

Hepatitis C Virus and Its Genotypes and Helicobacter Pylori in Pediatric Non-Hodgkin's Lymphoma: Could There be a Possible Etiologic Role?

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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). However, most of the studies were carried out on adult patients. On the basis of this observation, we sought to determine the prevalence of these infections in pediatric NHL patients at National Cancer Institute (NCI) and whether there is any clinical or histopathologic picture linked to the presence of these infectious agents.

Patients and Methods: The study was performed on 119 pediatric NHL patients, either as new or relapse cases, at the hematology out patient clinic of NCI from January 2002 to July 2003. Thirty apparently healthy children were studied as the control. We searched for H. pylori IgG antibodies using an enzyme immuno-assay (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 genotype.

Results: Antibodies for H. pylori were detected in 51/119 (43.2%) and in none of the control group, $p \leq 0.001$, whereas HCV antibodies were found in 21/119 (17.6%) of the NHL patients and in one of the controls (3.3%), $p \leq 0.001$. HCV RNA was detected in 18/21 (85.7%) anti-HCV positive patients analyzed, but was negative in the anti-HCV positive control. Viral genotype most frequently encountered was genotype 4; a mixed genotype of 1+4 was detected in four patients and only one patient showed genotype 1. No specific histological subtype, extra-nodal presentation, nor stage of disease was related to H. pylori or HCV positivity. Older age was significantly related to H. pylori positive NHL patients. A positive correlation between the presence of H. pylori antibodies and a complaint of vomiting and diarrhea was observed in our patients, $p \leq 0.001$.

Conclusions: We report a high prevalence of HCV and H. pylori infections in paediatric NHL patients at NCI. Concerning the hypothesis of their pathogenetic role

in lymphomagenesis, it is still unclear whether these agents have a direct role in malignant transformation in pediatric lymphoma because a typical NHL clinico-histological feature associated with HCV and H. pylori is lacking.

Key Words: Hepatitis C virus - Helicobacter pylori - Non-hodgkin's lymphoma - Pediatric lymphoma.

INTRODUCTION

The etiology of non-Hodgkin's lymphomas (NHL) 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. Several viruses have been identified as possible etiologic agents for NHL; one of the best studied is the Epstein-Barr virus, which was detected in cultures of tumor cells from patients with Burkitt's lymphoma. In addition, the human T-cell lymphotropic virus family (HTLV) was also recognized as possible etiologic agents for several lymphomas, such as cutaneous T-cell lymphoma and T-cell leukemia-lymphoma syndrome (HTLV-I), and T-cell hairy cell leukemia (HTLV-II) [1]. Recently, the presence of hepatitis C virus infection has also been recognized in several hematological malignancies such as mixed cryoglobulinemia, low-grade malignant lymphomas and Waldenstrom's disease [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.

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 and reactive oxygen species [3]. Numerous studies confirmed the crucial role of *H. pylori* in the pathogenesis of gastritis and peptic ulcers. Recent studies support the conclusion that the association of *H. pylori* with gastric cancer is causal [4]. Moreover, extra gastric 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 [5].

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 [6,7]. Therefore, we aimed to determine whether there is increased frequency of HCV and *H. pylori* infections in pediatric lymphoma patients when compared to healthy children. In addition, we looked for any clinical or histopathological distinctive features in those who are HCV and *H. pylori* positive in order to determine whether they have a role in lymphomagenesis.

PATIENTS AND METHODS

The present study was carried out on 119 pediatric lymphoma patients, diagnosed at the National Cancer Institute (NCI), Cairo University from January 2002 to July 2003. They were either new or relapse cases. Diagnosis was based on clinical, hematological and pathologic examination, as well as imaging. Thirty apparently normal children from Abo-El-Rish Children's hospital, not suffering from any malignancy, abdominal or hematological conditions, with matched age, sex and socio-economic status were studied as controls.

