). Also, Afsharpooyan and Mohammadian., showed that the predominance of *H. pylori* infection was 68.04 percent, and the mean level of the IgG was 67.35 percent (7). While the result of the current study disagreed with Mohammed *et al.*, who revealed that there were no significant differences between the patient and healthy individuals in the IgG levels ( $p = 0.53$ ) (31). Also, EL-Ageery *et al.*, showed that *H. pylori* IgG was high in both case and control groups ( $p = 0.346$ ). However, there have been conflicting reports regarding the relationship between serology and the occurrence of CAD. While some researchers found no correlation between *H. pylori* seropositivity and CAD, others found a significant correlation. These contradictory reports may be explained by the inability of antibody testing to distinguish between current and past infection (15). The IL-6 levels in healthy people's blood are between 1–5 pg/ml. IL-6 values rise several thousand folds during inflammatory conditions

(34). Numerous investigations established the function of interleukin-6 in the association between systemic inflammation and cardiovascular disease. The current study results agree with the results of a study in Saudi Arabia on a group of 100 patients which showed that there was a significant increase in the levels of IL-6 in the serum of patients with IHD than in the serum of the control group. The increase in IL-6 levels could be a result of the development and instability of atherosclerotic plaques via activation of leukocytes and endothelial cells, or it could be a result of the induction of various cytokines (25). Tomas *et al.*, reported that there is a significant variation ( $p < 0.001$ ) in the levels of IL-6 of people suffered from IHD (median of 33 pg/mL) from individuals without IHD (median of 3.8 pg/mL). As an inflammatory cytokine, IL-6, are known to increase the risk of CAD via the stimulation of the acute phase response and hepatic production of acute-phase proteins like the production of CRP and fibrinogen. This elevates blood viscosity and stimulates the proliferation and activity of the platelets. Following that, autocrine and paracrine activation of monocytes via IL-6 promotes fibrinogen deposition in the vessel wall. Then, IL-6 induces a reduction in the action and levels of plasma of the monomeric lipoprotein lipase, resulting in an increase in macrophage lipid uptake. As well as, IL-6 stimulates the hypothalamic-pituitary-adrenal

axis. Obesity, hypertension, and insulin resistance are all associated with this (41). Also, Furuto *et al* showed that *H. pylori* increase the levels of many inflammatory cytokines including IL-6 that directly or indirectly damage the vascular walls, thereby causing arteriosclerosis (14). Yildirim *et al.*, revealed that IL-6 levels were significantly higher in *H. pylori*-positive patients than in control ( $p < 0.05$ ). Infection with *Helicobacter pylori* results in the infiltration of macrophages into the gastric mucosa and subsequent secretion of the B-cell stimulatory cytokine IL-6. Additionally, they suggested that phagocytosis of the bacteria appears to be a requirement for induction of IL-6 in these cells (46). The current study disagrees with Afsharpooyan and Mohammadian., who indicates that there was no relationship between *H. pylori*-infected people and CVD because the IL-6 expression by the *H. pylori* effect has reported that the bacterium had no significant impact on it, and there were no main variations between the titer of *H. pylori*-positive and *H. pylori*-negative individuals and IL-6 (10).

#### Detection by molecular techniques

DNA quality and integrity were estimated through electrophoresis on 1% agarose for 1 hour. The bands appear sharp, single, not diffused and have no smear as shown in (Figure 1)



**Figure 1. Gel electrophoresis of genomic DNA of blood samples**

As mentioned below in (Table 2) results shows that there were a highly significant differences in PCR results between patients and healthy individuals ( $p = 0.0001$ ), DNA of *H. pylori* was recognized in the venous blood of 81 (81%) of 100 IHD patients by PCR using

*H.pylori* specific 16S rDNA (16s rRNA) primers and the amplified PCR products were ~ 400 bp (Figure 2). None of the healthy individuals gave a positive result for the *H. pylori* 16S rDNA gene.

