The Association between Hepatitis C Virus Infection and Lymphoma

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Abstract: Objectives: To identify the frequency of HCV infection among patients with Non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD). Subjects and Methods: The study involved 152 patients; 111 with NHL and 41 with HD proved by lymph node biopsy and a control group of 50 individuals attending the internal medicine clinic of Sohag University hospital. ELISA technique was used for detection of antibodies against HCV. HCV RT-PCR was used to detect the presence of viral RNA in blood. Results: HCV antibody positivity was significantly higher ($p = 0.017$) in NHL (36%) and HD (31.7%) groups compared to the control group (14%). There was no significant difference in HCV antibody positivity between NHL and HD groups ($p = 0.495$). Using RT-RCR, HCV RNA was detected in 8 control subjects, 44 patients with NHL and 12 patients with ($p = 0.011$). Conclusion: Despite the high prevalence of HCV in the general population in Egypt, HCV is significantly more frequent in patients with NHL and Hodgkin's disease. Further larger studies are needed to establish this epidemiological relationship between HCV and lympho-proliferative disorders.

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1. Introduction

Hepatitis C virus (HCV) currently infects around 2% of the world's population[1]. HCV infection most often leads to an asymptomatic chronic state, which can later progress to active liver disease, liver failure, or primary hepatocellular carcinoma. The Middle East and North Africa region (MENA) suffers from high prevalence of unnecessary medical injections and transfusions, reuse of needles and syringes, needle-stick injuries among health care workers, and skin scarifications[2-4].

Collectively, among all nations, the percentage positivity for HCV ranges from 0.01% in Scandinavia to 3% in North Africa, with a single unique exception, Egypt[1]. The recently published Egyptian Demographic Health Survey (EDHS) in 2009 was a national probability sample of the resident Egyptian population. This report estimated an overall anti-HCV antibody prevalence of 14.7%. The number of Egyptians estimated to be chronically infected was 9.8%[5].

This virus can be isolated from hepatocytes, hepatic lymphocytes, peripheral blood mononuclear cells, bone marrow and lymphoid tissues in infected people. Particles of viral genome were found in T and B cells of patients with chronic hepatitis and infected with HCV[6]. This is due to the fact that HCV is a hepatotropic and lymphotropic virus.

HCV infection was found in most of the type 2 mixed cryoglobulinemia patients. It is known that it progresses to B-cell non-Hodgkin lymphoma (NHL) in some cases[7]. In rare B lymphocyte neoplasms associated with monoclonal IgM production (Waldenstrom's macroglobulinemia), HCV infection is found in substantial proportion of cases, indicating a possible role for the virus in neoplastic IgM gammopathies[8].

Several papers were published concerning the possible relationship among HCV infection, mixed cryoglobulinemia (MC) and non-Hodgkin lymphoma (NHL)[9-12].

The aim of the current study was identification of the frequency of HCV infection among patients with Non-Hodgkin's lymphoma and Hodgkin's disease.

2. Subjects and Methods:

This study involved 152 patients with generalized lymphadenopathy. Diagnosis of lymph node biopsy proved non-Hodgkin's lymphoma in 111 patients and Hodgkin's disease in 41. A control group of 50 individuals attending the internal medicine clinic of Sohag University hospital for non-hepatological non-hematological complaints was included. All patients were recruited from the departments of surgery, internal medicine and medical oncology of Sohag University hospital between July 2007 and June 2009.

An informed consent was provided by each patient before history taking and complete physical examination. We stressed upon history of blood transfusions, jaundice or prior diagnosis of infective hepatitis. A venous blood sample of 10 ml was

obtained from each subject, centrifuged and stored at -20 ℃ until examined. Blood samples were then subjected to virological studies.

1. Detection of anti-HCV-Ab by ELISA:
Sera were tested for HCV-Ab using ELISA (Sorin Biomedica, Sallugia, Italy). It employs HCV polypeptides corresponding to highly antigenic determinants of both the structural and non-structural regions of HCV.

2. HCV RT-PCR:

a. Nucleic acid extraction: HCV was purified form 100 µl of serum or 100 ul of CIC precipitate as previously described by *Boom et al. (1990).*

b. Conversion to c-DNA (RT-Step): A cocktail of RT reaction was prepared with a final volume of 12.5 µl to which the 12.5 µl of the elution step was added and reaction for RT took place in the thermal cycler (Perkin-Elmer Cetus, type 480), where the tubes were incubated at 40 ℃ for 50 min. followed by another 15 min. at 70 ℃.

c. DNA amplification (PCR): A cocktail for PCR reaction was prepared with a final volume of 37.5 µl to which 12.5 µl of the RT reaction product was added and reaction of PCR took place in the thermal cycler: a. Samples were denatured at 95 ℃ for 5 min., and b. were subjected to 40 rounds of thermal cycling where a cycle consisted of denaturation for I min. at 95 ℃, annealing for 1 min. at 55 ℃, and extension for 2 min. at 72 ℃. After the cycling program, the samples were incubated for 10 min. at 72 ℃.

d. Detection: The RT-PCR amplicon was detected by 2% agarose gel electrophoresis using a Miniprep electrophoresis chamber (Pharmacia, LKB), and the gel was then examined under the UV light and photographed. The expected HCV-PCR band was at the level of 265 bp range.

3. Results:

Patients with NHL were significantly older than control and HD groups. There was no gender significant difference between the three groups. Only 7 cases (6.3%) of the NHL group had T-cell disease (Table 1).