Patients under study were investigated for history of complaints of vomiting and diarrhea and to clinical examination for detection of the site of the disease, being nodal or extranodal. Diagnosis of NHL was made by L/N biopsy according to the World Health Organization (WHO) classification [8]. Immunophenotypic analysis for surface B and T lymphocyte markers was done for 85 out of 119 cases. Laboratory investigations done included complete blood

picture and bone marrow examination for the diagnosis of hematological dissemination of lymphoma.

H. Pylori:

Serological detection of IgG antibodies to *H. pylori* was done using a bioelisa *Helicobacter* kit supplied by Biokit, Spain, in sera of both patients and controls. The interpretation of results was qualitatively determined as positive if samples showed an absorbance above the cut-off value. Qualitatively, samples with absorbance > the cut-off value (0.9) are considered to have *H. pylori* IgG antibodies. Then the concentration of antibodies was quantitatively measured in Au/ml by a semilog calculation for the positive samples.

Hepatitis C Virus:

Serological detection of IgG antibodies to hepatitis C virus was achieved in sera of both patients and controls by ELISA, using a kit supplied by Innogenetics N.V. Belgium.

I- RT-PCR of HCV:

HCV RNA was extracted from the sera using Quiagen kits. Oligonucleotide primers: RT-PCR were performed with a primer pair selected from the highly conserved 5'-UTR of HCV genome [9]. The following sequences were used as antisense primers for c-DNA synthesis HCV-6 (5'-ACC-TCC-3' nucleotides (NT) 319-324). The internal primers were RB6A and RB6B for amplification of 266 bp of the 5'-UTR, RB6A 5'-GTG AGG AAC TAC TGT CTT CAC G-3' (NT 47-68) and RB6B 5'-ACT CGC AAG CAC CCT ATC AGG- 3' (nt. 292-312).

Reverse transcription was performed in 25µl reaction volume containing the following: 20 U of Rnase inhibitor (Promega Biotec Madison, US), 67mM tris HCl (pH 8.8), 17mM ammonium sulfate, 1mM B-mercaptoethanol, 6uM EDTA (pH 8.0), 0.2mg/ml of bovine serum albumin (Boehringer, Germany), 6mM Mg Cl₂, 25ng of HCV primer, 0.6µl of 25mM (each) deoxynucleotide triphosphates, 11.5µl of the nucleic acid elute and 200U superscript-11 Rnase H⁺ reverse transcriptase (GIBCO-BRL, Gaithersburg, Md., Germany). The mixture was incubated at room temperature for 5 min and then at 42°C for 60 minutes. RT product was denatured by incubation for 5 minutes at 95°C. The PCR was performed in a 50µl volume containing the following: 2.5U Taq polymerase (Perkin-

Elmer Cetus, US), 50mM tris HCl (pH 8.3), 20mM KCl, 1.2mM MgCl₂, 1mg/ml BSA, 12.5µl of the RT reaction mixture, 200mM (each) deoxynucleotide triphosphate and 100ng of each RB-65A and RB-6B primers. The samples were denatured at 95°C for 5 minutes and subjected to 35 rounds of thermal cycling in a DNA thermal cycler (type 480, Perkin Elmer Cetus, US). Each cycle consisted of denaturation for 1 minute at 95°C, annealing for 1 min at 55°C, and extension for 2 minutes at 72°C. After the cycling program, the samples were incubated for 10 minutes at 72°C.

Upon completion of the amplification reaction, 10µl each PCR reaction product was analyzed by electrophoresis through a 1.2% agarose gel in tris-acetate-EDTA buffer (pH 8.0) and ethidium bromide staining. DNA was transferred from the gel onto nitrocellulose filter using 4N NaOH. The transferred DNA was cross linked by incubation for 2-3 hours at 80°C and the blot was then hybridized with an internal probe. Fig. (1) shows an example of the amplified product resulting from RT-PCR for HCV.