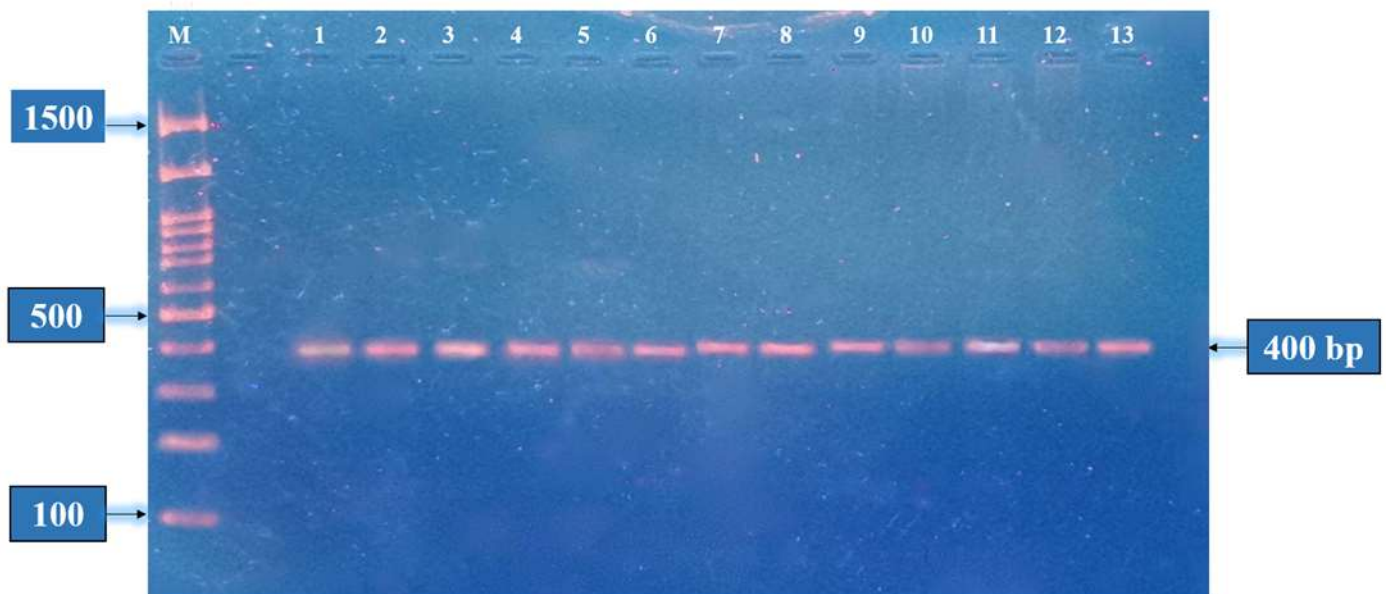
**Table 2. Distribution of sample study according to PCR results in patients and control.**

PCR	Group		p-value
	Patients No. (%)	Control No. (%)	
Positive	81 (81 %)	0 (0 %)	0.0001 *
Negative	19 (19 %)	60 (100 %)	0.0001 *
p-value	0.0001 *	0.0001 *	-----

\* ( $p \leq 0.01$ ).

A blood sample analysis by PCR is a faster and more accurate way to detect bacteremia than a blood culture. A bacterium can be recognized by looking for certain consensus sequences in its bacterial DNA, like the 16S rDNA gene. Therefore, Huang *et al.*, investigated *H. pylori* DNA in venous peripheral blood and gastric mucosa of individuals with chronic gastritis or peptic ulcer using PCR. They recognized the DNA of

*H. pylori* in blood in Three (15%) out of 20 patients while they recognized *H. pylori* in fifteen (75%) out of twenty gastric specimens, and Nine (45%) of those samples were positive for *H. pylori* culture. None of the healthy individuals were positive for Helicobacter genes. It's believed that *H. pylori* infection raises the risk of a variety of extra-intestinal diseases, such as coronary heart disease and autoimmune thrombocytopenia, as well as skin and hepatobiliary diseases (21). Abd-Alrahman *et al.*, used PCR technique to detect 16S rRNA for *H. pylori* in the DNA samples of whole human blood and they reported that 66.67 % of chronic urticarial patients (CU) and 70.37 % of atopic dermatitis patients (AD) were positive to 16S rRNA. Only 1 person out of 22 healthy persons gave a positive result to 16S rRNA. A highly significant difference ( $p < 0.01$ ) was shown through the statistical analysis (6).

**Figure 2. PCR result of the *H. pylori* 16S rDNA (16S rRNA) gene in IHD patient blood samples**

When infected with *H. pylori*, it can reduce the production of gastric mucus, cause a significant inflammatory response, and alter microcirculatory variables like blood flow and leukocyte activities. It can also alter vascular endothelial lining. As a result of these actions, the stomach mucosal membrane may be compromised, which could lead to mucosal damage leading to *H. pylori* penetrating the bloodstream. Several circumstances are expected to cause the enhanced translocation of microorganisms over the gastro-intestinal

barrier. These involve disturbance of the ecology of the indigenous gut microbiome resulting in bacterial overgrowth, reduced immunity of the host, and physical disruption of the intestinal mucosal lining via direct chemical damage, hemorrhagic shocks, and endotoxin (21). AL-Jobori *et al.*, when using the PCR for detection 16S rRNA gene which was specific for *H. pylori*, reported that 20 (100 %) of patients were positive for PCR from peripheral blood (13). The study result was in agreement with saeideh *et al.*, who

