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RESEARCH PAPER

Association of H. pylori infection indicated by their serum IgG antibodies with lymphoma malignancy

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A B S T R A C T:

Lymphoma is a type of lymphocyte malignancy that develops in different types of lymphoid tissues, many pathogens are expected to be implicated in the establishment of the disease including *Helicobacter pylori* (*H. pylori*). A total of 64 B-cells lymphoma patients recruited to oncology-hematology unit in Azadi Teaching Hospital in Duhok city and 60 sex and age matched apparently healthy individuals were involved in the current study. Serum samples were collected from all subjects and tested for detecting IgG antibodies against *H. pylori* as an indicator for *H. pylori* infections. The age average was (52.5±12.4) years for lymphoma cases and (56±12.5) years for controls. IgG anti-*H. pylori* antibodies were found in 11/64 (17.9%) of the lymphoma patients and in 8/60 (13.3%) of the control subjects. Lymphoma cases younger than 80 years had higher prevalence of IgG anti *H. pylori* antibodies (28.6%) as compared with all other age categories with a significant increase compared to both age groups <40 years (p= 0.008), 51-60 years (p=0.04) and 61-70 years (p=0.02) respectively. No significant difference was found in the IgG anti *H. pylori* antibodies prevalence between the lymphoma and control subjects (p=0.087), the gender had no significant effect on the IgG anti *H. pylori* antibodies prevalence in both of the lymphoma and control subjects respectively and between the two groups. Based on OR=1.34 (95% CI= 0.49-3.42) a very weak association of IgG anti *H. pylori* antibodies prevalence was observed with an overall increased risk of lymphoma. In conclusion, a week association of *H. pylori* infection with lymphoma was found due to non categorization of the lymphoma cases pathologically, the association might increases significantly if being categorized pathologically.

KEY WORDS: Lymphoma, Helicobacter pylori, MALT malignancy, H. pylori antibodies
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1. INTRODUCTION

Lymphoma malignancy includes a large group of malignancies that usually develop from the lymph nodes (Kuppers 2009). The lymphocytes in the lymph nodes, undergo mutations or changes that lead in uncontrollable cell proliferation resulting in tumorigenesis. The cause of lymphoma remains argued, certain individuals are more susceptible to establishing the cancer. Some pathogens are reported to be significantly associated with the disease.

HIV-positive patients and people infected with several other viruses or bacteria including *Helicobacter pylori*, Epstein-Barr virus, and human T-lymphotropic virus are found to be more likely developing the disease (Engels 2007), in addition a genetic link or familial connection in lymphoma development has been suggested by Cerhan and Slager 2015. Also it has been speculated that gut colonizing fungi might have an role in some cancers, in a study conducted by Khidir A.K. and colleagues they retrieved Malssezia genus of fungi in high frequency from fecal samples of cancer patients (Khidir A.K. *et al*. 2017). Lymphoma can be