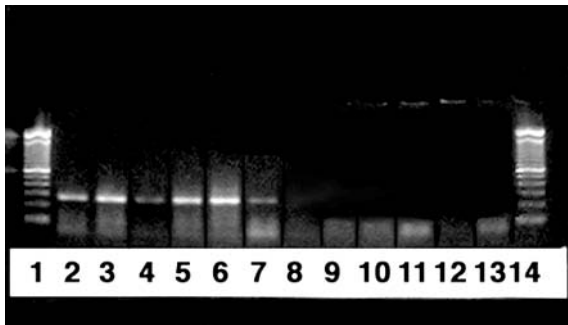


Fig.(1): Ethidium bromide stained gel electrophoresis of HCV RT-PCR product showing positive (lanes 2-7) and negative cases (lanes 8-13). Lanes 1 and 14 are showing 100 base pair ladder. Positive signals are 265 base pairs.

II- HCV Genotyping:

HCV-RNA positive samples were genotyped with both the LiPA and TRUGENE 5'NC kit from aliquots of a single RT-PCR sequence analysis.

1-INNO LiPA II, Genotyping: The line probe assay was used to assess HCV genotypes using kits provided by Innogenetics, N.V., Belgium. The 5-UTR region was amplified using nested PCR with biotinylated primers. The labeled amplicon was allowed to hybridize and mounted on a strip. After stringent washing, streptavidin labeled with alkaline phosphatase was used to

trace the hybridized products and nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl-phosphate were used as a substrate according to the manufacturer's instructions. The probe reactivity patterns were interpreted using the chart provided by the manufacturers and as described above [10].

2- HCV Genotyping by Sequencing: The TRUGENE HCV 5' NC genotyping kit, Visible Genetics Inc. (USA), was used in conjunction with the OpenGene DNA sequencing system. All HCV PCR products were run on a 1% agarose gel electrophoresis. The specific band was cut from the gel and purified by silica, and sequenced by CLIP sequencing. This allows both directions of the target amplicon to be sequenced simultaneously in the same tube using two different dye-labeled primers (Cy5.0 and Cy5.5) for each reaction. A set of four reactions was prepared containing standard dideoxynucleotide primers, one primer labeled with Cy5.0 and one with Cy5.5. A PCR reaction was performed for 45 cycles from each primer. The reaction products were detected in four lanes of a polyacrylamide gel using a two-dye automated OpenGene DNA sequencing system. This method provides sequence information for both positive and negative DNA strands from a single reaction.

The forward and reverse sequences are combined to form a query sequence. The query sequence is then compared to previously characterized isolates in the TRUGENE HCV 5' NC Module of the OpenGene software system in order to determine the HCV genotype of the sample. Gene Objects software analyzes chromatograms from each sample; the final 5'UTR sequence was obtained from the comparison of both sequenced strands. This information is compared with deposited HCV sequences by Gene Librarian™ software with a minimal concordance of 98%. The genotype assignments of these samples were confirmed by BLAST searches.

Sequencing was carried out to detect the genotypes of 10 cases with their gene numbers deposited in the National Center for Biotechnology Information/National Institute of Health Gen Bank nucleotide sequence data base with accession number AY707623- AY707637. Fig. (2) shows the results of the sequence analysis of one of the cases.

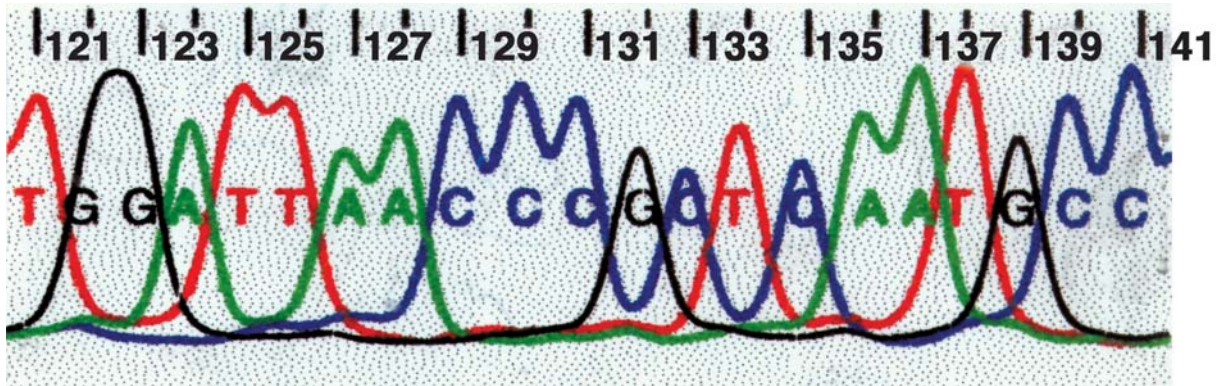


Fig. (2): A photo of the HCV sequence analysis of HCV in a NHL case positive for HCV-RNA.

