Clinical and Epidemiologic Study of Hepatitis C Virus Genotype 4 Infection among Patients with B cell non Hodgkin's Lymphoma

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Background and study aim: Many recent studies showed that chronic infection with hepatitis C virus (HCV) is associated with increased risk for B-cell non-Hodgkin's lymphoma (NHL). The aim of this study is to evaluate the frequency of HCV infection in a series of de novo B cell non Hodgkin's lymphoma (NHL) patients and to correlate virological findings with clinico-histological features.

Patients and methods: 50 patients with B cell NHL diagnosed by histopathology and immunophenotyping were recruited from Tropical medicine department and Oncology unit affiliated to Internal medicine department, Zagazig University hospitals. Gender and age matched controls (N = 50) were volunteers selected from relatives of patients. Study participants were subjected to history taking, clinical examination, routine and specific laboratory tests. Anti-HCV antibody was determined by ELISA for all study participants. HCV RNA PCR was done for all cases and HCV antibody positive controls. Appropriate radiologic examinations were performed.

Results: Frequency of HCV infections were statistically significantly higher in B cell NHL patients than in controls ($p = 0.004$). ALT levels were statistically significantly higher in HCV positive patients than in HCV positive controls ($p < 0.001$) and HCV negative patients but without statistically significant difference ($p = 0.067$). There was no statistically significant difference in histologic types, grades and stages of NHL between HCV positive patients and HCV negative patients. Cryoglobulinemia showed no significant difference between studied groups.

Conclusion: HCV has a strong association with de novo B cell NHL, not complicating essential mixed cryoglobulinemia (EMC).

INTRODUCTION

The World Health Organization (WHO) estimates that 170 million people are infected with hepatitis C virus (HCV) [1]. An estimated 12–15% of Egyptians, have serological evidence of HCV infection (up to 99 % genotype 4), with higher rates in older age groups and residents of rural areas in lower and middle Egypt. There is evidence for a large-scale iatrogenic transmission of HCV during the parenteral anti-schistosomal treatment campaign carried out from the 1920s through the 1980s [2]. Continued transmission in Egypt has been associated with transfusion of unscreened blood, invasive surgical procedures, including Caesarean section and abortion; injections by informal health care providers and haemodialysis [3, 4].

Since its identification, HCV has been added to the roster of tumour-associated viruses because of its role in hepatocarcinogenesis. It has also been linked to extrahepatic disease manifestations [5]. One of the extrahepatic diseases in which HCV has been implicated is B cell non Hodgkin's lymphoma (NHL). HCV associated lymphomas have been observed, but whether they are caused by HCV remains to be shown definitively.

There is a suggestion that some B-cell NHL associated with HCV arise from clonal expansion of B-cells with particular immunoglobulin gene rearrangements specific for the E2 protein of the HCV envelope [6]; which is consistent with the hypothesis that lymphomas develop when B cells proliferate in response to antigen. However, no biological mechanism of HCV-associated lymphoma genesis has been definitively elucidated [7].

Most of the studies reported to date failed to find an association of HCV with NHL were conducted in areas where the prevalence of HCV was extremely low, leaving open the possibility that such an association actually exists but could not be detected because neither cases nor controls had adequate opportunity for exposure [8,9,10]. Working in a population with high prevalence of HCV allowed us to conduct a case-control study with adequate statistical power to assess the question of whether there is an association of chronic HCV infection with NHL [11].

Some studies investigated the sequel of HCV infection on the liver of patients with B cell NHL [12,13]. In the present study, we try to evaluate the frequency of HCV infection in a series of *de novo* B-cell non-Hodgkin's lymphoma (B NHL) patients and to correlate virological findings with clinico-histological features.

PATIENTS AND METHODS

50 patients with B cell NHL were collected from Tropical medicine department and Oncology unit affiliated to Internal medicine department. The diagnosis of B cell NHL was based on histopathology and confirmed by immunophenotyping. Of these cases, 25 were admitted at Tropical medicine department, because of generalized lymphadenopathy (7 patients), FUO (5 patients), anaemia (5 patients), persistent vomiting (3 patients), ascites (2 patients), anorexia and weight loss (2 patients) and bleeding tendency (1 patient). The other 25 cases were patients already diagnosed as B cell NHL and coming to receive chemotherapy in the Oncology unit.

The control group included 50 volunteers selected from relatives of patients admitted at Tropical medicine department and the Oncology unit. Controls were frequency-matched to cases by the 5-year age category and gender. Control subjects were representative of the source population of cases by region; since all cases and controls were from the region of Sharkia governorate. Controls were matched to cases as regard other risk factors of NHL; namely smoking and occupational exposure to industrial or agricultural pesticides. That is to have a more accurate assessment of HCV infection as a risk factor for NHL.