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divided into two major categories, including non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL), they can be further categorized into more than 30 types of NHL and five types of HL (WHO Classification of Tumours, 2017). *Helicobacter pylori* (*H. pylori*), is a Gram negative, spiral-shaped, microaerophilic bacterium that inhabits the human stomach. According to records, it has been estimated to colonize more than half of the world's human population (Hatakeyama M . 2004). Due to its pathogenesis and multiple virulence factors, it has been recognized as a carcinogen and classified to be class I carcinogen and led to a new method for classifying gastric carcinoma (Wotherspoon *et al*. 1991). It has been shown that *H. pylori* infections trigger the gastric associated lymphoid tissues responses in a way to be a potential oncogenic factor leading to the development of gastric associated malignant lymphomas like mucosal associated lymphoid tissues (MALT) lymphoma and DLBCL (Amieva *et al*. 2016; Lee *et al*. 2016; Suzuki *et al*. 2006). The virulence factors of H. pylori involved in the mechanism of pathogenesis and potential ontogenesis are CagA, VacA and OipA, they have a significant role in lymphomagenesis which includes also host factors and environmental conditions. Cytotoxin-associated gene A (CagA) protein is the *H. pylori* virulence factor most intensively studied as an oncogen factor, it has the ability to cross the host cell membrane and induce intracellular cell signaling that might lead to oncogenesis (Murata-Kamiya et al, 2010). Evidences have proven that gastric associated lymphoma patients that are *H. pylori* sero-positive may show long-term survival and better prognoses ((Meimarakis *et al*. 2006; Marrelli *et al*. 2009; Postlewait *et al*. 2016). Because DLBCL (MALT) fails to respond to anti- *H. pylori* therapy, it is thought to be *H. pylori* status independent according to the WHO (World Health Organization) classification that differs from low-grade and *H. pylori*-dependent MALT lymphomas (MALT lymphoma) (Hussell *et al*. 1993; Neubauer *et al*. 1997; Swerdlow *et al*. 2008). Many other studies have demonstrated that an elevated rate of gastric DLBCL (MALT) is associated with *H. pylori* infections through responding effectively to *H. pylori* eradication (Chen *et al*. 2001; Morgner *et al*. 2001). The current study has aimed at estimating the prevalence of IgG anti- *H. pylori* antibodies among lymphoma patients as an indicator for H. pylori infection among them thereafter, the association of the *H. pylori* infection with the lymphoma malignancy.

2. SUBJECTS AND METHODS

2.1 **Subjects**

Patients involved in the current study were recruited to oncology-hematology unit in Azadi Teaching Hospital in Duhok city from January 2018 to February 2019. All of the cases were patients diagnosed with a lymphoid malignancy. The diagnosis of lymphoma was done locally based on serial complete blood count, peripheral blood smear examination, flourescent in situ hybridization, and bone marrow examination, at baseline in addition to histology and immunohistochemistry. Subjects with a diagnosis of uncertain malignant potential were excluded. Controls were apparently healthy individuals with age and sex matched. Severe immune-suppressed patients systemic infections, other than *Helicobacter pylori infection*, were excluded. Data on demographic, medical and family history, and environmental exposures were collected from each subject. . Informed consent was obtained from all subjects before enrollment. Blood samples were taken from the patients and controls.

2.2. Methods

serum anti- H. pylori IgG anti bodies

From each enrolled subject, 200 ul of serum sample was collected and preserved at -20 ^{o}C until processing in the laboratory. All samples were tested for in vitro qualitative and quantitative detection of IgG antibodies against *H. pylori* in duplicate using (MyBioSource, Inc. San Diego, USA) kit according to the manufacturer instructions, the sera samples were tested in duplicates. The enzyme immunoassay plate spectrophotomer reader was used to read the at absorbance of 450 nm. According to the kit supplier, the cut off value for the assay was 8 U/mL.

2.3. Statistical analysis

The comparison between lymphoma cases and IgG anti-*H. pylori* antibodies prevalence was done with a X^2 test. P values at level 0.05 and less were considered statistically significant. Regression was used to estimate the odds ratios and 95% confidence intervals (OR, 95% CI) to

measure association between anti-H. pylori IgG antibodies and the risk of lymphoma. The SPSS software was used for data analysis.

3. RESULTS AND DISCUSSION

There are scanty previous studies systematically reporting the potential role of *H. pylori* in lymphomagenesis, most of those studies have estimated no increased risk of lymphomas other than MALT associated lymphoma in the presence of *H. pylori* infection. In the current study, the IgG anti *H. pylori* antibody was estimated by serologic method as an indicator for the *H. pylori* infection of the subjects involved in the study, since IgG anti *H. pylori* has been demonstrated to be the best performance overall other serologic noninvasive diagnostic test for the detection of the *Helicobacter pylori* infection (Rosemary et al. 2009). A total of 64 B-cells lymphoma patients and 60 sex and age matched apparently healthy individuals were involved in the current case control study. Table 1 shows the demographic characteristics of the study subjects including the age and sex. The age average was 52.5 years for cases and 56 years for controls. There was no statistical difference in the distribution of the demographic characteristics (age and gender) between cases and controls (P value = 0.11), which is consistent with findings of Silvia *et al*. 2004 when they reported that no statistical differences were observed in the distribution of these characteristics between patients with lymphoma types and control subjects. The prevalence of IgG anti-*H. pylori* antibodies is indicated in table 2, the antibodies were found in 11/64 (17.9%) of the lymphoma patients, were as among the control subjects the IgG anti-*H. pylori* antibodies were detected in 8/60 (13.3%).

