Evaluating the role of the hepatitis C virus in the pathogenesis of Hodgkin's lymphoma in Egyptian patients

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Received 15 May 2012
Accepted 15 July 2012

Egyptian Journal of Internal Medicine
2012, 24:43–46

Background
Hepatitis C is an infection caused by a virus that attacks the liver and leads to inflammation. Several studies from Europe have reported a high prevalence of hepatitis C virus (HCV) infection in patients with non-Hodgkin's lymphoma. It has been suggested that HCV plays a role in the pathogenesis of B-cell non-Hodgkin's lymphoma (B-NHL). The aim of our study was to determine the prevalence of HCV infection in patients with B-NHL in the Egyptian population and to compare it with apparently healthy volunteers (as a control group).

Patients and methods
The current study was carried out on 50 patients diagnosed with B-NHL (as a patient group) as well as 35 healthy individuals (as a control group). HCV status was evaluated by the detection of anti-HCV antibodies using the enzyme-linked immunosorbent assay (ELISA) technique as well as the detection of HCV RNA by a reverse transcription PCR (RT-PCR).

Results
In terms of the results of anti-HCV antibodies by ELISA, 26 of 50 patients (52%) were positive in patients with B-NHL compared with 10 of 35 cases (28.6%) in the control group ($P=0.0541$). HCV RNA detection by RT-PCR was positive in 30 of 50 patients (60%) with B-cell lymphoma compared with 15 of 35 patients (42.9%) in the control group ($P=0.1823$).

Conclusion
In conclusion, the results of our study show that there is a higher incidence of HCV infection in B-NHL patients compared with apparently healthy individuals. This supports the suspected role of HCV in the pathogenesis and etiology of B-NHL.

Keywords:
anti-hepatitis C virus antibodies, hepatitis C virus, hepatitis C virus RNA, non-Hodgkin's lymphoma, reverse transcription polymerase chain reaction

Egypt J Intern Med 24:43–46

1110-7782

Introduction

Hepatitis C virus (HCV) is a hepatotropic flavivirus that persistently infects hepatocytes and some lymphocytes [1]. HCV was first identified by molecular cloning of the virus genome in 1989 [2]. The majority of cases of HCV infection give rise to an acute illness, but up to 80% may then develop into chronic hepatitis. Almost all patients develop a vigorous antibody-mediated and cell-mediated immune response that fails to clear the virus infection, but may contribute to liver damage. Spontaneous resolution of chronic liver disease is very rare (<2%) and patients with chronic disease are at risk of developing hepatocellular carcinoma. However, some studies have suggested that infection may have a more benign outcome, at least in some populations [3]. Besides being a hepatotropic virus, HCV is also lymphotropic and its infection affects the B-lymphocyte compartment, with the occurrence of B-cell proliferative disorders. Accordingly, HCV infection is strongly associated with mixed cryoglobulinemia (MC) [4], a benign disorder characterized by the proliferation of B lymphocytes producing polyclonal IgG or monoclonal IgM with rheumatoid factor activity [5] that may characteristically precipitate at low temperatures. This condition develops in around 10% of the patients into an overt lymphoma [6,7]. In addition, HCV has also been suggested to play a role in the pathogenesis of B-cell non-Hodgkin's lymphoma (B-NHL) outside the context of MC [8–10], as several distinct types of NHL can be associated with HCV infection. HCV infection is also associated with an overall increased risk of NHL development in individuals without MC, although with a lower incidence than in individuals with MC. Many studies provide evidence that, in both NHL groups, HCV infection is associated with the development of both indolent and aggressive B-NHL, lymphoplasmacytoid lymphoma (immunocytoma), diffuse large B-cell lymphoma, and splenic lymphoma with villous lymphocytes, but it is not clear whether HCV-associated NHL with and without MC are two distinct clinical entities [11]. The role of HCV infection in lymphomagenesis may be related to chronic antigenic stimulation of HCV, similar to that reported for *Helicobacter pylori* infection in the development of gastric

mucosa-associated lymphoid tissue lymphoma [12–14]. The causal role of HCV in lymphomagenesis is also supported by the regression of indolent NHLs after eradicating the infection [15,16]. Recent retrospective studies have confirmed a high HCV prevalence also among patients with diffuse large B-NHL [17,18].

The aim of the present study was to determine the prevalence of HCV infection in Egyptian patients with B-NHL and to compare them with apparently healthy volunteers.

