

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
 Open Access Macedonian Journal of Medical Sciences. 2020 May 10; 8(B):172-175.
<https://doi.org/10.3889/oamjms.2020.3199>
 eISSN: 1857-9655
 Category: B - Clinical Sciences
 Section: Endocrinology



Interleukin-6, Tumor Necrosis Factor- α , and High-sensitivity C-reactive Protein in Diabetic Patients with *Helicobacter pylori* in Kosovo

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Abstract

Helicobacter pylori is a Gram-negative spiral-shaped bacterium that infects from 30% to 50% of the world's population and it is one of the most important in dyspeptic syndrome causes of gastritis and peptic ulcer. *H. pylori* is one of the most common chronic bacterial infections especially in the development countries because the socioeconomic contribute to chronic disease. The infection induces an acute polymorphonuclear infiltration in the gastric mucosa. Infection with *H. pylori* has been epidemiologically linked to some extra digestive conditions, including ischemic heart disease, diabetes mellitus (DM), and others. The patients with DM are at risk for *H. pylori* infection, since they have coupled susceptibility of to a wide range of infections as a result of chronic elevation of blood glucose level and impairment of immune functions. Chronic inflammation is a risk factor for coronary heart disease, because inflammation, vascular injury and thrombosis are considered to cause atherosclerosis. The risk of cardiovascular events is associated with increased levels of the acute phase proteins, C-reactive protein (CRP), and pro-inflammatory cytokines. Interleukin 6 (IL-6), a major pro-inflammatory cytokine is produced in a variety of tissues, including activated leukocytes, adipocytes, and endothelial cells. CRP is the principal downstream mediator of the acute phase response and is primarily derived through IL-6-dependent hepatic biosynthesis. Tumor necrosis factor- α (TNF- α), as an important inflammatory factor, has been shown to play a central role in the pathogenesis of diabetes. CRP and IL-6 were determinant of risk for the development of type 2 DM in apparently healthy middle-aged women. Since the prevalence of infected persons with *H. pylori* in Kosovo is high, the aim of this study was the evaluation of cytokines (IL1, TNF- α) and CRP in diabetic type 2 patients with positive *H. pylori*.

Edited by: Sasho Stoleski
Citation: Begolli-Stavileci G, Begolli G, Begolli L. Interleukin-6, Tumor Necrosis Factor- α , and High-sensitivity C-reactive Protein in Diabetic Patients with *Helicobacter pylori* in Kosovo. Open-Access Maced J Med Sci. 2020 May 10; 8(B):172-175. <https://doi.org/10.3889/oamjms.2020.3199>
Keywords: *Helicobacter pylori*; Interleukin-6; Tumor necrosis factor- α ; High sensitivity C-reactive protein; Coronary heart disease
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Received: 11-Jun-2019
Revised: 10-Feb-2020
Accepted: 05-May-2020
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Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
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Introduction

Helicobacter pylori is one of the most common chronic bacterial infections in the world, especially in the development countries because the socioeconomic status contribute to chronic disease [1], [26]. Infection with *H. pylori* (*H. pylori* is a Gram-negative bacteria) has been epidemiologically linked to some extra digestive conditions, including ischemic heart disease, diabetes mellitus (DM), neurological disease, gynecological disease, ophthalmology, skin, and oral mucosa disease, respiratory, ear, nose and throat disease, and hematologic disease [2].

The patients with DM are at risk for *H. pylori* infection [3], since they have coupled susceptibility of to a wide range of infections because of chronic elevation of blood glucose level and impairment of immune functions [4].

Chronic inflammation is a risk factor for coronary heart disease, because inflammation, vascular injury and thrombosis are considered to cause

atherosclerosis [5], [6], [7], [8]. The risk of cardiovascular events is associated with increased levels of the acute phase proteins, C-reactive protein (CRP), and pro-inflammatory cytokines [9].

Interleukin 6 (IL-6), a major pro-inflammatory cytokine, is produced in a variety of tissues, including activated leukocytes, adipocytes, and endothelial cells. CRP is the principal downstream mediator of the acute phase response and is primarily derived through IL-6-dependent hepatic biosynthesis. Tumor necrosis factor- α (TNF- α), as an important inflammatory factor, has been shown to play a central role in the pathogenesis of diabetes [10].

CRP and IL-6 were determinant of risk for the development of type 2 DM in apparently healthy middle-aged women [11].

Since the prevalence of infected persons with *H. pylori* in Kosovo is high, the aim of this study was the evaluation of cytokines (IL-6, and TNF- α) and CRP in diabetic type 2 patients with positive *H. pylori*.

