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Immunological Detection of Toxoplasma Gondii Among Patients Suffering Helicobacter Pylori Gastritis and Gastric Ulcer

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Abstract
Simultaneous infection with H. pylori and T. gondii is common and occurs via the transmission of fecal matter to the mouth. Both infections are associated with gastritis and peptic ulcer disorders. ***Objectives:*** *Both H. pylori infection and Toxoplasmosis symptoms are very common illnesses that are transmitted by the fecal-oral channel. The primary objective of this research was to investigate the link between these two diseases* ***Materials****: In order to conduct upper and lower bronchoscopy, as well as gastrointestinal endoscopy, the patients were transported to the gastroenterology clinic at Azadi Teaching Hospital as well as the private Kirkuk clinic in Kirkuk city. Both of these clinics are located in an educational institution.* ***Methods:*** *This particular research had been conducted during the months of November 2021 and April 2022. This patient's group consisted of 148 individuals in total. The ages of the patients ranged from 20 to 40 years. Stool antigen test and ELISA (IgM and IgG) for Toxoplasma species were performed on a subset of patients who were eligible for the study. The examination was performed according to non-invasive procedures.* ***Result:*** *In the current research, 78 patients (female) exhibited a positive result for H. pylori, which had been found by stool antigen test. Additionally, 31 patients (39.7%) showed positive results for T. gondii by utilizing ELISA technique since they had a positive result in H. pylori.* ***Conclusion:*** *When H. pylori was paired with toxoplasmosis, it resulted in the return of the latter, which in turn caused a more severe case of gastritis and a stomach ulcer.*
Key words: - Toxoplasma gondii, Helicobacter pylori, gastritis, gastric ulcer, stool antigen, ELISA.

1. INTRODUCTION

Bacteria belonging to the genus Helicobacter are Gram-negative, have the ability to move around, and are found in the digestive tracts of both humans and animals. The species of Helicobacter that lives in the human stomach that has received the most attention from researchers and is the most common is called Helicobacter pylori (1). Peptic ulcers, mucosa-associated lymphoid tissue (MALT)-lymphoma, and/or adenocarcinoma are some of the significant gastrointestinal disorders that have been shown to be connected with H. pylori infection. Additionally, there have been reports of co-infections with H. pylori, which have been linked to a higher occurrence of peptic ulcers (2, 3).

The zoonotic illness known as toxoplasmosis is caused by the protozoan parasite Toxoplasma gondii, which is the only species in the Toxoplasma genus. Toxoplasmosis is a disease that may be found in any region of the globe. Toxoplasma has the capacity to infect almost any creature with a warm-blooded circulatory system, including humans and animals, which are referred to as intermediate hosts (IH)(4). Domestic and wild felids are the only two kinds of hosts that are capable of releasing oocysts into the environment due to their ability to discharge them. Cats like these are the definitive hosts (DH) for the disease. Toxoplasma gondii is commonly considered to be one of the most important parasites that may be transmitted via the consumption of food and water, according to the disciplines of veterinary medicine and medicine (5).

In particular, immunocompromised individuals and pregnant women are at risk for contracting toxoplasma gondii, which is a matter for worry for public health. Despite the fact that healthy people are often asymptomatic, severe clinical illness, which may potentially be deadly, can be found mostly in newborns and immunocompromised persons (such as those who have HIV infection, are using long-term corticosteroids, have hematologic cancers, or have had a transplant) (6,7). The ocular manifestation of toxoplasmosis is another significant clinical symptom of the disease. Research has shown that the incidence of the disease in infected individuals is around 2% in Europe and 17% in South America8.