Participants and methods

The current study was carried out on 50 patients diagnosed with B-NHL (as a patient group), as well as 35 apparently healthy individuals who were age and sex matched with the studied group. All the participants included in the study gave their written informed consent before participation in the study. All of the participants included in the study were recruited from the clinical hematology unit in the internal medicine department, Kasr El Eini School of Medicine, in the period between August 2010 and May 2011.

Both patient and control groups were subjected to the following: full history taking and clinical examination, complete blood count with a differential white cell count, determination of the erythrocyte sedimentation rate, assessment of complete liver and kidney functions, serum uric acid, serum LDH, β2 microglobulin, abdominal ultrasound for the detection of organomegaly and lymphadenopathy, CT abdomen and pelvis for proper diagnosis and staging, determination of anti-HCV antibodies using the commercially available enzyme-linked immunosorbent assay (ELISA) kits, (Diagnostic Automation, Inc., Calabasas, California, USA) reverse transcription PCR (RT-PCR) for HCV RNA detection, immunophenotyping, lymph node biopsy for the diagnosis of non-Hodgkin's lymphoma, bone marrow biopsy, and immunohistochemistry for staging.

Two milliliter of venous blood samples were collected on plain tubes for anti-HCV antibodies detection using the commercially available ELISA kit; also 2 ml of venous blood was collected in a sterile EDTA vacutainer. Separation of mononuclear cells was carried out by Ficoll-density centrifugation; a part of this sample (RNA) was reverse transcribed and amplified by the single-step RT-PCR using the QIAGEN One-Step RT-PCR Kit (QIAGEN Sciences, Inc., Germantown I, USA) (catalogue number; 210212).

HCV RNA was determined by RT-PCR. A portion of RNA solution (obtained from mononuclear cell separation) was reverse transcribed and amplified by the single-step RT-PCR (Qiagen One-Step RT-PCR Kit) for 30 cycles with appropriate primers ′5-CGC GCG ACT AGG AAG ACT TC-3′ and ′5-ATA GAG AAA GAG CAAC CA GG-3′ as forward and reverse primers, respectively. Denaturation: for 30 s to 1 min at 94°C, annealing: for 30 s to 1 min at 50–68°C, extension: for 1 min at 72°C, and a final extension for 10 min at 72°C. Negative-strand HCV RNA was determined by the strand-specific RT-PCR. A portion of total RNA was reverse transcribed and amplified by the single-step PCR for 25 cycles with the sense primer (′5-AGC ATC GGG CCA GAA GTG TCC-3′) and an antisense primer (′5-CGT TCA TCG GTT GGG GAG CAG G-3′). Initial denaturation was carried out for 35 s at 94°C, annealing at 64°C for 40 s, and extension at 72°C for 45 s, followed by a final extension at 72°C for 5 min. The 174 bp second PCR product was subjected to electrophoresis using a 1.5 agarose gel and was visualized by ethidium bromide staining under ultraviolet light.

Results

The current study was carried out on 50 patients diagnosed with B-NHL (as a patient group), as well as 35 apparently healthy individuals (as a control group) who were age and sex matched with the studied group.

Patients included in the study were 24 women (48%) and 26 men (52%). Their ages ranged between 18 and 68 years, with a mean value of 45.8 ± 12.7.

In the control group, there were 10 women (28.6%) and 25 men (71.4%); their age ranged between 10 and 58 years, with a mean value of 27.9 ± 12.2.

There were no statistically significant differences between the two groups in terms of the sex ($P = 0.1160$); there were highly statistically significant differences between the two groups in terms of age ($P \leq 0.0001$).

The clinical data of the patients and control groups are shown in Table 1.

There were statistically significant differences between the two groups in terms of hepatomegaly ($P = 0.0216$) and splenomegaly ($P < 0.0001$), but no statistically significant difference between the two groups in lymphadenopathy ($P = 0.2934$).

The laboratory data of the patients and control groups are also shown in Table 1. There were no statistically significant differences between the two studied groups in terms of hemoglobin ($P = 0.0905$) and platelet count ($P = 0.0665$), but there was a statistically significant difference between the two groups in the total leukocytic count (TLC) ($P < 0.0001$).