Methods

Study subject

The study was an observational study performed through case-control method in which was two identify groups one as a study group and one as a control group conducted from January 2017 to January 2019.

The patients diagnosed with diabetes type 2 were selected in the Internal Clinics of University Clinical Centre of Kosovo, whereas the samples analysis took place in the Medical Laboratory “Bioticus” in Kosovo.

The following subjects were excluded from this study: Pregnant woman, patients with antibiotic treatment or any other medication known to affect the inflammatory markers and those with any diagnosed systematic disease. The study was performed in compliance with human – studies guidelines and all participants were informed about the study and the nature of procedure was explained.

Detailed analysis

Blood was obtained from each participant subject by venipuncture using tubes without anticoagulant. After this, the tube was immediately transferred for centrifugation at 2000 rpm for 10 min and transferred into Eppendorf tubes and stored at temperature -20°C.

Serum samples were obtained for the determination of *H. pylori* antibodies, specific immunoglobulin G (IgG) antibodies with a specific high sensitivity method using the enzyme-linked fluorescent assay technique in an automated instrument of the Vidas Family a Mini Vidas (bioMerieux, Vittek, France).

For IL-6, TNF -α, and high sensitivity CRP (hs-CRP) serum samples were assessed using a kit with a specific high sensitivity methodology – ELISA test, according to the manufacturer’s instructions (IBL International GmbH, Hamburg, Germany).

Statistical analysis

Statistical analysis was analyzed with the Vassar-Stats system. T-test was conducted and the average, correlation (r) and standard deviation were counted. These results are shown in the table presentation. The differences in control group and study group were compared using the Student’s t-test of mean value. The average and standard deviation were assessed for IL-6, TNF-α, and hs-CRP. The correlation of IL-6, TNF-α, and hs-CRP was compared between diabetic patients with *H. pylori* positive and healthy subject. Differences were considered significant when $p < 0.05$.

Results

Eighty participants were enrolled in this study aged between 40 and 60 years old both gender.

All participants were divided in two groups. The first study group contains 50 patients, 30 male and 20 female who were identified as a diabetic type 2 patients with regular oral or parenteral therapy and also positive *H. pylori* IgG antibodies and control group which contains 30 subjects which were identified as healthy subject with no disease.

Control group was healthy subjects without any clinical signs related to diabetes and *H. pylori*.

Mean values in diabetic patients and control group are shown in Figure 1.

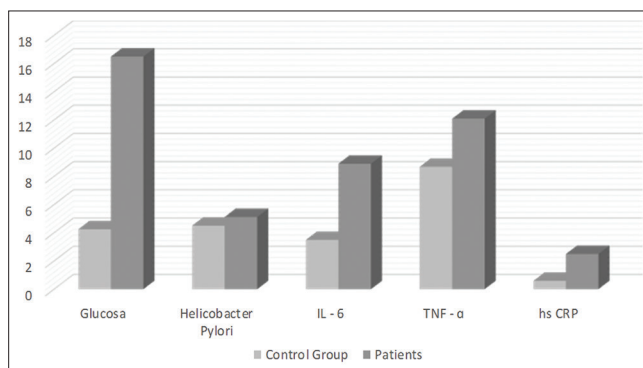


Figure 1: The comparison of mean values in patients and control group

Comparisons of inflammatory values in diabetic patients with *H. pylori* positive and control group are summarized in Table 1.

Table 1: Inflammatory markers in patients and control groups

Parameters	Healthy n=30	Diabetic with <i>H. pylori</i> n = 50	p-value
Age (years, mean±SD)	42.13±1.92	52.22±4.66	
Gender n %			
Female	14 (47)	20 (40)	
Male	16 (53)	30 (60)	
Glucose			
Mean±SD	4.26±0.47	16.5±5.53	
<i>H. pylori</i> IgG			
Mean±SD	4.51±1.55	5.12±4.3	
IL-6			
Mean±SD	3.5±1.1	8.9±14.3	0.02*
TNF-α			
Mean±SD	8.68±6.19	12.1±8.7	0.04*
hs-CRP			
Mean±SD	0.6±0.5	2.5±2.6	0.001*

*Significant difference (Mann-Whitney test). IL-6: Interleukin 6, TNF-α: Tumor necrosis factor-alpha, hs-CRP: High sensitive C-reactive protein, *H. pylori*: *Helicobacter pylori* IgG antibodies.