revealed that *H. pylori* can secrete DNA in peripheral blood besides its existence in gastric mucosa, and it is possible for the infection of *H. pylori* to cause bacteremia or inject DNA to blood by type IV secretory system. They tested *H. pylori* DNA in (18%) of all 100 serum samples of peptic ulcer and gastritis patients by PCR. Septicemia is not promoted by *H. pylori* but it does enable secretion of DNA into blood and host cells (38). The existence of *Helicobacter pylori* DNA has been proved in atherosclerotic plaques and aortic tissues of most of CHD patients. Of the 40 aortic wall samples from coronary patients, 32 (80%) were positive for *H. pylori*, whereas 17 of 20 (85%) specimens from valve patients were positive for *H. pylori*. This provides important evidence for the direct involvement of bacteria in disease pathogenesis. As a result, *Helicobacter pylori* can cause a direct effect on the induction of the inflammation within atherosclerotic plaques (36).

#### REFERENCE

1. Abbas, L. M. R., A. J. Hashim and W. Al-Hadban 2020. Identification local isolates of *Trichophyton mentagrophytes* and detection of keratinase gene using PCR technique. Iraqi Journal of Agricultural Science, 51(6), 1534-1542. <https://doi.org/10.36103/ijas.v51i6.1181>
2. Abd Al Kareem, R. M. and W. S. Abd 2020. Impact of EBV on multiple in a sample of Iraqi females: Immunological and molecular study. Iraqi Journal of Science, 61(5): 1008-1015
3. Abd, W. S. and R. M. A. A. Kareem 2020, December. Impact of EBV on multiple sclerosis in some of the Iraqi males: Immunological and molecular study. In AIP Conference Proceeding, 2290(1): 020023. AIP Publishing LLC
4. Abd, W.S. and N.S. Abd 2021. Ischemic Heart Disease in correlation with some immunological parameters, the Fifth Scientific Conference for Scientific and Human Studies 2021, AIP conference proceeding. AIP Conference Proceedings 2414, 060005 (2023); <https://doi.org/10.1063/5.0116787>. Published Online: 13 February 2023
5. Abd W.S. Incidence of Psoriatic Arthritis in Psoriatic Patients in Some of Iraqi Patients according to some serological Tests. 2019 Journal of Pakistan Association of Dermatologists. 30(1): 72-80. <https://www.jpap.com.pk/index.php/jpap/article/view/1304/1350>.
6. Abd W.S. Abd N. S. Celiac and Thyroid Diseases in Some of Iraqi Patients with Juvenile Rheumatoid Arthritis. Indian Journal of Public Health Research & Development. 10(10).2072-2075. <https://web.p.ebscohost.com>.
7. Abd-Alrahman, T. Z., R. S. Aboud and S. D. Al-Ahmer 2017. Immunological and molecular detection of *Helicobacter pylori* in patients clinically diagnosed with chronic urticarial and atopic dermatitis. Current Research in Microbiology and Biotechnology, 5(3), 1082-1087
8. Abd Mohsen , W.S. 2005. ANA,RF and CRP in patients with some of rheumatic symptoms. Baghdad Science Journal. 2(4),638-644. <https://www.iasj.net/iasj/article/67947>
9. Abdullah, D., & Aloubaidy, R. M. . 2022. Genetic Polymorphism of Caspase 8 and 9 in Iraq. Iraqi Journal of Agricultural Science, 53(3),505–514. <https://doi.org/10.36103/ijas.v53i3.1558>
10. Afsharpooyan, S. and T. Mohammadian 2019. Seroepidemiology study of *Helicobacter pylori* infection and its effect on cardiovascular diseases in Karaj, Iran. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy, 38(6), 277-281
11. Ahmed N.A. S.S. Mahmood, and A.H. Abbass. 2019. A comparative study of some virulence factors and phylogenetic characterization of escherichia.coli isolation causing urinary tract infection and The commensal gut microbiota. Iraqi Journal of Agricultural Sciences, 50(4):1193-1198. <https://doi.org/10.36103/ijas.v50i4.763>
12. Atta, R. Z. . and R. M. Aloubaidy. 2022. Genetic polymorphism of asthma in Iraq. Iraqi Journal of Agricultural Science: 53(2):288–296. <https://doi.org/10.36103/ijas.v53i2.1536>
13. AL-Jobori, M.B., M. T. AL-Oqaili and E. M. Abdulla 2011. Detection of 16S rRNA gene of *Helicobacter pylori* in patients with peptic ulcer and gastric carcinoma: molecular and bacteriological study. Egypt. Acad.J. Biology. Sci. 3(1): 95-104
14. AL-Obeidy, E. S. and B. N. Saeed 2011. *H. Pylori* infection in Iraqi patients with

ischemic heart diseases. Journal of the Faculty of Medicine Baghdad, 53(1), 29-31

