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ORIGINAL ARTICLE

Relation between Hepatitis C Virus and *Helicobacter Pylori* in Non-Hodgkin's Lymphoma Patients

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ABSTRACT

Background*: It has been recently hypothesized that Helicobacter pylori (H. pylori) and Hepatitis C virus (HCV) might be involved in the pathogenesis of malignant non-Hodgkin's lymphoma (NHL).* ***Objective****: is to assess if there is a relationship between Helicobacter pylori and Hepatitis C virus infection and malignant non-Hodgkin's lymphoma.* ***Methodology:*** *The study was performed on 100 adult NHL patients, newly diagnosed cases, at clinical pathology department; Faculty of medicine, Al-Azhar university from December 2015 to July 2017. Fifty apparently healthy relative individuals were studied as the control. We searched for H. pylori stool antigen using an enzyme immunoassay (EIA) procedure. HCV was investigated by EIA to detect its antibodies, reverse transcriptase polymerase chain reaction (RT-PCR) for the presence of its RNA and viral sequencing for the determination of the viral vireamia.* ***Results:*** *H. pylori stool antigen detected by the ELISA technique was found in 54/100 of the cases (54.0%) and (28.0%) of the controls with a p value <0.001. Correlation between H. pylori and different clinical and pathological data revealed that; there was a statistical significant correlation between patients complaining of fatigue, fever and the positivity of H. pylori (p-value <0.001). HCV antibodies detected by the ELISA technique were found in 35/100 patients and in 10/50 of controls with a p-value <0.001. Hepatitis C Virus antibodies and Helicobacter pylori stool Antigen were equally distributed among clinical and pathological parameters in Non-Hodgkin's Lymphoma cases. There was an overlap between both infections in eleven (11) of the 100 patients reactive for HCV antibodies were also positive for H. pylori stool antigen.* ***Conclusion****: H.pylori and HCV infection may have a role in the pathogenesis of non- Hodgkin lymphoma*

INTRODUCTION

The term lymphoma describes a heterogeneous group of malignancies of B cells, T cells, and rarely natural killer (NK) cells and their precursors that usually originate in the lymph nodes, but they may originate in any organ of the body [1].

The non-Hodgkin's lymphomas (NHL) etiology remains a controversial matter, but, in the last few years, considerable evidence suggests that aberrations of the immune system and infections may act as etiologic agents, in at least some cases of NHL [2].

Thus, it is evident that other infections rather than the well-known EBV model may play a pathogenetic role in the occurrence of lymphoma, due to its heterogeneity in site and different cell types, several infectious agents may contribute to this nature of lymphoma [3].

The presence of hepatitis C virus (HCV) infection has also been recognized in several hematological disorders such as mixed cryoglobulinemia, low-grade malignant lymphomas and Waldenstrom's disease [4].

Helicobacter pylori is one of the most frequent causes of gastroduodenal infections worldwide, resulting in the release of various bacterial and host dependent cytotoxic substances including ammonia, platelet activating factor, interleukins 1 and 12, tumor necrosis factor (TNF) and reactive oxygen species [5,6].

Numerous studies confirmed the crucial role of *H. pylori* in the pathogenesis of gastritis and peptic ulcers. Some studies support the conclusion that the association of *H. pylori* with gastric cancer is causal [7].

Moreover, extra gastric mucosa associated lymphoid tissue (MALT) lymphoma has been also linked to *H. pylori* infection based on the observation that early eradication of this infection in low-grade tumors leads to complete remission [8].

A pathogenetic role of HCV has been hypothesized for a subset of B-cell NHL and of *H. pylori* with MALT lymphoma; but this has been emphasized in adult NHL patients [9]

Moreover, both *H. pylori* and HCV have pathogenic influence on gastric and liver epithelium, including the risk of malignant transformation [10]

Aim of the work: is to assess if there is a relationship between *Helicobacter pylori* and Hepatitis C virus infection and malignant non-Hodgkin's lymphoma.

METHODOLOGY

Subjects:

The present study was carried out on 100 lymphoma patients, diagnosed at El-Hussein university hospital, clinical pathology department Faculty of medicine, Al-Azhar University from December 2015 to August 2017. Diagnosis was based on clinical, hematological and pathologic examination, as well as imaging. Fifty apparently normal subjects (patient's relatives) not suffering from any malignancy, abdominal or hematological conditions, with matched, socio-economic status were studied as controls.

