INVESTIGATING THE CORRELATION BETWEEN LEVEL OF IGG ANTIBODIES AGAINST H.PYLORI AND ACUTE NON-CARDIOEMBOLIC ISCHEMIC STROKE IN ADULTS

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A R T I C L E   I N F O

A B S T R A C T

Introduction: Acute ischemic stroke that includes thrombotic and embolic types is one of the major causes of morbidity and mortality worldwide. Identifying risk factors and the possibility of intervention in its modifiable varieties is useful in its incidence rate and its clinical manifestations intensity. The objective of this study was to evaluate serum levels of IgG against H. pylori in patients with non-cardioembolic ischemic stroke compared with healthy people.

Methods: In this study, 140 patients were selected, which they were divided into control and experimental groups (each group containing 70 patients), who were hospitalized in the Neurology Ward of Imam Khomeini Hospital in Urmia in Iran in the three last months of 2012 and three first months of 2013. Serum IgG levels in both groups were examined using ELISA test by one device and one kit (Leading made in Germany). Finally, the SPSS20 software was used to analyze the data.

Results: In this study, significant difference was found between anti-H. pylori IgG antibodies level of patients in two groups and high blood pressure (P=0.03). In addition, there is significant difference between anti-H. pylori IgG antibodies level of the two groups of diabetic patients (P=0.001). The significant difference was also found between the anti-H. pylori IgG antibodies level of two groups and high blood fat (P=0.002).

Conclusion: According to the results of this study, H. pylori infection can be involved in patients with cerebral ischemic. In this regard, risk factors for CVA (high blood pressure, diabetes, high blood fat) can be effective factors.

Introduction

Helicobacter pylori (H.pylori) are spiral microaerophilic gram-negative bacilli exclusively colonized in the human stomach and especially in the gastric antrum (1 and 2). The bacterium infects about 50% of world population and its prevalence increases with increased age and reduced socio-economic status of humans (1). It also has a high prevalence in developing countries (3). The transmission of the infection is oral-oral or fecal-oral depending on environmental conditions and lifestyle including health, living conditions, and supply of drinking water (3, 4).

This bacterial infection is associated with chronic gastritis, peptic ulcer, gastric cancer, and gastric lymphoma (5). The infection is diagnosed by biopsy of the stomach, culture, PCR, and positive urease reaction (6). Many studies have stated that this organism acts as a possible risk factor for coronary artery and cerebrovascular diseases (7 and 8). In several studies, the

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role of this infection has been considerable in coronary artery diseases and in more than 90% of cases, IgG titer was higher in patients group compared to control group, but after adjustment of other risk factors, its role was undermined. Its reason is due to multiplicity of risk factors of cardiovascular diseases. Although the pathogenesis mechanism of H. pylori in vascular diseases is still unknown, its role in the pathogenesis of atherosclerosis along with several other infectious factors such as Chlamydia pneumoniae has been proven. In one study, it was stated that infection with this bacterium leads to homocysteine, followed by formation of atherosclerotic blood vessels. In most studies, the role of reactions mediated with immune system including increased acute phase reactants (including CRP), increased fibrinogen and increased lipids in chronic infection with H.pylori and their effects on the vessel wall in order to justify the pathogenicity of organism has been emphasized (9-12). In addition, it has been stated that helicobacter leads in heart disease increased thrombosis and stroke by increasing the levels of blood fibrinogen and increasing the risk of thrombosis (8-10). Cerebral stroke refers to acute sensory, motor, perceptual, cognitive and speech impairment (13). Type of its ischemic is created due to impaired blood flow of part of the brain that leads to sudden dysfunction in that part of brain (14). Vascular cerebral ischemic events, despite limited developments in treatment area, are one of the most common causes of death and disability in the world, which is due to limited capacity of central nervous system reconstruction (15). For this reason, it is one of the most important health problems in the world (16-18). In Iran, the death rate caused by cerebral stroke was estimated 8% in statistical investigations in 2003 and the rate of life lost years was estimated 4.4 (16, 19). In recent years, studies on risk factors for ischemic stroke have been increased and the role of factors such as oxidative stress and bacterial infections such as Chlamydia pneumoniae and H.pylori has been considered (19). These studies show the incidence of seropositivity for this bacterium in cases of thrombotic stroke was higher compared to embolic subtype (16, 17 and 20). Due to the lack of extensive study in this case, the study is one of the first controlled studies conducted to examine the relationship between H. pylori infection and non-cardioembolic ischemic CVA disease using serum markers. The importance of issue is revealed when we realize that H.pylori infection has known role in cardiovascular atherosclerosis in IHD disease, and considering the major several risk factors common between the disease and non-embolic cerebral stroke, it is supposed that H.pylori infection to be involved in the development of this disease (21, 22). On the other hand, as H.pylori infection is considered among modifiable risk factors for the disease, it can be eradicated using antibiotic treatment. The relationship between serum level of antibodies against H. pylori and the occurrence of non-cardioembolic ischemic stroke has not been examined in Iran and there is no adequate information in this regard. Therefore, this study was conducted to determine and compare the positive - negative presence of IgG against H. pylori in serum, to investigate antibody titer in positive cased between patients with non-cardioembolic ischemic stroke and healthy subjects, so that the relationship between the two issues to be determined.