Statistical Analysis:

Statistical analysis was done using the SAS system [11]. 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 1 to 18 years with a mean of 7.6 ± 4.2 years for patients, and 6.8 ± 4.6 years for controls. They were 69% males and 31% females with a ratio of 2.3:1, while the controls were 56% males and 44% females with a ratio of 1.3:1. Of the 119 cases, 79 were diagnosed as having localised NHL (66%) and 40 were diagnosed with disseminated lymphoma (34%). The main presentation was extranodal, as 43.24% of the patients had extranodal lesions (E), 21.62% had nodal and extra-nodal (E+N), whereas 35.14% presented with nodal disease (N). Twenty-nine of cases complained of vomiting and diarrhea. As for the site of disease, 40.74% of cases showed GIT mass, 16.67% showed mediastinal mass and 42.59% showed affection of other sites (e.g. bony NHL, eye, L/N, kidney, parotid and maxillary).

Staging and Histologic Subtype:

According to St. Jude staging system, 14.29% of the cases presented as Stage 1, 20.54% cases as Stage 2, 33.04% as Stage 3 and 32.14% as Stage 4. The pathologic subtypes were classified according to WHO into 37.6%

Burkitt's lymphoma (BL), 22.6% diffuse large cell lymphoma (DLCL) and 39.8% were lymphoblastic lymphoma (LBL). Immuno-phenotyping was done for 85 patients, and revealed 61% B-phenotype and 39% T-phenotype.

Helicobacter Pylori Seroprevalence:

H. Pylori antibodies detected by the ELISA technique was found in 51/119 of the cases (43.22%) and 0/30 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 vomiting and diarrhea and the positivity of H. pylori antibodies (*p*-value <0.001). H. pylori antibody was equally distributed among pathological subtypes, stages of the disease and immuno-phenotypes (Table 1).

H. Pylori Titer:

Among the 51 positive cases, high titer i.e. ≥ 120 Au/ml was found in 33/51; eight of them had a titer of 640Au/ml. Again, there was a statistical significant relation between the antibody titer and patients presenting with gastro-enteric manifestations mainly vomiting and diarrhea, as higher titers were detected in patients complaining with vomiting and diarrhea, (*p*=0.004).

Hepatitis C Virus Results:

HCV antibodies detected by the ELISA technique were found in 21/119 patients and in 1/30 of controls with a *p*-value <0.001. The only patient of the controls, who was anti-HCV reactive, was found negative by PCR. HCV

antibodies positivity was equally distributed among clinical and pathological parameters as summarized in Table (2). HCV-RNA was detected by RT-PCR in 18/21 (85.7%) anti-HCV antibody-positive patients analyzed. HCV genomic sequences to identify HCV genotype was found to be of type 4 in the majority of patients, mixed 1+4 in four patients and of type 1 in one

case. The INNO LiPA, genotyping gave the mixed genotypes of HCV, whereas sequencing gave pure 4 types, which were either 4a or 4c.

Overlap Between Both Infections:

Twelve of the 21 patients reactive for HCV antibodies were also positive for *H. pylori* antibodies.

Table (1): The correlation between *H. pylori* antibodies and different clinical and pathological parameters in 119 lymphoma patients.

