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ORIGINAL ARTICLE

Hepatitis C virus infection among patients with non-Hodgkin's lymphoma in northern India

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Abstract
Background and aim Hepatitis C virus (HCV) has been postulated to be an etiological agent for lymphoid malignancies. Whereas a high prevalence of HCV infection in non-Hodgkin's lymphoma (NHL) patients has been shown to exist in many geographical areas of high HCV prevalence, studies from other parts have not established any form of association. In India, there is a scarcity of data to show either a positive or a negative association between NHL and HCV infection. Therefore, we determined the prevalence of HCV infection in patients with NHL.
Methods A total of 228 subjects were included, out of which, the number of newly diagnosed consecutive patients with lymphoproliferative disorders (NHL and CLL) were 57 [mean age, 48.7 years (range: 18–80)] and the control group consisted of 171 subjects [mean age, 48.6 years (range: 18–80)]. We used third generation enzyme immunoassay to detect HCV antibodies. HCV RNA was detected by nested RT-PCR.
Results Among the 57 patients of NHL, 44 (77.2%) had high-grade disease (diffuse large B cell), 6 (10.5%) intermediate-grade (follicular lymphoma), and 7 (12.3%) low-grade (small lymphocytic); 26 patients had B symptoms at diagnosis. None of the patient tested positive for antibody to hepatitis C virus (anti-HCV) while 1 patient (1.75%) tested positive for HCV RNA. Among the age- and sex-matched controls, 2 (1.17%) subjects tested positive for anti-HCV; both were also positive for HCV RNA.
Conclusions HCV infection is unlikely to be associated with lymphoproliferative disorders in northern India and does not play a major role in the pathogenesis of lymphoproliferative disorders.

Keywords Hepatitis C virus · Non-Hodgkin's lymphoma · Lymphomagenesis

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Introduction

Viruses have been increasingly incriminated as contributing to lymphomagenesis. Yet, except for a few well elucidated examples, such as the endemic form of Burkitt's lymphoma associated with Epstein-Barr virus [1] (EBV) (especially in African children) and the adult T-cell leukemia lymphoma with human T-cell leukemia/lymphoma virus (HTLV-1), the majority of associations are not well understood. Of the other viruses, the hepatitis C virus (HCV) has been postulated to be an etiological agent for some types of lymphoid malignancies. However, considerable geographical variations have been noted in the possible role of HCV in lymphomagenesis. Whereas a high prevalence of HCV infection in non-Hodgkin's lymphoma (NHL) patients has been shown to exist in many geographical areas of high HCV prevalence, studies in many other areas have not established any form of association. According to a study, the etiologic fraction of NHL attributable to HCV varies greatly by country, and may be upward by 10% in areas where HCV prevalence is high [2]. In India, studies on blood donors at Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh and All India Institute of Medical Sciences (AIIMS),

Delhi have shown HCV prevalence of 0.91 and 1.47%, respectively [3, 4].A study by Duseja et al. [5] at PGIMER showed a prevalence of anti-HCV positivity to be 0.87% among the health care workers.

In India, there is a scarcity of data to show either a positive or a negative association between NHL and HCV infection. In a preliminary study from our centre, the only study from India, only one patient (1%) out of a hundred patients of NHL tested positive for antibody to HCV (anti-HCV), signifying the absence of an association in our country [3]. A possible weakness of the previous study was that these patients were tested for antibodies alone and not for the presence of the viral RNA. This study was therefore conducted to determine, prospectively, whether an association does exist between HCV and NHL among Indian patients and also whether HCV RNA testing would pick up a greater number of such patients than the serological test alone.

Methods

Patients

A total of 57 patients [Male 37 (64.9%), age 47.8 years (range: 18–80)] consecutive newly diagnosed patients with B cell NHL who came to seek treatment at the Hematology Clinic of the Post-graduate Institute of Medical Education and Research, Chandigarh, a tertiary care centre, during the study period were included in this study. The diagnosis was made by lymph node fine needle aspiration cytology, lymph node biopsy, or bone marrow examination. The B cell origin of the neoplasm was confirmed by immunophenotyping. The Ann-Arbor staging system was used for staging the disease. Patients with a recent history of jaundice, intravenous drug abuse, and prior interferon-α therapy were excluded. Patients with a prior history of blood/blood component transfusion and surgical intervention were noted. All patients were selected prior to the institution of definitive chemotherapy.

Using the Ann-Arbor staging, three of the 57 patients were in stage II disease [IIA-1(1.7%), IIB-2(3.5%)] at presentation to our clinic, 11 were in stage III [IIIA-5(8.7%), IIIB-6(10.5%)], and 43 were in stage IV [IVA-25 (43.8%), IVB-18 (31.5%)]. Of these, 44 (77.2%) were of the diffuse large B cell type (high grade), 6 (10.5%) of the follicular type (intermediate grade) and 7 (12.3%) were of the small lymphocytic type (low-grade), and 26 patients had B symptoms at diagnosis. Only one of these patients had a history of blood transfusion prior to attending our clinic. This patient tested positive for HBsAg but negative for anti-HCV.