15. EL-Ageery, S. M., N. S. Gouda, I. M Fawzy, A. Bahy-Eldeen and R. Mahmoud 2020. Serological evidence of association between *Helicobacter pylori* infection and coronary artery disease. African Journal of Clinical and Experimental Microbiology, 21(2), 88-96

16. Fox, J. G., F. E. Dewhirst, Z. Shen, Y. Feng, N. S. Taylor, B. J. Paster, ... and I. Roa 1998. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology, 114(4), 755-763

17. Furuto, Y., M. Kawamura, J. Yamashita, T. Yoshikawa, A. Namikawa, R. Isshiki, ... and Y. Shibuya 2021. Relationship between *Helicobacter pylori* infection and arteriosclerosis. International Journal of General Medicine, 2021(14): 1533–1540

18. Ghaleb, R., H. Mansour, M. O. Amer, A. Ismael, A. S. Bhagavathula and A. E. Abd Elrazek 2017. Is there a role of *Helicobacter Pylori* infection in incidence of acute coronary syndrome?. Int. J. Adv. Biomed, 2(2), 25-33

19. Ghufran, Q. A. A. and W. S. Abd 2020 Immunological study on some of psoriatic iraqi patients treated with etanercept. International Journal of Psychosocial Rehabilitation, 24

20. Hasegawa, H., I. Mizoguchi, Y. Chiba, M. Ohashi, M. Xu and T. Yoshimoto 2016. Expanding diversity in molecular structures and functions of the IL-6/IL-12 heterodimeric cytokine family. Frontiers in Immunology, 7, 479

21. Huang, Y., X. G. FAN, Z. S. TANG, L. Liu, X. F. TIAN and N. Li 2006. Detection of *Helicobacter pylori* DNA in peripheral blood from patients with peptic ulcer or gastritis. Apmis, 114(12), 851-856

22. Hussein, A. M., and R. Al Sahlanee. 2022. Serological and molecular evaluation of MRP14 in thyroiditis and Its role in pro-inflammatory chemokines activation. Iraqi Journal of Agricultural Science: 53(6), 1368–1376. <https://doi.org/10.36103/ijas.v53i6.1652>

23. Ismael, M. K., M. R. Al-Haleem and R. S. Salman 2015. Evaluation of anti-*Helicobacter pylori* antibodies in a group of Iraqi patients with atherosclerosis and coronary artery

disease. Iraqi Journal of Science, 56(1A), 81-88

24. Izadi, M., M. Fazel, S. H. Sharubandi, S. H. Saadat, M. M. Farahani, M. H. Nasserri, ... and S. Taheri 2012. Helicobacter species in the atherosclerotic plaques of patients with coronary artery disease. Cardiovascular Pathology, 21(4), 307-311

25. Jabir, N. R., C. K. Firoz, M. A. Kamal, G. A Damanhour, M. N. Alama, A. S. Alzahrani, ... and S. Tabrez 2017. Assessment of genetic diversity in IL-6 and RANTES promoters and their level in Saudi coronary artery disease patients. Journal of Clinical Laboratory Analysis, 31(5), e22092

26. Jafarzadeh, A., A. Esmaeeli Nadimi, M. Nemati, M. Tahmasbi and P. Ahmadi 2010. Serum concentrations of *Helicobacter pylori* IgG and the virulence factor CagA in patients with ischaemic heart disease. EMHJ-Eastern Mediterranean Health Journal, 16 (10), 1039-1044, 2010

27. Jamkhane, P. G., S. G. Gattani and S. A. Farhat 2016. *Helicobacter pylori* and cardiovascular complications: a mechanism based review on role of *Helicobacter pylori* in cardiovascular diseases. Integrative medicine research, 5(4), 244-249

28. Jha, H. C., J. Prasad and A. Mittal 2008. High immunoglobulin A seropositivity for combined Chlamydia pneumoniae, *Helicobacter pylori* infection, and high-sensitivity C-reactive protein in coronary artery disease patients in India can serve as atherosclerotic marker. Heart and vessels, 23(6), 390-396

29. Khan, M. A., M. J. Hashim, H. Mustafa, M. Y. Baniyas, S. K. B. M. Al Suwaidi, R. AlKatheeri, ... and S. N. A. H. Lootah 2020. Global epidemiology of ischemic heart disease: Results from the global burden of disease study. Cureus, 12(7).