Parameters	H. pylori		p-value
	+ve (n=51)	-ve (n=68)	
Age (years)	9.1±4.2	7.4±4.5	0.034
Sex:			
F	13 (35%)	24 (65%)	0.230
M	38 (46%)	44 (54%)	
Type of disease:			
DL	17 (42.5%)	23 (57.5%)	0.864
NHL (localised)	34 (43%)	45 (57%)	
Extranodal (E)	20 (42.6%)	27 (57.4%)	0.557
Extranodal and nodal (E,N)	13 (54.2%)	11 (45.8%)	
Nodal (N)	16 (41.1%)	23 (58.9%)	
Site of disease:			
GIT	20 (46.5%)	23 (53.5%)	0.883
Mediastinal	8 (44.4%)	10 (55.6%)	
Others	19 (41.3%)	27 (58.7%)	
Vomiting and diarrhea:			
Negative	22 (31%)	49 (69%)	<0.001
Positive	21 (72.4%)	8 (27.6%)	
Pathologic subtypes:			
BL	18 (51.4%)	17 (84.6%)	0.362
DLCL	7 (35%)	13 (65%)	
LBL	20 (54.1%)	17 (45.9%)	
Immunophotyping:			
B-phenotype	21 (40.4%)	31 (59.7%)	0.314
T-phenotype	17 (52%)	16 (48%)	
Stage of disease:			
Stage 1 + 2	16 (42.1%)	22 (57.9%)	0.755
Stage 3 + 4	33 (45.2%)	40 (54.8%)	

Table (2): The correlation between HCV antibodies and different clinical and pathological parameters of 119 lymphoma patients.

Parameters	HCV		p-value
	+ve (n=51)	-ve (n=68)	
Age (years)	7.6±4.4	8.3±4.4	0.535
Sex:			
F	7 (18.9%)	30 (81.08%)	0.806
M	14 (17.07%)	68 (82.93%)	
Tupe of disease:			
DL	8 (20%)	32 (80%)	0.654
NHL (localised)	14 (17.7%)	65 (82.3%)	
Extranodal (E)	10 (20.8%)	38 (79.2%)	0.782
Extranodal and nodal (E,N)	5 (20.8%)	19 (79.2%)	
Nodal (N)	6 (15.4%)	33 (48.6%)	
Site of disease:			
GIT	12 (27.27%)	32 (72.73%)	0.137
Mediastinal	4 (22.3%)	14 (77.7%)	
Others	5 (10.9%)	41 (89.1%)	
Vomiting and diarrhea:			
Negative	12 (16.9%)	59 (83.1%)	0.654
Positive	6 (20.7%)	23 (79.3%)	
Pathologic subtypes:			
BL	5 (14.3%)	30 (85.7%)	0.644
DLCL	5 (23.8%)	16 (76.2%)	
LBL	6 (16.2%)	31 (83.8%)	
Immunophotyping:			
B-phenotype	12 (23%)	40 (77%)	0.373
T-phenotype	5 (15%)	28 (85%)	
Stage of disease:			
Stage 1 + 2	7 (18%)	32 (82%)	0.874
Stage 3 + 4	14 (19.1%)	59 (80.8%)	

DISCUSSION

Several recent 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 histologic lymphoma subtypes, in particular MALT lymphomas and it has recently been speculated that HCV might be involved in the multistep process of gastric lymphoma genesis, in addition to the well established role of chronic *H. pylori* infection [12].

In the present study, a high prevalence of HCV (17.6%) and *H. pylori* infections (43.2%) was detected in pediatric NHL patients when

compared to the control group, 21/119 vs. 1/30 and 51/119 vs. 0/30, respectively. 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 between 1990 and 1995 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%) [7].

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 adult Egyptian NHL patients, almost one third of patients showed evidence of HCV infection, 32% for HCV-antibodies and 28% for HCV-RNA [13]. These results corre-

sponded with another study from Italy in which HCV prevalence was 17.5% among 400 lymphoma patients and 5.6% among 396 controls [14]. Furthermore, HCV infection was detected in 17/100 patients with B-cell NHL versus 0/25 patients with non-B-cell NHL ($p=0.023$) and in 34 patients (6.6%) in the control group with miscellaneous diseases ($p=0.0011$) [15]. On the other hand, other studies done in areas where HCV is rare, failed to find an association between HCV and NHL [16].