IgG anti *H. pylori* antibodies prevalence varied by age, subjects older than 80 years having higher prevalence of antibodies (28.6%) as compared with all other age categories. Regarding the age groups in lymphoma subjects, there was a significant increase in the IgG anti-*H. pylori* antibodies prevalence when the age group >80 years compared to both age groups <40 years (p= 0.008), 51-60 years (p=0.04) and 61-70 years (p=0.02) respectively. No significant difference was found in the IgG anti *H. pylori* antibodies prevalence between the lymphoma and control subjects (p=0.087). Also the gender had no significant effect on the IgG anti *H. pylori* antibodies prevalence in both of the lymphoma and control subjects respectively and between the two groups. Epidemiological studies on the general populations show a male preponderance in the infection rate by H pylori, although there are controversial reports representing comparable rates (Shi R *et al*. 2008; Dore MP *et al*. 2012), but Agah S *et al*. 2016 found that females are more vulnerable to develop gastric cancers after getting *H pylori* infection, in a time that males have shown higher risk of developing other related side effects associated with *H pylori* infection, including cancer, though more future prospective studies with large patient population are still needed to explain this disparity. As shown in table 3, a very weak association of IgG anti *H. pylori* antibodies prevalence was observed with an overall increased risk of lymphoma (OR=1.34, 95% CI= 0.49-3.42), of the 64 lymphoma patients, 11 had detectable IgG anti *H. pylori* antibodies in their sera. To some extent, these findings are consistent with those reported by Silivia *et al*. (2004), they found that *H. pylori* infection was not associated with an overall increased risk of lymphoma, within all lymphoma categories, they found that *H. pylori* was associated with an almost 4-fold increased risk of splenic MZL (OR = 3.97, 95% CI = 0.92-17.16, P value = 0.065). In contrast, in a study conducted on stomach cancer patients in Erbil city, Sulaiman K., found that most of the stomach cancer patients had *H. pylori* infection (Sulaiman K. 2016). This inconsistency in the results could be due to the lymphoma stratification, in the current study the lymphoma cases are not stratified into nodal, extranodal and MALT lymphomas, however, in a future study plan o the same samples of the same patients, the stratification will be considered and will be compared with the current findings to see the significance of lymphoma stratification. In the study conducted by Silivia *et al* (2004), the cases were stratified and the strongest association of *H. pylori* was found with MALT lymphomas, and they identified that 100% of the subjects with a gastric lymphoma categorized as MALT or as DLBCL histology had antibodies against *H. pylori* in agreement with the data reported by Nakamura *et al*. 2003. In the present study, if the MALT

associated lymphoma would of been studied separately, the association of *H. pylori* with the lymphoma might be much more stronger, this is supported by Anttila *et al.* (1998), they did not identify an increase in the seroprevalence of IgG anti-H. pylori among patients with non-Hodgkin's lymphomas (OR = 0.8 95% CI = 0.4-1.9). No data were presented by lymphoma subtype, and in contrast, when stratifying and categorizing the lymphoma cases the association will be increased as reported by Cuttner *et al*. (2001) when they found that *H. pylori* seroprevalence was significantly higher for MALT lymphomas as compared with other lymphoma types