Method

This research is an analytical (case-control) and prospective study. The total study population included 140 patients who were divided into control group and experimental groups (each containing 70 people). The patients were selected among the patients hospitalized in Imam Khomeini Hospital of Urmia in Iran in the last three months of 2012 and the first three months of 2013 (among patients hospitalized in Neurology Ward) by using simple random sampling. Selecting the control group was based on a homogeneity of background and confounding factors (age, gender) between the two groups. Demographic data including age and gender were collected through checklist. Laboratory data that are the serum levels of anti-H. pylori IgG antibodies were collected through taking 2 cc of blood for necessary tests during hospitalization and all samples underwent antibody investigation after collection by ELIZA method. The authorities of experiments were quite unaware if samples are control and experimental. In all of these experiments, one type of device and kit (leading made in Iran) was used. In this study, to determine the effect of IgG level of H. pylori as an independent risk factor in the development ischemic stroke, the known risk factors of this disease were excluded from study, unlike other studies. Accordingly, a series of selection criteria was defined for control and experimental group as follows:

 The inclusion criteria of study included:

Inclusion criteria of patients were:

1. The first time for stroke

2. Being in the age range between 50 and 75 years

3. Hospitalization within 72 hours of the incident

Exclusion criteria of study included:

1. Previous history of stroke or ischemic heart disease

2. Approved history of PUD

The collected data were entered to statistical software of SPASS 20 and

Logistic regression test, t- test, and Chi- square test were used to analyze the data.

Results

Among study group patients, 32 patients (45.7%) were males and 38 (54.3%) of them were female. In the control group, 31 patients (44.3%) were male and 39 (55.7%) of them were female. Additionally, in this study, the mean age of the patients in the study group was 66.70 ± 12.99, while it was 65.98 ± 10.38 in the control group.

Results in this study showed that the average anti-H. pylori IgG antibodies in the study group was 64.53 ± 45.19, and it was 35.50± 32 in control group, that this difference was statistically significant (P=0.0001)

Out of 70 patients in the study group, 9 patients (12.9%) had negative H. pylori infection while 61 patients (87.1%) had positive H. pylori infection

In the control group, 19 patients (27.1%) had negative H. pylori infection while 51 (72.9%) of them had positive H. pylori infection. According statistical test, there is significant difference between the two groups in terms of H.pylori infection (P=0.02).

Comparing risk factors for CVA (high blood pressure, hyperlipidemia and diabetes), results showed that in the study group, 56 (80%) patients had positive CVA risk factors and 14 patients (20%) had negative CVA risk factors.

In the control group, 45 patients (64.3%) had positive risk factors for CVA and 25 patients (35.7%) had negative CVA risk factors. According to the results, statistically significant difference was found between the study group and the control group in terms of CVA risk factors (P=0.004).