Research was carried out with the purpose of determining whether or not there is a connection between two widespread infectious conditions, namely gastritis and the formation of peptic ulcers, which are both brought on by the gram-negative bacteria H. pylori and T. gondii (10).
It is likely that the manifestations of chronic infections with Helicobacter pylori and T. Gondii rely on the immunological response of the host, and it has been shown that individuals who suffer from chronicity carry more than one pathogen (11).
Other pathogenic agents and H. pylori might be nothing more than a signal for other occurrences. For instance, early-life antibiotics have the potential to eradicate H. pylori (12), in addition to other bacteria that have the potential to operate as protective agents that are really present. This article discusses the significance of a number of infections, including Ascaris lumbricoides, T. gondii, HSV, and EBV, in the prevention of atopy (13). Moreover, interactions between H. pylori and sex as well as interactions between H. pylori and race were shown to be predictive of cognitive performance (14). Additionally, substantial relationships between toxoplasmosis and educational attainment, race-ethnicity, and economic position were shown to be predictive of cognitive function in young and middle-aged individuals (15).
Invasive diagnostic procedures are not only costly and time-consuming, but also often call for a number of further tests to confirm the diagnosis. On the other hand, non-invasive methods are able to identify both active and passive H. pylori infections (16). According to the findings of a large number of researches, stool antigen tests are both highly sensitive and high-specificity (17). A diagnostic method that does not include any invasive procedures is recommended by the European Helicobacter pylori research group. This method is known as the stool antigen test (18). Non-invasive methods that may be used for the detection of H. pylori antigens include the H. pylori stool antigen-lateral flow immunochromatography assay (HpSA-LFIA) (19,20) and enzyme immunoassays (EIA), which include the semiquantitative Enzyme-Linked Immunosorbent Assay (ELISA). Both of these methods do not include any invasive procedures (21,22). Due to its speed, application, reliability, and longer shelf life at room temperature (12-24 months), the HpSA-LFIA, which is a point-of-care test, is the one that is considered to be the most preferred option (23).

MATERIALS AND METHODS

Patients

In Kirkuk city, we carried out clinical trial research that was done in a random fashion. The study included 148 female patients who were referred to the gastrointestinal clinic of Azadi Teaching Hospital as well as the private Kirkuk clinic for the purpose of upper and lower bronchoscopy fiberoptic GIT endoscopy. The research was conducted for the goal of accomplishing the aforementioned objectives. Data were gathered on clinical symptoms that included pain or discomfort in the upper abdomen, nausea, vomiting, loss of appetite, weight loss, and black or tarry stool, or red or maroon blood mixed feces. These symptoms were collected between November 2021 and April 2022. It was observed that individuals who were also undergoing weight reduction had these symptoms. All of the information was gathered from people who had previously experienced these symptoms. Patients who were between the ages of 20 and 40 years old were considered for inclusion in the study.

Questionnaire

A questionnaire was designed to involve the relationship and risk factors between H. pylori and toxoplasmosis in the females. Demographic parameters and factors were as follows: age, case history of serious disease, and previous history of toxoplasmosis.

Samples

Collecting the stool and placing it in containers that were clean, dry, and watertight was the protocol that was followed. After careful consideration, the H. pylori Antigen fast test cassette was selected as the instrument of choice for the stool assay in order to ensure its usefulness. Following coagulation and centrifugation, blood samples were taken from individuals who had positive findings for the stool Ag test. The samples were five milliliters in volume. Eppendorf tubes were used to separate the serum that was collected from the blood sample, and the serum was then kept at a temperature of –20 degrees Celsius.

Rapid test for Helicobacter pylori (Stool Antigen test)

To carry out the H. pylori antigen quick test cassette (feces), which is a rapid chromatographic immunoassay for the qualitative detection of H. pylori antigens in human feces specimens, a kit manufactured by Linear in Spain with the catalog number 5094503 is employed. This kit is used for the purpose of performing the test. The conduct in question is carried out in order to be of aid in the process of diagnosing H. pylori.

One of the goals of the H. pylori Antigen quick test, which is a qualitative lateral flow immunoassay, is to evaluate whether or not individual samples of human feces contain H. pylori antigen. This is one of the aims of the test. The test line section of the membrane is pre-coated with antibodies that are specific to H. pylori. These antibodies are located on the membrane. Within the framework of this specific examination, this is carried out. A interaction between the specimen and the H. pylori antibody-coated particle is activated when the specimen comes into contact with the particle. It is the capillary mobility that allows the mixture to rise higher on the membrane, where it then interacts with anti-H. pylori antibodies that are already present on the membrane, which ultimately results in the formation of a red line. Capillary motion is the mechanism that is responsible for this response. In order to extract the greatest possible quantity of antigens, it was necessary to collect a sizeable quantity of feces (between one and two milliliters or between one and two grams) in a specimen collecting container that had been well cleaned and dried. At ten minutes after the specimen was dispensed, the findings were made available for reading.

Enzyme linked Immunosorbent Assay (ELISA) IgG and IgM

It is the purpose of this kit to perform qualitative analysis on human serum in order to determine the levels of Toxo IgG and IgM. For the purpose of carrying out the test, it is necessary to make use of a fully-automatic chemiluminescence immunoassay (CLIA) analyzer known as MAGLUMI. This analyzer includes the Maglumi 600, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 3000, and Maglumi 4000.