It has been found that Gastric and MALT lymphoma is a rare type of non-Hodgkin lymphoma . This cancer represents approximately 12 percent of the extranodal (outside of lymph nodes) non-Hodgkin (Wu XC et al. 2009). On the other hand, even though the lymphoma patients are not categorized into extranodal lymphomas, the week association of the *H. pylori* infection with lymphoma in the current study could be due to the small sample size studied compared to others, because in some investigations it has been accepted that MALT lymphoma cells may disseminate into the splenic marginal zone through homing mechanisms since there has been no evidence of *H. pylori* playing a role in the development of lymphomas localized in the spleen with no evidence of gastric lymphoma (Cavalli *et al*. 2001). *H. pylori* virulence factors (e.g., CagA, VacA and OipA) have a significant role in lymphomagenesis which includes also host factors and environmental conditions. Cytotoxin-associated gene A (CagA) protein is the *H. pylori* virulence factor most broadly studied, it has the ability to cross the host cell membrane and induce intracellular cell signaling that might lead to oncogenesis (Murata-Kamiya et al, 2010). In other studies, researcher explored the significance of H. pylori infection in lymphoma oncogenesis and the significance of *H. pylori* eradication in lymphoma remission. It has been reported that *H. pylori* infection is significantly associated with lymphoma specifically Gastric lymphoma and MALT lymphoma, medicines used for the eradication of H. pylori are usually used as the first-line treatment for this disease particularly during the early stage of the disease (Nakamura et al. 2012; Fischbach et al. 2007), these data have been supported by researchers when they found a complete remission of diffused large B cell lymphoma DLBCL after *H. pylori* eradication (Sugimoto et al, 2003; Alsolaiman et al, 2003). Also, a large cohort study has validated the association of *H. pylori* infection with the de novo DLBCL (Kuo SH et al. 2012). Also it has been demonstrated that de novo gastric DLBCL *H. pylori*-positive is less aggressive than *H. pylori* negative and patients with primary gastric de novo DLBCL without *H. pylori* infection are more likely to have poor prognoses than patients with the infection; therefore, the patients without *H. pylori* may benefit from more aggressive treatment and more systematic follow-up (Cheng et al. 2019).

Table1. The demographic characteristics of the subjects included in the study

	Controls	**Lymphoma patients**	***P* value**
	***n* (%)**	***n* (%)**	
Age (years)			
<40	5(8.3)	6(9.4)	
41-50	9(15)	10(15.6)	
51-60	9(15)	9(14.1)	
61-70	13(21.7)	14(21.9)	0.11

71-80	10(16.7)	11(17.1)	
>80	14(23.3)	14(21.9)	0.23
Gender			
Males	32(53.3)	37(57.7)	
Females	28(46.7)	27(42.2)	
Total	60	64	

Table2. Seroprevalence of IgG anti H. pylori by demographic characteristics in Patients and control subjects

	Controls	**Lymphoma patients**	***P***
	IgG Anti- H. pylori+/Total *n* (%)	**IgG Anti- H. pylori+/Total *n* (%)**	**value**
Age			
(years)	1/5(20)	0/6(0)	
<40	0/9(0)	2/10(20)	0.008
41-50	2/9(22.2)	1/9(11.1)	
51-60	2/13(15.4)	1/14(7.14)	0.04
61-70	1/10(10)	3/11(27.3)	0.02
71-80	2/14(14.3)	4/14(28.6)	
>80			
Gender	4/32(12.5)	6/37(16.2)	
Males	4/28(14.3)	5/27(18.5)	
Females	8/60(13.3)	11/64(17.9)	
Total			

Table3. Association of IgG anti- *H. pylori* antibodies prevalence with the lymphoma cases.

	IgG Anti- H. pylori+/Total *n* (%)	%	OR (95% CI)
Controls	8/60	13.3	Reference
Lymphoma patients	11/64	17.9	1.34(0.49-3.42)

4. CONCLUSIONS

A week association of *H. pylori* infection with lymphoma was found due to pathologically non stratified and categorized cases of the lymphoma, if being categorized pathologically, the association might increased significantly since persistent infection of *H. pylori* has been reported to be associated with some types of lymphoma specifically gastric and MALT lymphomas. Clinically it is important to explore the *H. pylori* infection among lymphoma patients since eradication of the bacteria could improve the treatment because when caught early, lymphoma is highly treatable and often curable.

