Prevalence of Hepatitis C Virus in Lymphoproliferative Disorders

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Abstract

Objective: To study the prevalence of hepatitis C virus in lymphoproliferative disorders.

Methods: A case control prospective study was performed on 143 patients with lymphoproliferative disorders and 29 patients with non-hematological malignancies were taken as controls. All the patients in both groups were analyzed for various risk factors for infection with hepatitis C virus and were tested for the presence of hepatitis C virus antibody (anti HCV), cryoglobulins and rheumatoid factor antibody. Hepatitis C viremia was documented by detection of HCV RNA by polymerase chain reaction.

Results: There was no significant difference for risk factors for hepatitis C virus infection in both the groups except for the increase in number of surgical procedures being carried out in the control group. There was no significant difference in the presence of rheumatoid factor antibody in both the groups and cryoglobulins were not positive in any individual. Five percent patients with lymphoproliferative disorders and 3.4% with non-hematological malignancies were positive for anti HCV. HCV RNA was detected in 29.2% cases and 31.0% in controls.

Conclusion: There was no association between hepatitis C virus infection and lymphoproliferative disorder in our population. However, further studies are required from this region to establish any causal relationship between hepatitis C virus infection and lymphoproliferative disorder (JPMA 54:202;2004).

Introduction

Hepatitis C virus has recently been implicated in the etiology of lymphoproliferative disorders. Hepatitis C virus (HCV) is an RNA virus that belongs to the family of flavivirus. The natural targets of HCV are hepatocytes and possibly B-lymphocytes.[1] In infected patients, HCV-related antigens have been found in peripheral blood B and T lymphocytes, lymph nodes and lymphocytes infiltrating the liver.[2] Eli Zukerman et al have reported a higher prevalence of monoclonal Ig H rearrangement and bcl - 2 translocation in patients with hepatitis infection than in patients with chronic liver disease of other etiologies. These data also suggest that HCV may play a role in the multistep mechanism of lymphomagenesis by inducing proliferation of B cell and inhibition of apoptosis.[3]

It has also been postulated that the association between HCV infection, mixed cryoglobulinemia and lymphoplasmacytic lymphoma raises the possibility that HCV may be initiating agent or a cofactor in the pathogenesis of non-Hodgkin's Lymphoma.[2] Previous studies clearly show a relationship between HCV and mixed cryoglobulinemia, a condition that is strongly associated with lymphoplasmacytic lymphoma, a low grade B cell NHL.[4] Study conducted by Linda et al found that HCV 2 a and 3 genotype was detected with higher prevalence in patients with mixed cryoglobulinemia than in controls.[5] It has also been found that haplotype HLA - B8 and DR 3 is strongly associated with the development of HCV - related mixed cryoglobulinemia.

There is no published data regarding association between Hepatitis C virus and the lymphoproliferative disorders from Pakistan. The aim of this study was to find out the prevalence of hepatitis C in patients with lymphoprolierative disorders in our population.

Patients, Methods and Results

This prospective case control study was carried out from June 1998 to June 2001 at Aga Khan University and Hospital on diagnosed patients of lymphoproliferative disorders and non -hematological malignancies.

Patients with various lymphoproliferative disorders attending hematology oncology wards and outpatient clinics at Aga Khan University and Hospital were included in the study as cases. Patients with non-hematological malignancies were chosen as controls.

The only exclusion criterion was patients who did not consent to give information and blood sample for the study. All patients with lymphoproliferative disorders were diagnosed according to the criteria set by REAL (Revised European American Lymphoma) classification[6], using histological and immunologic techniques along with their clinical presentations. Two pathologists performed the microscopic examination of the material provided for the diagnosis.

Table 1. Distribution of patients with lymphoproliferative disorders (n=143).

Types of lymphoproliferative disorder	Frequency	%
Hodgkin's disease (Nodular sclerosis)	7	4.9
Hodgkin's disease (Mixed cellularity)	8	5.6
Diffuse non - Hodgkin's lymphoma	27	18.9
Follicular / Low grade NHL	8	5.6
Small lymphocytic lymphoma	3	2.1
T cell non - Hodgkin's lymphoma	6	4.2
Marginal zone lymphoma	1	0.7
MALToma	1	0.7
Multiple myeloma	24	16.8
Waldernstroms macroglobulinemia	2	1.4
Chronic lymphocytic leukemia	28	19.6
Hairy cell leukemia	2	1.4
Acute lymphoblastic leukemia	26	18.2

All of the non-hematological malignancies were diagnosed on histological examination of the excised tumor mass. Diagnosis of hepatitis C virus infection was made if patients were tested positive for the hepatitis C antibody by ELISA and for hepatitis C RNA by PCR (polymerase chain reaction). Sample size was calculated by using Epi Info program.