A test based on the principle

In order to coat nano-magnetic microbeads, anti-human IgG and IgM from mice were used for the purpose of labeling ABEI and pure Toxo antagonist. The sample, the calibrator, or the control is thoroughly mixed with buffer (goat anti-human IgM, goat anti-human IgG, and goat anti-human IgA) and nanomagnetic microbeads coated with Toxo antigen. This is done in order to ensure that the resulting mixture is accurate. After that, the mixture is subjected to an incubation period at 37 degrees Celsius, and it is rinsed through the cycle once. Following the completion of this process, the sandwich is assembled by including the ABEI Label, which had been incubating previous to this operation. After that, the sandwich is washed for the second time. In the next step, the starting reagents were introduced, and a flash chemiluminescent reaction was started. A photomultiplier was used to quantify the light signal as RLU within three seconds. The light signal is proportional to the concentration of Toxo IgG and IgM that has been present in samples.

Statistical analysis

The SPSS (16) program and Microsoft excel were applied for statistical analysis. The chi-square (x2) test, p-value <0.05 was considered as significant.

RESULT

Seventy-eight of the individual patients out of a total of one hundred forty-eight showed positive results for H. pylori, which was discovered by the use of stool antigen in the testing process. The ages of the patients served as the distinguishing element, and they were divided into four unique groups respectively. Twenty-five to thirty years of age was the age group that reached its highest point, with the age range extending from twenty to forty years of age. As can be seen in Table (1), the statistical differences between older and younger age groups were not statistically significant ($p = 0.240$). Also showed 31 (39.7%) of women infected with T. gondii and H. pylori out of the total positive patients, as shown in Figure (1).

Table (1) Patient categorization based on age for females utilizing the H. pylori Stool Antigen test

Patients (female)	Frequency of age			
	20-24	25-30	31-35	36-40
Positive 78 (52.7%)	18 (12.2%)	28 (18.9%)	21 (14.2%)	11 (7.4%)
Negative 70 (47.3%)	13 (8.8%)	17 (11.5%)	24 (16.2)	16 (10.8%)
Total 148 (100%)	31 (21%)	45 (30.4%)	45 (30.4%)	27 (18.2%)
P= 0.240				

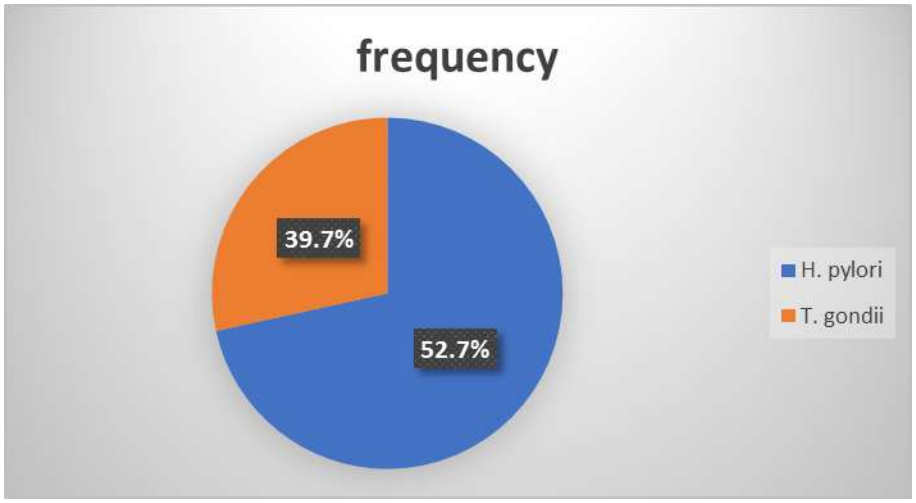

Figure (1) Patients infected with Helicobacter pylori were found to have positive results for Toxoplasma gondii, as shown by the statues of these individuals.

The largest proportion of H. pylori and T. gondii infections was found in 11 patients (35.5%) between the ages of 20 and 24. This was determined by utilizing a serological test, which was performed on 31 patients. The rate of infection was determined according to age groups. There was statistically highly significant (p= 0.00004), as shown in Table (2).

Table (2) Categorizing patients as positive for H. pylori and T. gondii based on their age.