In terms of liver functions, there were no statistically significant differences between the two studied groups in aspartate aminotransferase (AST) ($P = 0.0888$) and alanine aminotransferase (ALT) ($P = 0.8919$), but there were statistically significant differences between the two groups in total bilirubin ($P = 0.0280$) and total proteins ($P < 0.0001$) (Table 2).

In terms of the results of anti-HCV antibodies by ELISA as shown in Table 3, 26 patients(52%) were positive and 24 patients were negative (48%), whereas for anti-HCV RNA detection by RT-PCR, 30 patients (60%) were positive and 20 patients (40%) were negative.

In the control group, anti-HCV antibodies were positive in 10 cases (28.6%) and negative in 25 cases (71.4%), whereas

Table 1 Summary of the clinical and laboratory data of the patients and control groups

	N (%)				
	Patients (*N*=50)		Controls (*N*=35)		
	Females	Males	Females	Males	*P*-value
Sex (years)	24 (48%)	26 (52%)	10 (28.6%)	25 (71.4%)	0.1160
Hepatomegaly	25 (50%)		8 cases (22.9%)		0.0216
Splenomegaly	42 (84%)		8 cases (22.9%)		<0.0001
Lymphadenopathy	27 (54%)		14 (40%)		0.2934
	Range	Mean ± SD	Range	Mean ± SD	
Age (years)	18–68	45.8 ± 12.7	10–58	27.9 ± 12.2	<0.0001
Hemoglobin (g%)	6.5–16	10.6 ± 2.5	5.4–16	9.8 ± 1.4	0.0905
Platelet count ($\times 10^3$ mm^3)	21–567	172.7 ± 104.8	15–394	135.7 ± 65.7	0.0665
Total leukocytic count ($\times 10^3$ mm^3)	1.2–33	9.9 ± 2.5	0.2–40	22.3 ± 2.5	<0.0001

Reference values for hemoglobin, men: 13–17 g%, women: 12–15 g%; platelet count, 150 000–450 000/mm^3; total leukocytic count, 4000–11 000/mm^3.

Table 2 Summary of liver functions for the patients and control groups

	Patients (*N*=50)		Controls (*N*=35)		
	Range	Mean ± SD	Range	Mean ± SD	*P*-value
AST (IU/dl)	11–192	41.9 ± 38.0	10–80	29.7 ± 21	0.0888
ALT (IU/dl)	7–133	34.1 ± 29.9	12–85	33.3 ± 21	0.8919
Bilirubin (mg/dl)	0.10–2.80	0.75 ± 0.64	0.1–3	1.1 ± 0.8	0.0280
Total protein (mg/dl)	2.8–6.5	3.22 ± 1.04	3.5–8	4.9 ± 0.2.0	<0.0001

Reference values for AST, 10–35 IU/dl; ALT, 10–45 IU/dl; total bilirubin, 0.1–1 mg/dl.
ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 3 Summary of correlation of clinical and laboratory data with the results of anti-HCV antibodies and HCV RNA by RT-PCR in the patient group

	HCV antibodies		HCV RNA	
Parameters	*r*	*P*-value	*r*	*P*-value
Age	0.091	0.528	0.082	0.571
Hb (g%)	0.023	0.877	0.086	0.552
Platelets ($\times 10^3$ mm^3)	0.007	0.960	0.016	0.913
TLC ($\times 10^3$ mm^3)	0.074	0.608	0.118	0.413
AST (IU/dl)	0.122	0.398	0.046	0.753
ALT (IU/dl)	0.021	0.884	0.229	0.110
Bilirubin (T) (mg/dl)	0.059	0.682	0.018	0.901

ALT, alanine aminotransferase, AST, aspartate aminotransferase; Hb, hemoglobin; HCV, hepatitis C virus; TLC, total leukocytic count.

Table 4 Summary of the correlation of clinical and laboratory data with the results of anti-HCV antibodies and HCV RNA by RT-PCR in the control group

	HCV antibodies		HCV RNA	
Parameters	*r*	*P*-value	*r*	*P*-value
Age	0.200	0.250	0.140	0.422
Hb (g%)	0.059	0.736	0.138	0.429
Platelets ($\times 10^3$ mm$^{3)}$	0.078	0.654	0.143	0.412
TLC (mm^3)	0.074	0.673	0.069	0.694
AST (IU/dl)	0.079	0.652	0.369	0.029
ALT (IU/dl)	0.080	0.647	0.191	0.273
Bilirubin (T) (mg/dl)	0.522	0.01	0.465	0.005

ALT, alanine aminotransferase, AST, aspartate aminotransferase; Hb, hemoglobin; HCV, hepatitis C virus; TLC, total leukocytic count.

for HCV RNA detection by RT-PCR, 15 cases were positive (42.9%) and 20 cases were negative (57.1%).