Questions asked for the assessment of risk of acquiring hepatitis C infection included history of blood transfusion, intravenous drug abuse, needle stick injury, history of surgical procedures, hepatitis C in sexual partner and also history of therapeutic injections. Patients were also inquired about the history of presence of autoimmune features such as dry eyes, dry mouth, purpura, arthralgias and numbness of limbs. Blood samples were tested for serum cryoglobulins, rheumatoid factor antibody, hepatitis C virus antibody and hepatitis C virus RNA by polymerase chain reaction.

Table 2. Distribution of patients with non-haematological malignancies (n=29).

Type of malignancy	Frequency	%
Ca Breast	9	31.6
Transitional cell Ca of Bladder	3	10.3
Ca Colon	3	10.3
Ca Caecum	1	3.4
Ca nasopharynx	1	3.4
Squamous cell Ca of Palate	1	3.4
Osteogenic Sarcoma	1	3.4
Adeno Ca of Stomach	1	3.4
Choroid Carcinoma	1	3.4
Ca Ovary	1	3.4
Ca Parotid	1	3.4
Ewing's Sarcoma	1	3.4
Ca Lung	1	3.4
Ca Esophagus	1	3.4
Ca Prostate	1	3.4
Abdominal Sarcoma	1	3.4
Embryonal Rhabdomyosarcoma	1	3.4

Cryoglobulins were performed by manual method.[7] Rheumatoid factor reagent[8] was performed by agglutination slide test (RF LATEX TEST, RANDOX laboratories Ltd. UK).There is an association between high rheumatoid factor (RF) activity and hepatitis C virus viral replication and selection of monoclonal or oligoclonal rheumatoid factor secreting B cells.[9] It has also been mentioned that rheumatoid factor are also induced during normal antiviral or antibacterial immune responses. In keeping with evidence given above we also looked for the presence of cryoglobulins and rheumatoid factor (RF) activity along with anti HCV antibodies and HCV RNA by PCR in our patients.

Table 3. Analysis of variables related to the risk of hepatitis C infection.

	Cases (n=143)	Control (n=29)	P value
History of blood transfusion			
Yes	49 (34.3%)	10 (34.5%)	1.000
No	94 (65.7%)	19 (65.5%)	
History of surgery			
Yes	24 (16.8%)	26 (89.7%)	0.000
No	119 (83.2%)	3 (10.3%)	
History of IV drug abuse			
Yes	0	0	NA
No	143 (100%)	29 (100%)	
History of needle stick injury			
Yes	0	0	NA
No	143 (100%)	29 (100%)	
History of hepatitis C in sexual partner			
Yes	0	0	NA
No	143 (100%)	29 (100%)	
History of therapeutic injections			
Yes	1 (0.7%)	1 (3.4%)	0.310
No	142 (99.3%)	28 (96.6%)	
History of features of autoimmune disease			
Yes	7 (4.9%)	1 (3.4%)	1.000
No	136 (95.1%)	28 (96.6%)	

Anti-HCV antibodies were performed using 3rd generation ELISA method (AXSYM HCV version 3.0, Abbott, Illinois, U.S.A.). Patients were also screened for hepatitis C viremia by PCR performed by reverse transcriptase polymerase chain reaction (AMPLICOR, ROCHE, Indianapolis, U.S.A.).

Statistical analysis was performed using risk estimate, calculated, by using odds ratio, Chi-square test and logistic regression for multivariate analysis.

Table 4. Results of RF antibody, cryoglobulins and hepatitis C antibody.

Test	Cases (n=143)	Controls (n=29)	Odds ratio (95% CI)	P value
Rheumatoid factor Antibody				
Positive	12 (8.4%)	0	NA	NA
Negative	131 (91.6%)	29 (100%)		
Cryoglobulins				
Positive	0	0	NA	NA
Negative	143 (100%)	29 (100%)		
Hepatitis C antibody				
Positive	7 (4.9%)	1 (3.4%)	1.441 (0.171, 12.18)	1.000
Negative	136 (95.1%)	28 (96.6%)		

Table 5. Results of hepatitis C virus detection by polymerase chain reaction.