Patients No (78)	**Frequency of age**				
	20-24	**25-30**	**31-35**	**36-40**	**total**
H. pylori + T. gondii	11 (35.5%)	9 (29%)	7 (22.6)	4 (12.9%)	31(100%)
H. pylori	18 (23.1%)	28 (35.9%)	21 (26.9%)	11 (14.1%)	78 (100%)
P= 0.00004					

Age Categories and Enzyme-Linked Immunosorbent Assay (ELISA) IgM

For T. gondii, the age group of 25-30 years had the highest accuracy rate of ELISA (IgM), which was 6 (66.7%). This was the case for the age group. Following this was the age group of 20-24 years, followed by the age group of 31-35 years, and finally the age group of 31-35 years, which had accuracy rates of 5 (45.5%), 2 (28.6%), and 0 (0%). The results that were positive and those that were negative within age groups did not vary significantly from one another (p = 0.331 and p = 0.678), which shows that there was no significant difference between the age group and ELISA (ILM). For the most part, the outcomes were all favorable.

Table (3) Importance of age group in relation to ELISA (IgM)

Age	**ELISA (IgM)**		
	+ve (%)	**-ve** (%)	**Total** (%)
20-24	5 (45.5%)	6 (54.5)	11 (100%)
25-30	6 (66.7%)	3 (33.3%)	9 (100%)
31-35	2 (28.6%)	5 (71.4%)	7 (100%)
36-40	0 (0%)	4 (100%)	4 (100%)
Total (%)	13 (41.9%)	18 (58.1%)	31 (100%)

P value	P= 0.331	P= 0.678	

Enzyme linked Immunosorbent Assay (ELISA) IgG and Age Groups

The current research related to the ELISA (IgG) among T. gondii infection according to age groups showed a higher proportion of positive patients within age groups 31-35. The relation between age group and positive cases is statistically significant (p=0.05), and the negative relationship between age and ELISA was statistically non-significant, as shown in Table (4).

Table (4) Toxoplasmosis and age-related ELISA (IgG) relevance

Age	ELISA (IgG)		
	+ve (%)	-ve (%)	Total (%)
20-24	8(72.7%)	3 (27.3%)	11 (100%)
26-30	8 (88.9%)	1 (11.1%)	9 (100%)
31-35	7 (100%)	0 (0%)	7 (100%)
36-40	2 (50%)	2 (50%)	4 (100%)
Total (%)	25 (80.6%)	6 (19.4%)	31 (100%)
P value	P= 0.05	P= 0.679	

Methods for detection of T. gondii

The identification of T. gondii was accomplished via the use of two different approaches in this investigation. As can be seen in figure (2), the rate of infections in the IgG was shown to be 25 (80.6%) among the positive cases in the H. pylori stool antigen test, whereas the rate of infections in the IgM was 13 (41.9%). This information was obtained from the observations that were made. The findings of the diagnostic test provided the basis for the acquisition of this information.

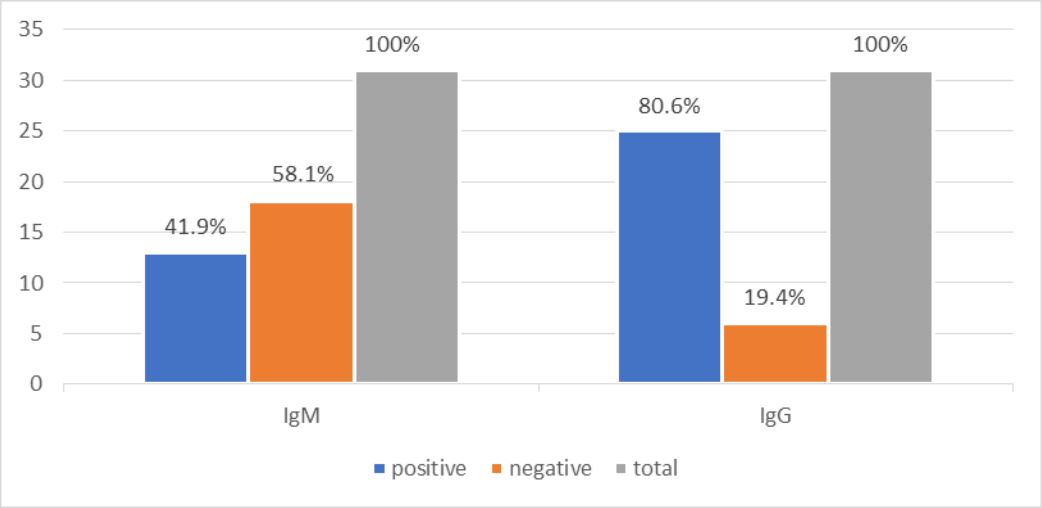

Figure (2) Comparative between ELISA (IgM and IgG) for detection of T. gondii statues with all positive to H. pylori