There were no statistically significant differences between the results of anti-HCV antibodies between the two studied groups ($P = 0.0541$) and the results of HCV RNA detection by RT-PCR ($P = 0.1823$).

According to Pearson's correlation, there were no correlations between age, TLC, AST, and total bilirubin, whereas there were positive correlations between Hb, platelet count, ALT, and the results of anti-HCV antibodies in the patient group (shown in Table 4).

Also, there were no correlations between age, Hb, TLC, and ALT, whereas there were positive correlations between platelet count, AST, total bilirubin, and the results of HCV RNA by RT-PCR (shown in Table 4).

There were no correlations between age, Hb, platelets, TLC, AST, ALT, total bilirubin, and the results of anti-HCV antibodies and HCV RNA by RT-PCR in the control group (shown in Table 4).

Statistical analysis

Data were summarized and presented in the form of mean, range, percentage, and SD as descriptive statistics. Descriptive statistics and statistical comparisons were carried out using the statistical software program SPSS (version 16) (IBM SPSS Inc., Chicago, Illinois). Comparison of clinical data was carried out using the χ^2-test, whereas for the laboratory data, the independent *t*-test and ANOVA test were used. Pearson's correlation was used for comparison of ouantitative measurement data. A *P*-value less than 0.05 was considered to be statistically significant.

Discussion

HCV infection is endemic in some countries such as Japan, Italy, and Egypt. The role of HCV infection in lymphomagenesis may be related to chronic antigenic stimulation of

HCV [12]. The causal role of HCV in lymphomagenesis is also supported by the regression of indolent NHLs after eradication of the infection. Recent retrospective studies have confirmed a high HCV seroprevalence also among patients with diffuse B-NHL [16,17].

The aim of this work was to study the prevalence of HCV infection in Egyptian patients with B-NHL and to compare this with apparently healthy individuals.

It has been shown epidemiologically that HCV induces a number of extrahepatic manifestations [19,20], of which lymphoproliferative disorders is related most closely to HCV infection [21]. Accordingly, it has been accepted that chronic infection with HCV can lead to the clonal expansion of B cells and that the sustained proliferation of B cells would promote the occurrence of genetic mutations.

The results of our study are consistent with the study of Zuckerman *et al*. [22], who found that the incidence of HCV infection among patients with B-cell lymphoma was higher than that in two groups of controls that included patients with other malignant hematologic conditions and patients with general medical conditions.

The association of HCV infection and B-cell lymphoma that we found raises the possibility that HCV plays a pathogenic role. Additional support for the possible association of clonal B-cell expansion and HCV infection has been provided by Franzin *et al*. [23], who found a high frequency of clonal B-cell expansion in HCV-infected patients, even in the absence of cryoglobulinemia. On the basis of these observations, it may be speculated that chronic HCV infection alone or in the presence of other factors may lead to B-lymphoid expansion. The occurrence of a subsequent transforming event may lead to malignant lymphoma [24].

Also, Ferri *et al*. [25] and Pozzato *et al*. [26] found a positive association between HCV and NHL, a finding that has now been confirmed in a large number of studies.

In contrast to our study, Brind *et al*. [27] failed to detect antibodies to HCV in 60 patients with B-cell lymphoma. These contrasting findings may reflect the different geographic origins and ethnicity of the populations studied. An alternative explanation for the low prevalence of HCV in the lymphoma group in this study is that not all lymphoma types are associated with HCV infection.

The prognosis of NHL in HCV patients is still debated. Some studies show that the prognosis of HCV-positive aggressive NHL is similar to HCV-negative aggressive NHL [28]. However, other studies have shown that diffuse large B-cell lymphomas in anti-HCV-positive patients have a worse outcome in terms of overall survival and disease-free survival compared with HCV-negative patients [17].

Conclusion

The current study found a higher incidence of HCV infection in patients with B-NHL if compared with the control group; this confers a high possibility for the possible etiological role of HCV infection in the pathogenesis of B-NHL.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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