HCV infection among cases and control was defined as: positive HCV RNA PCR test with or without positive HCV antibody test. Positive HCV antibody test alone was not adopted to, minimize the false positive results of HCV infection among cases and controls in patients who catched the virus and cleared it, and minimize the false negative results of HCV infection among cases in patients who are immunocompromised and can not sustain antibody response to HCV infection. In order to further minimize the false negative results of HCV infection among cases, cases were selected at the time they were diagnosed before starting treatment, so, immunocompromisation complicating chemotherapy excluded.

After an informed consents were obtained from all subjects before enrollment, study participants were subjected to: detailed history taking and thorough clinical examination, routine laboratory. investigations **(**CBC, LFT, KFT, INR and urine analysis) and special lab. investigations(detection of anti-HCV antibodies using a third generation enzyme-linked immunosorbent assay (ELISA),determination of HCV RNA PCR in cases and HCV positive controls using COBAS® AmpliPrep/COBAS® Taqman® HCV Test. Detection of cryoglobulinin in cases and HCV positive controls, [14] and determination of LDH, in all patients [15]. Pelviabdominal ultrasonography was done for all cases and for HCV positive controls to determine the sonographic state of the liver, spleen, abdominal lymph nodes and to detect the presence of ascites. Pelviabdominal and chest CT and MRI of brain and spinal cord were done for all cases for staging [16]. Histopathologic examination and immunophenotyping were performed at Pathology department, faculty of medicine, Zagazig University. The slides were reviewed blindly without knowledge of virologic state of the patient. Formalin-fixed tissues were stained with haematoxylin and eosin and examined by light microscopy. Type and grade of B cell NHL were defined according to the 2008 World

Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues[17]. Low grade B-NHL included: marginal zone lymphoma (nodal, splenic and extranodal), lymphoplasmocytoid lymphoma and grade 1-2 follicular lymphoma. Intermediate and high grade B-NHL included: diffuse large cell lymphoma, mantle cell lymphoma, Burkitt's lymphoma and grade 3 folliclar lymphoma.

All slides were subjected to immunophenotyping for B- and T-cell markers. Identification of B- and T-cell surface markers was carried out using pan-B (CD-20) and pan-T (CD-45) monoclonal antibodies with the DAKO EnVision System (Code No. K4006, DAKO, Carpinteria, CA, USA). Patients with samples that tested positive for the B-cell marker were considered cases, while those positive for the T-cell marker were dropped from the study, regardless of previous classification based on histological examination of haematoxylin and eosin-stained slides.

HCV positive cases and controls with no clinical, laboratory and sonographic evidence of liver cirrhosis underwent a liver biopsy taking. Hepatitis grading and staging were evaluated according to the METAVIR scoring system [18, 19].

Patients were excluded form the study if the had any of the following criteria: patients with B-cell NHL who started treatment for patients collected from the oncology unit, patients who turned out to be T-cell lymphoma by immuno phenotyping, for those collected from tropical medicine department, age less than 15 years old, patients not physically and mentally capable of understanding and completing interview, hepatitis B surface antigen positive patients and HIV patients.

Statistical Analysis

Data were checked, entered and analyzed using SPSS version 15 for data processing and statistics. Data were expressed as numbers, and percentage for qualitative variables and mean (x) ± standerd deviation (SD), and range for quantitative variables. Student, t, test, and chi-square (X^2) were used when indicated to assess significance, $p < 0.05$ was considered significant and highly significant if $p < 0.001$.

RESULTS

The present study included 50 patients with B-cell NHL and 50 age and sex matched control.

Characteristics for studied groups were represented in table (1). There was statistically significant increase in the percentage of NHL patients who had positive HCV Ab and HCV RNA than control (p, 0.02, p, 0.004 respectively), also viral loads were higher in HCV infected patients than HCV infected control without statistically significant difference (p, 0.79) table (2). Liver enzymes and serum bilirubin were significantly increase in HCV positive cases when compared with HCV positive control ($p < 0.001$, P, 0.005 respectively), table (3). Elevated LDH levels were more common in HCV positive patients (14 patients out of 18 patients) than HCV negative patients (20 control out of 32 control) without statistically significant difference (p, 0.26 data not shown). Apart from, lymphomatous infiltration in 3 HCV positive patients, and one HCV positive patient with liver cirrhosis, no significant differences in necro inflammatory activity grading and staging of fibrosis between HCV positive patients and HCV positive control were recorded (p, 0.24 data not shown).