Controls

Patients admitted in or attending the out-patient clinics of the Post-graduate Institute of Medical Education and Research for non-hematological conditions in the other departments of the hospital, such as Departments of Ophthalmology, Otorhinolaryngology, Dermatology, and Internal Medicine, served as controls. A total of 171 age- and sex-matched controls [Male 111 (64.9%), age 48.6 years (range: 18–80)] were included in this group, so as to keep the ratio of patients to controls as 1:3. Controls with a history of jaundice, intravenous drug abuse, prior interferon-α therapy, immunosuppression, such as steroid therapy and HIV infection, were excluded.

Serology

All patients and controls were tested for anti-HCV. All patients' sera were tested for the presence or absence of serum HCV RNA. However, HCV RNA testing was not done as a screening test in controls under the assumption that these were immunocompetent and should provide a serological response against HCV infection, if it existed. A third generation ELISA was used to detect antibodies against HCV using cut-off OD value (0.450) calculated from negative and positive controls as described by the manufacturer (LG HCD 3.0 Plus; LG Chemical Ltd., Pharmaceutical division, Seoul, Korea). The test detected antibodies against three kinds of fusion proteins which are constituents of the HCV nucleocapsid; core 518, E1E2NS4, and NS5.

For the detection of hepatitis B surface antigen (HBsAg), the method used in our study was a direct immunoenzymatic assay of the "sandwich" type (Bioelisa HBsAg colour; Biokit, S.A., Barcelona, Spain).

HCV RNA detection

HCV RNA was detected by nested reverse transcriptase polymerase chain reaction. The primers were chosen from the conserved 5′ UTR region of the HCV genome [6]. The primer sequences that were used in this study were:

Outer sense primer (nucleotide position −297 to −278)
5′-CTG TGA GGA ACT ACT GTC TT-3′
Outer antisense primer (position 8–36)
5′-ATA CTC GAG GTG CAC GGT CTA CGA GAC CT-3′
Inner sense primer (position −279 to −260)
5′-TTC ACG CAG AAA GCG TCT AG-3′
Inner antisense primer (position −29 to −4)
5′-CAC TCT CGA GCA CCC TAT CAG GCA GT-3′

Statistical analysis

The data are presented as number (%) and odds ratio (OR) with 95% confidence interval (CI). Fisher's exact test was used to compare the prevalence of HCV infection among patients and controls. A p value <0.05 was considered significant.

Results

None of the patients in the B cell NHL group tested positive for anti-HCV while one patient in the same group (1.75%) tested positive for HBsAg. Among the age- and sex-matched controls taken, one tested positive for HBsAg (0.58%) and two tested positive for anti-HCV antibody (1.16%) (Table 1).

The prevalence of anti-HCV was not different between controls and patients [OR 0; 95% CI (0–12.40)]. The prevalence of HBsAg was also not different between controls and patients [OR 1.51; 95% CI (0.13–17.05)]. All the 57 patients of B cell NHL were subjected to serum HCV RNA testing. One patient tested positive for HCV RNA (genotype 1); however he had tested negative for anti-HCV. Two of the control subjects who tested positive for the anti-HCV antibody subsequently tested positive for the HCV RNA (genotype 3) confirming infection.

Discussion

This prospective study confirms our previous observation that HCV infection is not associated with lymphoproliferative disorders in northern India [3]. HCV prevalence varies considerably in different areas of the world. In general, most of the studies to date are retrospective leaving space for the argument that the association between HCV and NHL may be related to the increased risk that exists for NHL patients to get infected by the virus than the viral infection leading on to oncogenesis. Prospective studies are few and far between.

An obvious argument is that the association between HCV and lymphoma can be explained in two ways: HCV infection increases the risk of developing NHL; conversely, that patients with NHL have an increased risk of developing HCV owing to frequent hospitalizations, intravenous injections, need for blood transfusions, and other such accepted risk factors for HCV infection.

The strongest argument for the role of HCV infection in NHL comes from interventional studies. The regression of gastric MALT lymphoma after eradication of *Helicobacter pylori* with antibiotics has been repeatedly reported [7]. Therefore, it could be that therapy against HCV is associated with regression of NHL. In fact, antiviral treatment appears to be effective in eliminating the clonal proliferation of B cells in patients with chronic HCV infection and may prevent the subsequent development of lymphoma [8]. Furthermore, encouraging results emerge from a recent report in which most patients with HCV and splenic lymphoma entered complete remission upon treatment with interferon [7–9].