DISCUSSION

The treatment and accurate identification of H. pylori is a crucial technique for tackling the problem that is provided by this bacterium. This is because H. pylori have a significant connection with gastroduodenal diseases and a high incidence of infectiousness, particularly in poor nations. This is the reason why this is the case. There are a number of diagnostic tests that have been developed for the goal of diagnosing H. pylori; however, each of these tests has both advantages and disadvantages. For the goal of determining whether or not an infection with Helicobacter pylori was present, the stool antigen of the infected individual was used. The results of a great number of examinations carried out on a worldwide scale have shown that it was a test that was reliable and provided correct results. A large population density and a low socioeconomic class are two factors that have been shown to be associated with significant prevalence of H. pylori infections in some places. These regions have also been shown to have a high population density. Furthermore, the prevalence of the bacterium varies from country to country (24).

The findings presented here demonstrated that the use of SAT resulted in a frequency of detection of H. pylori that was 52.7%. According to the findings, the prevalence of positive H. pylori infection using stool antigen test was found to be 54% (25). This finding was found to be in accordance with the findings. This was shown to be the case after investigation. Further, the findings were in accordance with Huwiage et al. (26), as well as with Thapa et al. (27), which demonstrated that the level of disagreement was lower than the predicted numbers given by other regions of our nation (67%) and countries that are next to us (28-29). It is possible that the decreased prevalence that was discovered in this research might be attributed to a number of variables, including a better quality of life, an increased income, an increased knowledge of health issues, and an improvement in sanitation.

According to the findings of a recent research, the age group of 25-30 years old has been the one that has had the highest prevalence of H. pylori infection (18.9%) among females. There was no statistically significant variation in the distribution of the individuals based on their age. Huwiage et al. (26-30) and Alhashimi et al. (31) both reported the same result in their respective studies. In contrast to the findings that were published by Mawlood et al (32-33) and Majeed et al (34), the results presented here are presented here. It was noted that women were responsible for the majority of the housework, food preparation, and cooking, which is another aspect that requires more investigation (35).

The study focused mainly on the relationship between those who had a previous infection with toxoplasma gondii and H. pylori infection (39.7%) of positive patients infected. When distributing them according to age groups, we found that the peak incidence of H. pylori and T. gondii occurred among age group 20-24 (35.5%). An very high level of statistical significance was found in the distribution of differences across age groups. Ghazy et al. (36), as well as Mahmood et al. (37), came to the same result, which was that there was a substantial correlation between the rising incidence of H. pylori infection and the intake of food from street vendors. This was the conclusion that was reached by both groups. Patients who rejected consuming food from street sellers and preferred eating fruits, on the other hand, did not exhibit any signs of getting an illness associated to street vendors.

According to the findings of the present research, patients who were infected with both H. pylori and T. gondii had severe gastritis. Furthermore, the detection of IgM antibodies for toxoplasmosis in gastric ulcers indicated a result of 41.9%, which was not statistically significant when compared to other age groups. That result was in agreement with Ghazy et al [(36)], and Vomero et al [(38)]. Also, the results were disagreements to another study in Tehran [(39)], and Majeed et al [(40)] who found a significant between age group and IgM for toxoplasmosis and high rate in age group (15-25). The reason might be attributed to hyperactivity of age in the life, lifestyle of eating, or due to contact with animal specially cat.

In the current study, there was a significant correlation between positive IgG ELISA and anti-toxoplasmosis. In this research, there was no significant correlation between negative IgG ELISA and anti-toxoplasmosis with a higher prevalence rate (88.9%). This finding similarity was consistent with our finding in Kirkuk [(41)], where they found the following rate respectively (91.6%), and in Ethiopia [(42)], with whom reported (81.6%). In contrast, the lower seroprevalence of IgG toxoplasmosis was reported in Palestine [(43)], Saudi Arabia, Sudan, Morocco, and China [(44)]. In addition to the limited number of samples, there is a dearth of scientific study on the connection between H. pylori and toxoplasmosis, which may be the cause of this disparity in the findings.

CONCULSION

When H. pylori was paired with toxoplasmosis, it resulted in the return of the latter, which in turn caused a more severe case of gastritis and a stomach ulcer.

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