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Conflict of interests

Nothing to declare

REFERENCES

Agah S, Khedmat H, Ghamar-Chehreh ME, et al.2016. Female gender and Helicobacter Pylori Infection,the most important predisposition factors in a cohort of gastric cancer. A longitudinal study. *Caspian J Intern Med*. 7(2):136-141.

Alsolaiman MM, Bakis G, Nazeer T, et al. 2003. Five years of complete remission of gastric diffuse large B cell lymphoma after eradication of helicobacter pylori infection. *Gut.* 52:507–9.

Amieva M, Peek RM. 2016. Pathobiology of helicobacter pylori-induced gastric Cancer. *Gastroenterology*. 150:64–78.

Anttila TI, Lehtinen T, Leinonen M, et al. 1998. Serological evidence of an association between chlamydial infections and malignant lymphomas. *Br J Haematol*.103:150-6.

Cavalli F, Isaacson PG, Gascoyne RD, Zucca E. 2001. MALT lymphomas. *Hematology* (Am Soc Hematol Educ Program). 1:241-58.

Cerhan JR, Slager SL. 2015. Familial predisposition and genetic risk factors for lymphoma. *Blood.* 126(20):2265–2273. [PMC free article] [PubMed] [Google Scholar]

Chen LT, Lin JT, Shyu RY, et al. 2001. Prospective study of helicobacter pylori eradication therapy in stage I(E) high-grade mucosa-associated lymphoid tissue lymphoma of the stomach. *J Clin Oncol*. 19:4245–51.

Cuttner J, Werther JL, McGlynn P, et al. 2001.Seroprevalence of Helicobacter pylori infection in patients with lymphoma. *Leuk Lymphoma*. 40:591-7.

Dore MP, Fanciulli G, Tomasi PA, et al. 2012. Gastrointestinal symptoms and Helicobacter pylori infection in school-age children residing in Porto Torres, Sardinia, Italy. *Helicobacter*.17:369-73.

Engels EA. 2007. Infectious agents as causes of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev*. 16(3):401–404. [PubMed] [Google Scholar]

Fischbach W, Goebeler ME, Ruskone-Fourmestraux A, et al. 2007. Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of helicobacter pylori can be managed safely by a watch and wait strategy: experience from a large international series. *Gut.* 56:1685–7.

Hatakeyama M. 2004. Oncogenic mechanisms of the helicobacter pylori CagA protein. *Nat Rev Cancer*.4:688–94.

Hussell T, Isaacson PG, Crabtree JE, et al. 1993.The response of cells from lowgrade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to helicobacter pylori. *Lancet.* 342:571–4.

Khidir A.K, Ibrahim Hamad and Hiwa A. Ahmad. 2017. Fungal Diversity in Gut Microbiota in Patients with Cancer. *ZJPAS*. 29(5): 55-65

Kuo SH, Yeh KH, Wu MS, et al. 2012. Helicobacter pylori eradication therapy is effective in the treatment of early-stage H pylori-positive gastric diffuse large B-cell lymphomas. *Blood*. 119:4838–44.

Kuppers R. 2009. The biology of Hodgkin's lymphoma. Nat Rev Cancer. 9(1):15–27. [PubMed] [Google Scholar]

Lee YC, Chiang TH, Chou CK, et al. 2016. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. *Gastroenterology*. 150:1113.

Marrelli D, Pedrazzani C, Berardi A, et al. 2009. Negative helicobacter pylori status is associated with poor prognosis in patients with gastric cancer. *Cancer*. 115:2071–80.

Meimarakis G, Winter H, Assmann I, et al. 2006. Helicobacter pylori as a prognostic indicator after curative resection of gastric carcinoma: a prospective study. *Lancet Oncol*.7:211–22.