Lymphomagenesis is considered to be a multifactorial process in which genetic, environmental, and infectious factors are involved. Studies from different parts of the United States have themselves yielded inconsistent results. In general, studies from the southern United States have showed an association, whereas those from the Northern part have not [10]. Researchers have tried to explain this variable association seen in the United States on the basis of the fact that the United States is a racially heterogenous society with distinct regional demographics. In different studies from different parts of the country, the ethnic make-up of the patient populations may have been varied contributing to the inconsistent results. For instance, Zuckerman et al. [11] reported prevalence of HCV in association with B cell NHL of 22% from a Los Angeles centre for which the patient population was 78% Hispanic and only 6% non-Hispanic white, whereas within other areas of the same city, the proportion of Hispanics in the study group was 25%, the HCV prevalence among B cell NHL was only 11.5%. It is clear that genetic and racial factors play a role in lymphomagenesis in addition to environmental factors. These factors could play a role in lymphomagenesis, not only in the United States, but all over the world and serve as confounding factors when HCV and its role in lymphomagenesis is studied. We studied a homogenous population of Indian patients and found no association between HCV infection and NHL.

Table 1 Prevalence of HCV infection among patients with NHL

Test	Patients ($n = 57$)	Controls ($n = 171$)	OR (95% CI)	P value
Anti-HCV	0 (0%)	2 (1.17%)	0 (0–12.40)	>0.05
Serum HCV RNA	1 (1.75%)	2 (1.17%)	1.51 (0.13–16.95)	>0.05
Anti-HCV and/or HCV RNA	1 (1.75%)	2 (1.17%)	1.51 (0.13–16.95)	>0.05

OR odds ratio, *CI* confidence interval

Gisbert et al. [12] in a systematic review and meta-analysis showed that there is a role of HCV in the pathogenesis of B cell NHL. The striking geographic association in this relation suggests that genetic and/or environmental factors are also involved in the pathogenesis of this disorder. Various studies in this review showed that there is a greater association of HCV with B cell NHL in countries, like Italy, Brazil, Spain, Saudi Arabia, Japan, and some parts of the United States. However, there are studies which have shown negative association between HCV and NHL in Canada, Turkey, and the UK. In a few countries, like France, and USA, there are data regarding positive and negative association from different studies [12].Whereas a high prevalence of HCV infection in NHL patients has been shown to exist in many geographical areas of high HCV prevalence such as Brazil [12], Italy [13, 14], Israel [15], Yemen [16], Spain [17], Canada [18], Egypt [19], and parts of the USA [12, 20]. Many other areas studies have not established any form of association as in Greece [6], Mexico [11], France [12], the UK [21], Thailand [22], The Netherlands [23], Turkey [24, 25], and Korea [26]. However, a study in Denmark [27], which is an area of low HCV prevalence, has shown association of HCV and lymphoma. In a Swedish study, Duberg et al. [28] reported the incidence of several malignancies in a nationwide cohort of HCV-infected persons. This study showed significantly increased risk of NHL and multiple myeloma in HCV-infected patients.

According to a study by Okan et al. [24], the seropositivity of HBsAg and/or anti-HCV was 8.7% (29/334), and among the controls 6.1% (49/802), however this difference did not achieve statistical significance [OR 1.36; 95% CI (0.82–2.26)]. Thus, they could not find any significant differences between the seropositivity rates of hepatitis B, C, or both in either NHL or Hodgkin's lymphoma and those of population-based controls.

The relationship between the hepatitis B virus (HBV) and lymphomas is even more nebulous than that of HCV and this study also attempted to clarify this association in Indian patients. Whereas a few studies have found a significantly increased prevalence of HBV surface antigen positivity in NHL patients [15, 29, 30], the relationship has been proposed to be coincidental rather than causal. This study also did not show any relationship between HBsAg positivity and NHL.

In the time that it has taken for us to complete this study, the relationship between HCV and B cell NHL has become more firmly established in countries where this relationship was already known. What has also become better known is the inconsistency of this relationship in different geographical areas including the complete lack of this association in certain countries. Even though a meta-analysis on the subject including 23 studies in it concluded that the association between HCV is consistent irrespective of the status of endemicity of the virus in the community, this study included as many as ten studies with a positive association from Italy alone. Also the authors themselves agree that the prevalence of HCV infection in the general population in many countries is not sufficiently known to draw this conclusion [31].

In this study, 57 patients and 171 controls were included and we did not find any significant association between HCV infection and NHL. Although it would require more studies with a larger number of patients to convincingly confirm this result in the Indian scenario, one suggestion that may emerge from our study is that HCV RNA testing would probably be needed to confirm or rule out this association in such patients owing to the possibility of a false negative serological test in them. As all our cases were selected prior to the initiation of chemotherapy, this can obviously be excluded from contributing to immunosuppression in these patients.

Our study suggests a lack of association between HCV infection and NHL in our country due to one or more of the reasons mentioned above. We also wish to propose that in order to set this controversy at rest in a vast country, such as ours with its profound racial, cultural, and geographical differences; larger prospective, multicenter studies which include the detection of HCV RNA in their protocols to diagnose infection would be needed. The need is not purely academic because treatment of HCV infection may alter the incidence of lymphoma if such an association is found in any region of the country.

In conclusion, this study does not find any significant association between HCV infection and NHL in our country. However, more prospective studies from different regions with a larger sample size which include HCV RNA detection in their protocols would be needed.

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