30. Knuuti, J., W. Wijns, A. Saraste, D. Capodanno, E. Barbato, C. Funck-Brentano, ... and J. J. Bax 2020. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes: the Task Force for the diagnosis and management of chronic coronary syndromes of the European Society of Cardiology (ESC). European heart journal, 41(3), 407-477



31. Mohammed, S. A., D. A. Kadhum and M. K. Abbas 2019. Serologic study to detect a relation between myocardial infraction diseases and *Helicobacter pylori* infections in Baqubah City, Iraq. *Indian Journal of Public Health*, 10(8), 101
32. Morieri, M. L., A. Passaro and G. Zuliani 2017. Interleukin-6 “Trans-Signaling” and ischemic vascular disease: the important role of soluble gp130. *Mediators of inflammation*, 2017
33. Nakagawa, H., T. Tamura, Y. Mitsuda, Y. Goto, Y. Kamiya, T. Kondo, ... and N. Hamajima 2013. Significant association between serum interleukin-6 and *Helicobacter pylori* antibody levels among *H. pylori*-positive Japanese adults. *Mediators of inflammation*, 2013:5
34. Piko, N., S. Bevc, R. Hojs, F. H. Naji and R. Ekart 2021. The association between pulse wave analysis, carotid-femoral pulse wave velocity and peripheral arterial disease in patients with ischemic heart disease. *BMC Cardiovascular Disorders*, 21(1), 1-9
35. Rasmi, Y. and M. H. Seyyed-Mohammadzad 2012. Frequency of *Helicobacter pylori* and cytotoxine associated gene A antibodies in patients with cardiac syndrome X. *Journal of cardiovascular disease research*, 3(1), 19-21
36. Reszka, E., B. Jegier, W. Wasowicz, M. Lelonek, M. Banach and R. Jaszewski 2008. Detection of infectious agents by polymerase chain reaction in human aortic wall. *Cardiovascular Pathology*, 17(5), 297-302.
37. Rose-John, S. 2018. Interleukin-6 family cytokines. *Cold Spring Harbor perspectives in biology*, 10(2), a028415
38. Saeideh, H. E. Davoud, F. Jalil and R. Jafar 2013. The presence of cagA genome in blood of infected patient with *Helicobacter pylori* new marker. *J. Bacteriol. Res.* 5(4).46-50
39. Sampasa-Kanyinga, H. and R. F. Lewis 2015. Frequent use of social networking sites is associated with poor psychological functioning among children and adolescents. *Cyberpsychology, Behavior, and Social Networking*, 18(7), 380-385
40. Tabarek A. A., and M. A. Reema. 2023. Genetic Polymorphisms of HLA-G Gene in Rheumatoid Arthritis. *Iraqi Journal of Agricultural Science*, 54(2), 378–387. <https://doi.org/10.36103/ijas.v54i2.1712>
41. Tomas, M. J., P. M. Baluyot, A. L. D. Santos, J. Faustino, E. Licudine, E. M. Mendoza, ... & M. J. Tiburcio 2015. Interleukin 6 as a biomarker of ischemic heart disease. *International Journal of Scientific and Research Publications*, 5(7), 1-4
42. Villar-Fincheira, P., F. Sanhueza-Olivares, I. Norambuena-Soto, N. Cancino-Arenas, F. Hernandez-Vargas, R. Troncoso, ... and M. Chiong, 2021. Role of Interleukin-6 in vascular health and disease. *Frontiers in Molecular Biosciences*, 8, 79
43. Viswanath, V., S.R. Padmaraj and D. Roy 2015. Genetic instabilities in *H. pylori* infected cardiovascular diseases. *International Journal of Scientific & Engineering Research*, 6
44. Wang, B., P. Li, F. He, Y. Sha, X. Wan and L. Wang 2021. Spatiotemporal variations in ischemic heart disease mortality and related risk factors in China between 2010 and 2015: a multilevel analysis. *BMC Public Health*, 21(1), 1-10
45. Yakoob, J., S. Abid, W. Jafri, Z. Abbas, M. Islam, and Z. Ahmad 2006. Comparison of biopsy-based methods for the detection of *Helicobacter pylori* infection. *British Journal of Biomedical Science*, 63(4), 159-162
46. Yildirim, Z., B. Bozkurt, D. Ozol, F. Armutcu, R. Akgedik, H. Karamanli, ... and M. İkizek 2016. Increased exhaled 8-isoprostane and interleukin-6 in patients with *Helicobacter pylori* infection. *Helicobacter*, 21(5), 389-394.