Table 1: Characteristics of the three studied groups

	Control	NHL	HD	p value
Age, yrs, mean ±SD	42.3 ±11.3[a]	50.6 ±16.6[b]	39.4 ±13.5[a]	< 0.001
Gender, male/female	22/28	53/58	24/17	0.357
Disease Type, No. (%)				
B cell		50 (45.0)		
T cell		7 (6.3)		
Others		54 (48.7)		

Groups with different superscript letters are significantly different

HCV antibody positivity was significantly higher in NHL and HD groups compared to the control group. There was no significant difference in HCV antibody positivity between NHL and HD groups (p = 0.495). There was a significant difference between the three groups in frequency of HCV infection (p = 0.011). HCV was detection in 8 control subjects, 44 patients with NHL and 12 patients in Hodgkin's disease group (Table 2).

Table 2: Frequency of HCV antibody positivity and RNA detection by PCR in the three studied groups

	Control	NHL	HD	p value
HCV antibody positivity	7 (14.0%)	40 (36.0%)	13 (31.7%)	0.017
HCV RNA by PCR	8 (16.0%)	44 (39.6%)	12 (29.3%)	0.011

4. Discussion:

The results of this study demonstrated a significantly high frequency of HCV antibodies among patients with NHL (36%) and HD (31.7%). Control subjects had a high frequency of HCV antibody positivity (14%). However, there was no significant difference between patients with NHL and HD in HCV antibody positivity (p = 0.495).

Initial observational studies among unselected patients indicated a very strong association between HCV infection and NHL, while the viral infection in the patients with other haematological malignancies was similar to that observed in the general population[13,14]. Bellentani et al.[15] reported a prevalence of 28% among NHL patients between 18 and 65 years. These studies were Italian, where the

prevalence of HCV is particularly high, especially in the South (up to 12%)[16-18].

The relationship between HCV and NHL was considered secondary to the high prevalence of HCV in the general population and therefore, confined to some areas of the world[19]. This can apply to the current series, as Egypt is considered one of the areas with high prevalence allover the world. However, many papers coming from different areas of the world reported the higher HCV prevalence in cases of NHL compared to general population.

It was suggested that HCV and NHL seem to be closely associated only in some countries, independent of the crude HCV prevalence in the general population[20]. In Italy several studies reported a prevalence of HCV infection between 8.9 and 37.1%, with a mean of 19.8%[21-23]. On the contrary, studies from Northern Europe[24-25] did not show any HCV positive patient (0%). In Germany there was a low incidence of HCV infection in NHL (1.9-4.3%)[26,27] and a study from the south of Switzerland[28] indicated a high prevalence of 9.4%. Higher figures were reported from Hungary (23.8%) and Romania (29.4%)[29,30].

Studies from Canada reported a low incidence of HCV infection (0.0-2.3%)[31,32]. In the United States, the prevalence was variable, in Los Angeles, where at least 50% of the cases are in Hispanic people, the prevalence of HCV infection is rather high (11.5-21.7%)[33,34], likewise in New Orleans[35] (25%), while in Midwest[36] there was no association (1.4%). Papers from Japan documented a prevalence of HCV infection ranging from 5.7 to 22.2% in NHL patients[37,38].

The mechanism by which HCV can induce malignant lympho-proliferative diseases is the main issue in this respect. As an RNA virus, HCV cannot be integrated into the host genome. Other indirect mechanisms are still possible as oncogene activation, cellular growth induction or apoptotic cell death inhibition[39].

It is likely that persistence of the virus in lymphocytes may result in chronic stimulation of B cells, leading to polyclonal expansion of these cells that can evolve into an oligloclonal and finally monoclonal expansion. This is supported by finding of somatic hypermutations in immunoglobulin genes in some HCV-positive immunocytomas[40].

De Rosa et al. reported preferential expression of VH51p1 associated with the VLkv325 gene combination in a subtype of B-lymphocytes associated with chronic HCV infection[41]. bcl-2 protein overexpression is a marker of low-grade lymphomas. The overexpression of bcl-2 in low-grade follicular lymphoma is generally (80%) due to a reciprocal translocation (14:18)[42], while in some cases (20%) no chromosomal abnormalities are found[43]. It was found that one-third of HCV-positive cases affected by chronic hepatitis without cryoglobulins showed the same chromosomal translocation[44].

On the contrary, patients affected by HBV chronic hepatitis or by ethanol-induced chronic liver disease do not show any chromosomal abnormalities, indicating that HCV seems to be the agent of this translocation. This 14:18 translocation was detected in patients who relapsed after the interruption anti-viral therapy[45].

Since lymphomagenesis is a longstanding, multistep process, it is likely that different mechanisms at different times could be operating to influence the manifestation of various HCV-related B-cell diseases. This could also explain the wide geographical heterogeneity of MC and NHL.

Few papers reported the between HCV infection and Hodgkin's lymphoma. In a Hungarian Keresztes et al.[46] found 9% HCV positivity of HD patients which was 12 times higher that control. They supposed that HD and its treatment may result in a state of decreased immune reactivity, which may lead to the reactivation of the former hepatitis infection. On the contrary, other authors found no difference in HCV infection in HD patients and normal population[47-49].

Our results are extremely different from these figures, even the Hungarian study that reported 9% prevalence of HCV among HD patients. However, it is important to notice that they found 12-fold increased prevalence compared to controls. In the current study we observed only a 2-fold increased frequency. The exceptionally high prevalence of HCV in Egypt necessitates further larger, preferably multicenter, studies to establish this epidemiological relationship between HCV and lympho-proliferative disorders.

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