Methods:

Patients under study were already newly diagnosed NHL patients not received treatment. All cases and control were subjected to following tests: Complete Blood Count (CBC), Liver function tests, HCV Ab by ELISA and *Helicobacter pylori* stool Ag by ELISA. Cases were subjected also for bone marrow aspiration, flow cytometry for the diagnosis of hematological dissemination of lymphoma.

H. Pylori:

Detection of *H. pylori* stool antigen was done using ELISA technique.

Hepatitis C Virus:

Serological detection of IgG antibodies to HCV was achieved in sera of both patients and controls by ELISA, using a kit supplied by Innogenetics N.V. Belgium. HCV RNA PCR as confirmatory test.

Statistical Analysis:

It included frequency and percentage for categorical data and mean and standard deviation for quantitative data. p-values less than 0.05 were considered significant.

RESULTS

Clinical Characteristics of Patients: The age of the patients and controls ranged from 18-83 years with a mean of 57±2.1 years for patients, and 51 years for controls. They were 63.0 % males and 37.0 % females with a ratio of 2.3:1, while the controls were 64.0% males and 36.0 % females with a ratio of 1.3:1. Of the 100 cases, 61 were diagnosed as having localized NHL (61.0%) and 39 were diagnosed with disseminated lymphoma (39.0%). The main presentation was extranodal, as 32.0% of the patients had extranodal lesions (E) whereas 7.0 % presented with nodal disease (N). 81.0% and 76.0% of cases complained of Fatigue and fever respectively. As for the site of disease27.0% of cases showed GIT mass, 7.0% showed mediastinal mass and 5.0% showed affection of other sites.

Staging and Histological Subtype: According to St. Jude staging system, 12.0% of the cases presented as Stage I, 20% cases as Stage II, 42.0% as Stage III and 26.0% as Stage IV. The pathologic subtypes were classified according to WHO into (4.0%) Burkitt's lymphoma (BL), 47.0% diffuse large cell lymphoma (DLCL) and 4.0% were lymphoblastic lymphoma (LBL). Immunophenotype was done for 100 patients, and revealed 61% B-phenotype and 39% T-phenotype.

Helicobacter Pylori Results: *H. pylori* stool antigen detected by the ELISA technique was found in 54/100 of the cases (54.0%) and (28.0%) of the controls with a p value <0.001. Correlation between *H. pylori* and different clinical and pathological data revealed that; there was a statistical significant correlation between patients complaining of fatigue, fever and the positivity of *H. pylori* (p-value <0.001). *H. pylori* was equally distributed among pathological subtypes, stages of the disease and immunophenotypes. The results are shown in table (1).

Hepatitis C Virus Results: HCV antibodies detected by the ELISA technique were found in 35/100 patients and in 10/50 of controls with a p-value <0.001.

In the present study Hepatitis C Virus antibodies and *Helicobacter pylori* stool Antigen in Non-Hodgkin's Lymphoma cases equally distributed among clinical and pathological parameters

Overlap between both infections: eleven (11) of the 100 patients reactive for HCV antibodies were also positive for *H. pylori* stool antigen (table 2).

Table 1: Correlation between *H. pylori* and different clinical and pathological and residence parameters in the studied cases.

Parameters	***H. pylori* 65 cases**	P. value
	The cases for only *H. pylori* (54)and, the cases for both *H. pylori* and HCV (11)	
Age (years)	20+70 y	0.034
Sex ; Female Male	 20 45	0.230
Residence ; Rural Urban	 43 22	0.177
Commonest symptoms ; Fatigue Fever	 40 25	0.001
Nodal states; Nodal 0-1 Extranodal>1	 18 47	0.864
Site of disease ; Abdominal Axillary Others	 37 23 10	0.883
Stage of disease ; Stage 1+2 Stage 3+4	 26 39	0.755

Table 2: Correlation between HCV antibodies and different clinical, pathological and residence parameters of studied cases