Out of 70 patients in the study group, 47 patients (67.1%) had high blood pressure, and in the control group, 21 patients (30%) had high blood pressure, which statistically significant difference was found between control and study groups results in terms of high blood pressure (P=0.001).

In our study, Cardiac arrhythmia was not found in control and study groups. In addition, no case of smoking was reported in the study group patients.

Diabetes mellitus

In this study, 35 patients in study group (50%) had diabetes mellitus and in the control group, 12 patients (17.1%) had diabetes mellitus. According to statistical analysis, there is significant difference between the two groups in terms of diabetes mellitus (P=0.000).

Out of 70 patients in the study group, 12 patients (17.1%) had hyperlipidemia, while no hyperlipidemia was found in the control group. Statistically, significant difference was found between the two groups in terms of hyperlipidemia (p=0.01). The results of this study showed that level of anti-H. pylori IgG antibodies in patients with positive was 65.68 ± 51.27, and it was 18.82 ± 11.27 in patients with negative CVA. Significant difference was found between the patients with positive and negative CVA in terms of anti-H. pylori IgG antibodies level (P=0.003).

H.pylori infection was reported in 42 patients (89.4%) who had positive CVA, and it was found in 16 patients (69.6%) who had negative H. pylori. According to statistical analysis, significant difference was found between H. pylori infection and risk of CVA (P=0.04).

Table 1: frequency distribution of H.pylori infection in study group patients based on CVA

H.pylori infection	CVA		Total
	Positive	Negative	
Negative	(%25)14	(%75)42	(%100)23
Positive	(%50)7	(%50)7	(%100)14
total	(%30)21	(%70)49	(%100)70

H.pylori infection was reported in 28 male patients (59.6%) and 21 (60%) female patients. According to statistical analysis, significant difference was not found between H. pylori infection and gender in the study group patients (p=0.05).

Table 2: determination of H. pylori infection in study group patients based on gender

Gender	H. pylori infection		Total
	Negative	Positive	
Male	(%20)7	(%80)28	(%100)35
Female	(%40)14	(%60)21	(%100)35
Total	(%30)21	(%70)49	(%100)70

The examine the effect of risk factors for high blood pressure, diabetes and high blood fat, we divided the patients randomly to two groups and by investigating the mean level of H.pylori, we observed that:

anti-H. pylori IgG antibodies level in the study patients with high blood pressure was 71.31 ± 50.25, and it was 42.13 ± 32.49 in control group patients. There is significant difference between anti-H. pylori IgG antibodies of patients of two groups and high blood pressure (P=0.03).

Anti-H. pylori IgG antibodies level in study group patients with diabetes was 77.50 ± 38.47, while it was 50.25±2.32 in the control group, which significant different was found between anti-H. pylori IgG antibodies level of patients in two groups with diabetes (P=0.001).

anti-H. pylori IgG antibodies level in patients with high blood fat was 78.47 ± 67.7 and it was 55.51 ± 40.25 in the control group. According to statistical analysis, there is significant difference between anti-H. pylori IgG antibodies level in two groups with high blood fat (P=0.002).

According to the results of logistic regression model, the variables of age and range of anti-H. pylori IgG antibodies, DM were significant in the model, which the results are as follows.

By increasing one unit of age, by remaining the variables of anti-H. pylori IgG antibodies and DM constant, the ratio of absence of cerebral ischemic was 1.020.

By increasing one unit of anti-H. pylori IgG antibodies, by remaining the variables of age and DM constant, the ratio of absence of cerebral ischemic was 0.982.

anti-H. pylori IgG antibodies in increments of one age and DM odds ratio of having patients with stable ischemic brain was equal to 0.982.

The ratio of absence of cerebral ischemic in diabetic and non-diabetic people was 0.183, if the variables of anti-H. pylori IgG antibodies and age remain constant.

