RESEARCH PAPER

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

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ABSTRACT:
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 Malassezia genus of fungi in high frequency from fecal samples of cancer patients (Khidir A.K. et al. 2017). Lymphoma can be...
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; Morgen 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 °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 X² 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

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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=0.87). 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 Silvia 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 strong, 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 |
|---------------|----------------|-------------------|---------|
| Age (years)   | *n (%)*        | *n (%)*           |         |
| <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    |
Table 2. Seroprevalence of IgG anti H. pylori by demographic characteristics in Patients and control subjects

| Age (years) | Controls | Lymphoma patients | Controls | Lymphoma patients | P value |
|-------------|----------|-------------------|----------|-------------------|---------|
| <40         | 1/5(20)  | 0/6(0)            | 2/10(20) | 2/13(15.4)        | 0.008   |
| 41-50       | 2/9(22.2)| 1/9(11.1)         | 1/9(11.1)| 1/14(7.14)        | 0.04    |
| 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)       | 0.02    |
| >80         | 4/32(12.5)| 6/37(16.2)       | 0.02    |

| Gender      | Controls | Lymphoma patients | Controls | Lymphoma patients | P value |
|-------------|----------|-------------------|----------|-------------------|---------|
| Males       | 4/28(14.3)| 5/27(18.5)       | 0.02    |
| Females     | 8/60(13.3)| 11/64(17.9)      | 0.02    |

| Total       | Controls | Lymphoma patients | Controls | Lymphoma patients | P value |
|-------------|----------|-------------------|----------|-------------------|---------|
| 60          | 60       | 64                | 0.23     |
Table 3. Association of IgG anti- \( H. \text{ pylori} \) antibodies prevalence with the lymphoma cases.

|               | IgG Anti- \( H. \text{ pylori} \)+/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 \( H. \text{ pylori} \) 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 \( H. \text{ pylori} \) has been reported to be associated with some types of lymphoma specifically gastric and MALT lymphomas. Clinically it is important to explore the \( H. \text{ pylori} \) 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

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