Parameters	**HCV Ab (35)**	P . value
	The cases for only H. HCV (35) and, the cases for both *H. pylori* and HCV (11)	
Age (years)	20-70%	0.535
Sex ; F M	 11 24	0.806
Residence ; Rural Urban	 24 11	0.171
Commonest symptoms ; Fatigue Fever	 29 27	0.654
Nodal states; Nodal 0-1 Extrenodeal>1	 23 12	0.782
Site of disease ; Abdominal Axillary Others	 11 7 17	0.141
Stage of disease ; Stage 1+2 Stage 3+4	 14 21	0.817

DISCUSSION

Several studies have reported a high rate of prior hepatitis C viral infection in patients with non-Hodgkin's lymphoma (NHL). However, it appears that there are marked geographical differences in the prevalence of HCV among NHL patients. Further there is controversy concerning a possible pathogenetic link between HCV and certain histological lymphoma subtypes, in particular MALT lymphomas and it has been speculated that HCV might be involved in the multistep process of gastric lymphoma genesis[11], in addition to the well established role of chronic *H. pylori* infection [12].

In the present study, a high prevalence of HCV (35.0%) and *H. pylori* infections (54.0%) was detected in NHL patients when compared to the control group, 10/50 vs. 14/50. Similarly, a high prevalence of HCV and *H. pylori* infections was previously reported in 180 newly diagnosed HIV-negative B-cell NHL patients, consecutively seen at a referral oncology center in Southern Switzerland when prospectively studied. Infection with HCV was detected in 17/180 patients (9.4%), whereas, anti-Helicobacter antibodies were detected in 81/180 patients (45%) [13].

The relationship between HCV and NHL was previously reported in several studies and was found to be linked to geographical areas with high HCV prevalence among the general population. In Egyptian NHL patients, almost one third of patients showed evidence of HCV infection, 32% for HCV-antibodies and 28% for HCV-RNA[11].

The increased prevalence of HCV in our study might reflect the increased incidence of HCV in certain areas of the Egyptian population, as an incidence of 20% was previously reported in cases living in rural areas [14] and 23% by other investigators [15].

No specific characteristics, as a certain clinical feature, histological subtype or stage of disease, were encountered in our group of HCV positive NHL patients. Similarly, neither histological subtypes nor specific extra-nodal presentations of NHL that were associated with a higher prevalence of HCV were encountered in other studies [16,13].

Still, other studies reported a high prevalence of HCV in certain types of NHL as with low grade B-cell NHL [17,18], or with aggressive NHL [19]. The failure of correlation of a certain subtype of NHL in the present study with HCV positivity could be explained by the limited histopathological subtypes of NHL in patients, which usually falls in the high-grade category. In fact, it was lately recommended by hemato-oncologists from different geographic regions that the terms like low-intermediate and high-grade should no longer be used[20,21].

An increased positivity of *H. pylori* was observed in our NHL patients and the prevalence of *H. pylori* was previously found

to vary among different geographical areas, related to poor socioeconomic conditions, and to increase significantly with age [22,23].

In the present study, *H. pylori positivity* was significantly increased with older patients. For a better understanding of the significance of *H. pylori* in our NHL patients, the site of disease was considered. Although *H. pylori* showed more positivity in patients presenting with a gastrointestinal localization of disease, this relation still did not reach a significant level.

Whether this increased prevalence of HCV and *H. pylori* indicates an active role in lymphomagenesis or it just represents a failure of eradication of infection as a result of weak immune system is difficult to decide on the basis of our results. Moreover, failure to define a certain extra-nodal presentation or a specific histological subtype of NHL in our patients further denies a possible pathogenetic role of these agents in NHL. Actually, the possibility exists that these infections occurred as independent events on a previously disturbed immune system that is susceptible to both chronic infections and lymphomagenesis. Or, it is also possible that these infections were a previous event that led to immune suppression, making the patient more amenable for lymphoma to occur. In fact, the presence of both infections in a good percentage of our patients necessitates a careful search for the effects of infections in this population of patients.

CONCLUSION

From the present study, the following can be concluded:

- Egypt is one of the highest prevalence rates of HCV infection in the world, with high incidence of hepatic morbidity and mortality from the HCV late complications, such as chronic hepatitis , cirrhosis and hepatocellular carcinoma (HCC).
- *H.pylori* and HCV infection may have a role in the pathogenesis of Non hohgkin lymphoma.

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