Table 3: frequency distribution of H. pylori infection risk factors, CVA, high blood pressure, diabetes, and high blood fat among the studied groups

Group	H. pylori infection		Total
	Negative	Positive	
Study	(%12.9)9	(%87.1)61	(%100)70
Control	(%27.1)19	(%72.9)51	(%100)70
Total	(%20)28	(%80)112	(%100)140
	CVA risk factor		
Study	(%80)56	(%20)14	(%100)70
Control	(%64.3)45	(%35.7)25	(%100)70
Total	(%72.1)101	(%27.9)39	(%100)140
Study	High blood pressure		
Control	(%67.1)47	(%32.9)23	(%100)70
Total	(%30)21	(%70)49	(%100)70
Study	(%48.6)68	(%51.4)72	(%100)140
Control	Diabetes		
Total	(%50)35	(%50)35	(%100)70
Study	(%17.1)12	(%82.9)58	(%100)70
	(%33.6)47	(%66.4)93	(%100)140
	Blood fat		
Study	(%17.1)12	(%82.9)58	(%100)70
Control	(%4.3)3	(%95.7)67	(%100)70
Total	(%10.7)15	(%89.3)125	(%100)140

Discussion and conclusion

In our study, 61 patients (87.1%) in the study group had positive H-pylori infection, and 51 patients (72.9%) in the control group had positive H-pylori. This finding is in line with result of the study conducted by Moayyedi et al (23), which found that the number of positive cases in patient group was significantly higher than that in healthy people (69% versus 58.5%). This study is also consistent with the study of Markus and Mendall (22) who concluded that H.pylori seropositivity in the patient group was more than that in the control group (58.8% versus 5.44%). Infection with H. pylori can lead to vascular

disease through endothelial dysfunction, dyslipidemia, and increased LDL, chronic inflammation, and hyperhomocysteinemia, followed by increased cardiovascular disease and cerebral stroke risk (24). In one case study, it was concluded that infection with helicobacter, Mycoplasma pneumoniae, Chlamydia pneumoniae and Porphyromonas Gingivalis do not independently increase the likelihood of ischemic stroke, but they can increase this likelihood by creating vascular dysfunction (25). In our study, the average H.pylori in the study group was 64.53 ± 49.19, and it was 35.50 ± 32 in the control group.

As seen, the percentage of seropositivity in the study group was more than that in the control group. This finding is consistent with the result of study conducted by Sawayana et al, which found H. pylori infection could act as an independent risk for increased risk of stroke. Obtained results showed that seropositivity was higher in the case group compared to control group, and the difference was significant (26). In a prospective study conducted in China, it was showed that the relative risk of coronary heart disease and ischemic stroke in patients with H. pylori infection was 1.55 and 1.54, respectively (at the 95% level) (27). However, Jang SH et al concluded that there is no significant difference between H. pylori infection and cerebral and vascular disease (28). In addition, H.pylori infection does not lead to an increase in mortality rate caused by cardiovascular disease (29). After reviewing several prospective studies, Yu M et al concluded that there is no significant relationship between H.pylori infection and stroke (30), which this result is not in line with result of our study. WS Huang et al also reported that infection with this bacterium leads to non-embolic stroke, but it does not affect the embolic stroke (31). In our study, the mean age of the patients was in line with the study conducted by Masoud et al (32). In our study, total CVA in patients of both groups was not identical. In our study, anti-H. pylori IgG antibodies level in patients with positive CVA and in patients with negative CVA was significantly different. According to importance of stroke as one of the major health problems in the world, studying the risk factors of the disease, and predicting the stroke are very important in reducing the incidence of the disease. In our study, H.pylori infection was reported positive in 28 male patients (59.6%) and in 21 female patients(60%) that this study was in line with the study conducted by Masoud et al (17) who concluded that IgG seropositivity is associated with stroke risk and this risk is the same for both genders. According to the results of this study, H. pylori infection can be involved in patients with ischemic stroke. It should be noted that this study was the first study conducted in Iran in this regard, and reviewed studies (32 and 33) have found contradictory results on the impact of H. pylori infection on ischemic diseases. Therefore, it is recommended that more studies to be conducted so that the relationship between H. pylori infection and ischemic diseases to be announced confidently.

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