	Cases (n=48)	Controls (n=29)	Odds radio (95% CI)	P value
Positive	14 (29.2%)	9 (31.0%)	0.915	1.00
Negative	34 (70.8%)	20 (69.0%)	0.336, 2.049)	

One hundred and forty three patients with lymphoproliferative disorders were enrolled as cases and 29 patients with non-hematological malignancies taken as controls.

Distribution of patients with lymphoproliferative disorders are given in Table 1. There were 94 males (65.7%) and 49 (34.3%) females and 12 (41.4%) males and 17 (58.6 %).in the mean age non-lymphoproliferative group. Lymphoproliferative disorder was 43 years (range 5-85 years) and in non-lymphoproliferative group was 50 years (range 12-73). Difference was insignificant.

All the patients in both the groups were analyzed for various variables related to the risk factors for hepatitis C virus infection. History of surgery was the only risk factor for hepatitis C infection that was significantly different in both the groups. All the patients were also analyzed for rheumatoid factor antibody, cryoglobulins and hepatitis C

antibody. Other results also revealed that there is no significant difference in presence of rheumatoid factor antibody in both the groups and the risk of high titers of RF antibody was not increased in patients with lymphoproliferative disorder. Cryoglobulins were not positive in any individual.

Hepatitis C virus RNA was then detected by polymerase chain reaction (PCR). All the controls were analyzed for hepatitis C using this method while 48 patients with lymphoproliferative disorders were analyzed for hepatitis C by PCR. Results for hepatitis C by PCR are given in Table 3.

The results of analysis of patients for hepatitis C infection by polymerase chain reaction and by detection of hepatitis C antibody in both cases and control group showed that there is no statistically significant difference between both the groups for the presence of hepatitis C infection. Also the risk estimate calculated by odds ratio showed that there is no association between hepatitis C virus infection and lymphoproliferative disorder.

Since no disease association with hepatitis C infection was established and not more than one of the risk factors for hepatitis C infection was found to be significant, hence multivariate analysis was not done.

Comments

Our study suggest a low prevalence of hepatitis C virus infection in lymphoproliferative disorder in contrast to relatively high rates seen in Italian[10], Romanian[11] and Japanese population.[12]

In our study we have analyzed a broad and heterogeneous group of lymphoproliferative disorder along with a diverse group of non - hematological malignancies.

Rate of hepatitis C virus infection in Pakistan is 1 to 6 % as found in large population of healthy blood donors tested for hepatitis C virus antibody.[13-15] The rate of positivity for hepatitis C infection in our group of lymphoproliferative disorder was not found to be higher as compared to the rate estimated to be in our general population. Comparing the rate of hepatitis C virus antibody positivity and hepatitis C viremia in our patients with lymphoproliferative disorders and the control group, it was found that there is no significant difference between the two groups.

Our findings are more in agreement with that of Mc coll et al who have suggested no association between lymphoproliferative disorder and hepatitis C virus infection.[16]

Our results are however in contrast to that reported in Italy by Ferri et al.[17] The high prevalence of this infection in a corresponding Italian population does suggest a possible role. But the argument in relation to this finding was that since prevalence of hepatitis C virus infection was higher in this population, this hypothesis given by Ferri et al requires further explanation.

Studies done previously have mentioned that hepatitis C virus infection is associated with mixed cryoglobulinemia.[18] However in our study cryoglobulins were not detected in patients found to be positive for hepatitis C antibody or for hepatitis C viremia by polymerase chain reaction.

Recent literature also suggested that there is an association between hepatitis C viral replication and rheumatoid factor activity. In our study 12 (8.4%) patients with lymphoproliferative disorders were positive for rheumatoid factor antibody. None of these patients tested positive for hepatitis C viremia.

As far as various risk factors for hepatitis C are concerned, there was no significant difference between the two groups .The only significantly different risk factor was the higher number of surgical procedures being carried out in the control group. This could be explained by the fact that most of the patients in this category underwent surgery at the time of diagnosis of their malignancies.

We suggest that most probably geographical difference may be decisive in the etiopathogenesis of such heterogeneous and multifactorial malignancies. In particular, different infectious agent, genetic or environmental factors may variably be combined in patient population from different countries.

Further studies are required from our region and also from other parts of the world to establish the causal relationship between hepatitis C virus infection and lymphoproliferative disorder.

In view of our data we conclude that there seems to be no association between hepatitis C virus infection and lymphoproliferative disorder in our population. No major differences were observed for hepatitis C viremia in patients with lymphoproliferative disorder and with non-hematological malignancies.

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