Morgner A, Miehlke S, Fischbach W, et al. 2001. Complete remission of primary high-grade B-cell gastric lymphoma after cure of helicobacter pylori infection. *J Clin Oncol*. 19:2041–8.

Murata-Kamiya N, Kikuchi K, Hayashi T, et al. 2010. *Helicobacter pylori* exploits host membrane phosphatidylserine for delivery, localization, and pathophysiological action of the CagA oncoprotein. *Cell Host Microbe*.7:399–411.

Nakamura S, Matsumoto T, Jo Y, et al. 2003. Chromosomal translocation t(11;18)(q21;q21) in gastrointestinal mucosa associated lymphoid tissue lymphoma. *J Clin Pathol*. 56:36-42.

Nakamura S, Sugiyama T, Matsumoto T, et al. 2012. Long-term clinical outcome of gastric MALT lymphoma after eradication of helicobacter pylori: a multicentre cohort follow-up study of 420 patients in Japan. *Gut*. 61:507–13.

Neubauer A, Thiede C, Morgner A, et al. 1997. Cure of helicobacter pylori infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. *J Natl Cancer Inst*. 89:1350–5.

Postlewait LM, Squires MH, Kooby DA, et al. 2016. Preoperative helicobacter pylori infection is associated with increased survival after resection of gastric adenocarcinoma. *Ann Surg Oncol*. 23:1225–33.

Rosemary C. She, Andrew R. Wilson, and Christine M. Litwin. 2009. Evaluation of Helicobacter pylori Immunoglobulin G (IgG), IgA, and IgM Serologic Testing Compared to Stool Antigen Testing. *Clinical and Vaccine Immunology*. 16(8): p. 1253–1255

Shi R, Xu S, Zhang H, et al. 2008. Prevalence and risk factors for Helicobacter pylori infection in Chinese populations. *Helicobacter*. 13: 157-65.

S.H, Swerdlow,., E.Campo, , N.L.Harris, , E.S.Jaffe, , S.A.Pileri, , H.Stein, , J.Thiele, , J.W. Vardiman. 2017. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th edition(Vol 2). WHO Classification of Tumours.

Silvia de Sanjose, Andrew Dickie, Tomas Alvaro, Vicens Romagosa, Mercedes Garcia Villanueva, Eva Domingo-Domenech, Alberto Fernandez de Sevilla, and Emad El-Omar. 2004. *Helicobacter pylori* and Malignant Lymphoma in Spain. *Cancer Epidemiol Biomarkers Prev*. 13(6).

Sugimoto M, Kajimura M, Sato Y, et al. 2001. Regression of primary gastric diffuse large B-cell lymphoma after eradication of helicobacter pylori. *Gastrointest Endosc*. 54:643–5.

Sulaiman K. 2016. Cytogenetic study of Stomach cancer in Erbil City. ZJPAS. 28 (4): 56-65

Suzuki T, Matsuo K, Ito H, et al. 2006. A past history of gastric ulcers and helicobacter pylori infection increase the risk of gastric malignant lymphoma. *Carcinogenesis*. 27:1391–7.

Swerdlow SH, Campo E, Harris NL, et al. 2008. WHO classification of tumors of hematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press

Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, et al. 1991.Helicobacter pyloriassociated gastritis and primary B-cell gastric lymphoma. *Lancet*. 338:1175–6.

Wu XC, Andrews P, Chen VW, Groves FD. 2009. Incidence of extranodal non-Hodgkin lymphomas among whites, blacks, and Asians/Pacific Islanders in the United States: Anatomic site and histology differences. *Cancer Epidemiology*. 33(5):337–346.

Yuan Cheng, Yinan Xiao, Ruofan Zhou, Yi Liao, Jing Zhou and Xuelei Ma. 2019. Prognostic significance of helicobacter pylori-infection in gastric diffuse large B-cell lymphoma. *BMC Cancer*.19:842