There was no statistically significant difference in staging, grading and types of NHL between HCV positive patients and HCV negative patients table (4). As regard cryoglobulonimia and cryoglobulinemic manifestations, no significant difference between HCV positive patients and HCV positive control was recorded, table (5).

Table (1): Demographic data of both studied groups

Demographic character	Patients (N=50)		Controls (N=50)		X2	P value
	Number	%	Number	%		
Age						
15-30	8	16.0	9	18.0	0.07	0.79
31-45	15	30.0	16	32.0	0.08	0.84
46-60	21	42.0	20	40.0	0.08	0.86
≥61	6	12.0	5	10.0	0.1	0.74
Gender						
Male	31	62.0	26	52.0	1.02	0.31
Female	19	38.0	24	48.0		
Smoking						
Non	33	66.0	29	58.0	0.68	0.4
Active	15	30.0	18	36.0	0.41	0.52
Ex-smoker	2	4.0	3	6.0	0.0	1.0
History of occupational exposure to pesticide						
No	41	82.0	40	80.0	0.06	0.72
Yes	9	18.0	10	20.0		
History of blood transfusion						
No	41	82.0	46	92.0	2.21	0.13
Yes	9	18.0	4	8.0		
History of surgical intervention						
Non	21	42.0	26	52.0	1.0	0.31
Major operation	9	18.0	5	10.0	1.33	0.24
Minor operation (dental manipulation)	20	40.0	19	38.0	0.04	0.83

Table (2): Serology and viremia of HCV infection in both studied groups

Parameter	Cases (N=50)		Controls (N=50)		X^2	P value
	Number	%	Number	%		
HCV Ab						
-ve	32	64.0	42	84.0	5.2	0.02
+ve	18	36.0	8	16.0		
HCV RNA PCR						
+ve	18	36.0	6	12.0	7.89	0.004
Viral load (IU X 10^5)					**T test**	
$\overline{X} \pm SD$	2.61 ± 5.3		2.96 ± 3.8		0.25	0.79
Range	0.001-20.0		0.076 -9.5			

P < 0.05 significant P < 0.001 highly significant

Table (3): Comparison of lab. investigations of HCV positive patients and HCV positive controls

Lab. test	HCV positive cases (N=18)	HCV positive controls (N=6)	T test	P value
Liver function tests				
Total bilirubin (mg/dL)				
X ± SD	2.36 ± 5.2	0.67 ± 0.25	7.59	0.005
Range	0.3-23	0.3-1.2		
Direct bilirubin (mg/dL)				
X ± SD	1.47 ± 4.1	0.3 ± 0.19	5.12	0.02
Range	0.1-18	0.1-0.8		
S. Albumin (gm/dL)				
X ± SD	3.5 ± 0.7	3.9 ± 0.3	2.95	0.004
Range	1.7-4.6	3.2-4.5		
ALT				
X ± SD	68.3 ± 22	36.8 ± 12.5	6.45	<0.001
Range	33-120	18-77		
AST				
X ± SD	70.8 ± 22	35.8 ± 8.6	7.99	<0.001
Range	38-114	21-55		
CBC				
Hb (gm/dL)				
X ± SD	10.9 ± 2.1	12.2 ± 2	2.09	0.04
Range	6.1-12.8	8.6-14.1		
PLT count (x 10^3/mm^3)				
X ± SD	182 ± 75	186 ± 49	0.22	0.82
Range	72-290	97-310		
WBC count (x 10^3/mm^3)				
X ± SD	6.8 ± 2.6	7.1 ± 2	0.49	0.62
Range	3.5-12.5	3.9-10.2		
INR				
X ± SD	1.2 ± 0.2	1.03 ± 0.05	1.9	0.31
Range	1-1.8	1-1.2		

Table (4): Comparison of histopathologic types, grading and staging of B cell NHL in HCV positive patients and HCV negative patients

Type of NHL	HCV positive patients (N=18)		HCV negative patients (N=32)		X^2	P value
	Number	%	Number	%		
Biffuse large B cell lymphoma	6	33.3	15	46.9	0.87	0.35
Follicular lymphoma	3	16.7	7	21.9	0.01	0.94
Small cell lymphoma/ chronic lymphatic leukemia	2	11.1	2	6.3	0.01	0.94
Marginal zone lymphoma	2	11.1	2	6.3	0.01	0.94
MALT lymphoma	1	5.6	3	16.7	0.01	0.94
Mantle cell lymphoma	1	5.6	2	6.3	0.27	0.68
Lymphoplasmacytic lymphoma	2	11.1	0	0.0	1.38	0.24
Burkitt lymphoma	1	5.6	1	3.1	0.11	0.74
Grade of NHL						
Low	6	33.3	13	40.6	0.26	0.61
Intermediate	4	22.2	8	25.0	0.02	0.82
High	8	44.5	11	34.4	0.5	0.48
Staging of NHL						
I	4	22.2	5	15.6	0.04	0.56
II	5	27.8	12	37.5	0.49	0.48
III	3	16.7	10	31.3	0.63	0.42
IV	6	33.3	5	13.6	1.2	0.27