Statistical analysis was shown a significant difference of inflammatory cytokines in healthy subject and those diabetes patient with *H. pylori* positive with regard to the levels of IL-6 (3.5±1.1 vs. 8.9 ± 14.3, respectively, $p < 0.02$) and TNF-α (8.68 ± 6.19 vs. 12.1 ± 8.7, respectively, $p < 0.04$).

The mean serum level of hs-CRP was significantly higher $p < 0.001$ in diabetic patient with *H. pylori* than in healthy subject (2.5 ± 2.6 vs. 0.6 ± 0.5).

We found also the correlation and significance between parameters in patients and control group (Table 2).

Table 2: Correlation and significance between parameters in patients and control group

Correlation	Control group		Diabetic with <i>H. pylori</i>	
	R	p-value	r	p-value
HPY/IL-6	-	-	0.49	0.005*
HPY/TNF- α	-	-	0.02	0.44
HPY/hs-CRP	-	-	0.56	0.001*
IL-6/TNF- α	-0.06	0.37	0.23	0.11
IL-6/hs-CRP	0.07	0.34	0.48	0.004*
TNF- α /hs-CRP	-0.12	0.25	0.08	0.33

*Significant difference (Mann-Whitney test). HPY: *Helicobacter pylori* IgG antibodies, IL-6: Interleukin 6, TNF- α : Tumor necrosis factor-alpha, hs-CRP: High sensitive C reactive protein, *H. pylori*: *Helicobacter pylori*.

Discussion

H. pylori may have an impact on cardiovascular conditions and metabolic syndrome [12], [13], [14]. Several studies have evaluated the relation of *H. pylori* infection with coronary artery disease; a study done by Kowalski concluded that there is a significant link between coronary artery disease and infection with *H. pylori* [15]. A study by Bener *et al.* [16] suggested that there is a significant association between *H. pylori* infection and Type 2 DM and interestingly, this infection is significantly higher in diabetic obese patients.

H. pylori infection stimulates the production of proinflammatory cytokines [27] potentially mediated by elevated inflammatory markers such as reactive protein C (CRP) [17], [18], [19] and interleukin (IL)-6. Inflammation and activated innate immunity have also been implicated in the pathogenesis of diabetes through insulin resistance [20], [21]. For example, elevated levels of inflammatory cytokines may lead to phosphorylation of serine residues on the insulin receptor substrate, which prevent its interaction with insulin receptors, inhibiting insulin action. Lipopolysaccharides from pathogens in the gut, such as *H. pylori*, have also been linked to the activation of toll-like receptors, resulting in energy harvesting, fat accumulation and stimulation of the innate immune system, and consequent insulin resistance [22], [23].

Low socioeconomic status includes chronic infections such as infection with *H. pylori*. Other contributor as risk factors are poor diet, smoking and physical inactivity are well-known contributors to the disparity, but only partially explain the gap in health states.

Based in literature *H. pylori* infection caused increase of inflammatory cytokines and CRP [17], [18], [19].

The results of some studies have shown that there is a higher prevalence of *H. pylori* infection in patients with DM type 2 in comparison to control group [6], [8], [16], [24], [25], [26], [27].

Infection with *H. pylori* was found in the previous studies to be correlated with elevated levels of CRP, IL-6, and TNF- α which are markers of inflammation implicated in insulin resistance and development of diabetes [9], [21].

The goals of this investigation were to compare the mean levels of TNF- α , IL-6, and hs-CRP in serum samples of healthy subjects and diabetic subjects seropositive *H. pylori*. The studied cytokines were found to be increased in patient group compared to their levels in the healthy subjects. In addition, our study suggests that the gastric epithelial cells contribute substantially to the pro-inflammatory cytokine response to *H. pylori* infection, either by active cytokine production or by uptake of cytokines produced by the lamina propria or intraepithelial leukocytes [19]. It is very important to emphasize that this is the first study conducted in Kosovo estimating serum hs-CRP, IL-6, and TNF- α levels in diabetic patients with positive *H. pylori* and healthy persons, to have general information on the Kosovo population and connection with inflammatory markers.

Conclusion

H. pylori may have an impact on cardiovascular conditions and metabolic syndrome potentially mediated by elevations in inflammatory markers such as CRP and IL-6. Inflammation and activated innate immunity have also been implicated in the pathogenesis of diabetes through insulin resistance.

Acknowledgments

We thank the personnel of the Institute of Biochemistry-University of Prishtina and personnel of Medical Laboratory "Bioticus" for their support during this study.

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