Thus, it is clear that the apparent discrepancy between the various epidemiological studies performed so far could be explained by the geographical differences in infection patterns within the general population. It is noteworthy that many of the studies concerning these infections in NHL were done in adult patients. In some of these studies, HCV positivity was significantly associated with older age group [6]. The increased prevalence of HCV in our paediatric NHL patients might reflect the increased incidence of HCV in certain areas of the Egyptian population, as an incidence of 12% was previously reported in children living in rural areas [17]. This is supported by the finding of genotype 4 in most of our HCV-RNA positive patient, which is the predominant type found in our population [10]. Besides, children with NHL pass through a series of investigations before presenting to NCI, where they could have been exposed to infection.

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 some studies [7,18]. Still, other studies reported a high prevalence of HCV in certain types of NHL as with low grade B-cell NHL [19], or with aggressive NHL [14]. 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 the pediatric group of 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].

Serum HCV-RNA was used to detect active HCV infection. Of the 21 anti-HCV reactive patients, 18 (85.7%) were found positive for HCV-RNA. In contrast, the only patient of the controls, who was anti-HCV positive, was found negative by PCR. It is difficult to reach a conclusion that children in the control group could clear their HCV infections due to the infrequent positivity of HCV in the control group. HCV-RNA is more useful for the detection of HCV infection in the immuno-compromised patients. Simultaneous detection of anti-HCV antibodies and HCV-RNA is more informative in this population [18]. As regards the method used for genotyping of HCV-RNA, sequencing was more accurate in subtyping, as it gave a pure subtype in contrast to the INNO LiPA, genotyping which gave either mixed genotypes or a genotype without subtyping.

Not only increased positivity of *H. pylori* IgG antibodies was observed in our pediatric NHL patients, but also high titers of antibodies. The seroprevalence of *H. pylori* was previously found to vary among different geographical areas, related to poor socioeconomic conditions, and to increase significantly with age [21]. In the present study, *H. pylori* positivity was significantly increased with older patients and with a complaint of vomiting and diarrhea. 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. In our group of patients, among other abdominal lymphoma, two cases of gastric lymphomas were reported, that were positive for *H. pylori* antibodies. Similarly, Moschovi, et al, [22] recorded a 2% incidence of primary gastric lymphoma in childhood and suggested a causal link with *H. pylori* infection.

Several infectious agents have been identified as possible etiologic agents for NHL; in most cases, the presence of a particular agent increases the risk of developing cancer or speeds its progression. For example, HIV and other viruses that affect the immune system make infected individuals prone to a variety of cancers by weakening the body's natural defenses. But in other cases, there is now compelling evidence that certain agents may also play a critical role

in causing cancer. However, proving that a particular agent causes cancer is complicated. HCV and *H. pylori* are postulated to be involved in the pathogenesis of B-cell NHL. Yet both agents have not been linked to a known translocation, nor are they endowed with oncogenes. The exact mechanism of neoplastic transformation of both agents is still unknown [7]. Since oncogenesis is a multistep process, the persistence of these agents in the immune system with the consequent stimulation of clonal expansion of lymphocytes together with the combination of genetic and environmental factors may lead to transformation resulting in B-cell neoplasia [23].

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. Primarily, the young age of patients gives no enough time for the evolution of an established malignant disorder on top of an infectious disease, especially for an HCV infection. Although it was previously stated that HCV infection in NHL patients was associated with a shorter time to lymphoma progression [7], still this observation has yet to be verified in the pediatric group of patients. Moreover, failure to define a certain extra-nodal presentation or a specific histological subtype of NHL in our pediatric patients further denies a possible pathogenetic role of these agents in pediatric 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.

Thus, we report a high prevalence of HCV and *H. pylori* infections in pediatric NHL patients at NCI. Neither a specific histologic subtype nor a certain extra-nodal presentation could be linked to both infections. Older age group and gastroenteric manifestations were significantly related to *H. pylori* infections. A

causal relationship could not be postulated on the ground of the results of the present study. Further studies including the detection of the immune system components, in addition to investigating other infections are needed to clarify whether there is a defective immune system responsible for both chronic infections and lymphoma or that there is an actual relationship between these agents and pediatric lymphomas.

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