Table (5): Comparison of cryoglobulinemia among HCV positive patients and HCV positive controls

Parameter	HCV positive patients (N=18)		HCV positive controls (N=6)		X^2	P value
	Number	%	Number	%		
Cryoglobulinemia						
No	10	55.6	4	66.7	0.0	1.0
Yes	8	44.4	2	33.3		
Cryoglobulinemic manifestations in the cryo +ve patients	2	11.1	0	0.0	0.73	0.39

DISCUSSION

HCV association with B cell NHL is still a matter of debate, no association between HCV infection and B cell NHL was found [8, 9]. Most of the studies that failed to find an association of HCV with B cell NHL were conducted in areas where the prevalence of HCV is extremely low; leaving open the possibility that such an association actually exists but could not be detected because neither cases nor controls had adequate opportunity for exposure to the virus [10].

Working in a population with the highest prevalence of HCV allowed us to conduct a case-control study with adequate statistical power to assess the question of whether there is an association of chronic HCV infection with B cell NHL [11].

The present study shows that the incidence of HCV active infection among the control group is 12% (6 out of 50 control), 16 % of controls were HCV antibody positive, 2 HCV Ab positive control were HCV RNA PCR negative, that mean they cleared viremia. About 15% of the Egyptian had HCV positive anti bodies while 10% had active infection with positive HCV RNA PCR. [20, 21]

The incidence of HCV infection among cases was 36 % (18 out of 50 cases). All cases were positive for HCV antibodies and for HCV RNA PCR; that mean none of the infected cases could clear viremia. This finding can be explained by the fact that B cell NHL is a sequel of disordered

immune system which can't successfully clear viremia [11].

The occurrence of HCV active infection among cases was statistically significantly higher than in controls (p 0.004). These results suggest a positive association between HCV infection and B cell NHL [11, 22].

Studies which failed to find an association were carried out in communities with low prevalence of HCV infection. Their results may be explained by the smaller sample size of their studies than that required to obtain an adequate statistical power or by that the spread of HCV in those communities is relatively recent and not having enough time to be complicated by NHL [23].

HCV viral loads were higher in HCV associated B cell NHL patients compared to HCV infected controls without statistically significant difference (p, 0.79). This finding is in agreement with that obtained by Karavattathayyil et al., [24] who demonstrated actively replicating virus in HCV-associated lymphomas.

There was a statistically highly significant increase in liver enzymes levels between HCV positive patients and HCV positive controls (p value < 0.001). This finding may be due to:

Lymphomatous infiltration of the liver in HCV positive patients and Chemotherapy induced hepatotoxicity in HCV associated patients [12]. There was no statistically significant difference in liver enzymes levels between HCV positive patients and HCV negative patients. Liver enzymes levels were elevated in both groups but higher values occurred in HCV positive patients [12, 13].

There was no statistically significant difference between different histological types of HCV associated lymphomas. In the present study, the commonest types of HCV associated B cell NHL were diffuse large B cell lymphoma which is an aggressive lymphoma followed by follicular lymphoma which may be indolent or aggressive. This is in concordance with Goldman et al. [25], who found that HCV is associated with diffuse large B cell, marginal zone, and follicular lymphomas. While others, found that lymphoplasmacytoid lymphoma/immunocytoma and Waldenströmmacroglobulinemia, were the only NHL associated to HCV, because they studied patients with HCV associated essential mixed cryoglobulinemia (EMC) in whom that types of NHL are a common complication [22, 26, 27]. The results of the present study showed that HCV is associated with de novo NHL not complicating essential mixed cryoglobulinemia (EMC).

The percentage of HCV positive patients who had cryoglobulinemia (44.4%) was higher than those with HCV positive control (33.3%) without significant difference. 2 HCV positive NHL patients had cryoglobulinemic manifestations in the form of purpura, arthralgia and microscopic hematuria for many years before the diagnosis of lymphoplasmacytic lymphoma. However, others found that HCV associated lymphoma, were overt B cell lymphomas that complicate essential mixed cryoglobulinemia (EMC) with up to 30% of cases associated with hepatitis C [22, 26, 27]. Our results showed that HCV is linked to de novo B cell lymphoproliferative disorders not complicating mixed cryoglobulinemia (EMC). The difference form our result is attributed to difference in selection criteria as, they selected patients with EMC as an inclusion criteria.

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