

Hepatitis C Virus infection in Saudi Arab patients with B-cell non-Hodgkin's lymphoma

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ABSTRACT

Objective: The objective of the current study is to determine the prevalence of Hepatitis C virus infection in Saudi Arab patients with B-cell non-Hodgkin's lymphoma.

Methods: Fifty-six unselected Saudi Arab patients with B-cell non-Hodgkin's lymphoma were tested for the presence of Hepatitis C virus antibodies using Elisa immunoabsorbant assay 2.0. Positive and indeterminate results were subjected to confirmatory testing using RIBA-Hepatitis C virus 2.0. Two control groups were utilized for comparison; the first is a group of randomly selected general medical patients and healthy blood donors; and the 2nd is a cohort of patients with hematological neoplasms other than B-cell non-Hodgkin's lymphoma. Patients with previous history of blood transfusion or liver disease were excluded from the study.

Results: Twelve of the 56 B-cell non-Hodgkin's lymphoma patients (21%) tested positive for Hepatitis C virus antibodies. Only 3 out of 104 (3%) and 2 out of 41 (5%) patients tested positive for Hepatitis C virus antibodies in the first and 2nd control groups.

Conclusion: The results of this study indicate a higher prevalence of Hepatitis C virus infection in Saudi Arab patients with B-cell non-Hodgkin's lymphoma than in the control groups. The prevalence of Hepatitis C virus infection in the 2 control groups, in turn, seems to fall within the estimated prevalence in the general population.

Keywords: Hepatitis C, lymphoma, non-Hodgkin's, B cell, epidemiology.

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Hepatitis C virus (HCV) is the major etiologic agent of post-transfusion hepatitis and sporadic non-A, non-B hepatitis.^{1,2} Focus on HCV as a possible factor in the pathogenesis of non-Hodgkin's lymphoma (NHL) was prompted by the unequivocal association between HCV and type II mixed cryoglobulinemia (MC-II) which is a lymphoproliferative disorder that can evolve into frank B-cell lymphoma in a limited number of patients.³⁻⁸ Ferri and associates postulated that HCV could have an etiologic role to play in the pathogenesis of B-cell non-Hodgkin's lymphoma (B-NHL) after their detection of HCV antibodies or genome in 34% of 50 unselected patients with B-

NHL.⁹ Subsequently, there have been numerous reports describing the association between HCV and B-NHL in other cohorts of patients. Some of these reports related this association to certain types of lymphoma while others did not demonstrate any relation of this association to the type of B-NHL. Based on these observations, we conducted a controlled study to determine the prevalence of HCV infection in Saudi Arab patients with B-NHL. To the best of our knowledge, this is the first report to look into this association in Saudi Arab patients.

Methods. The study was conducted at King Fahad National Guard Hospital in Riyadh which is a

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tertiary care facility primarily serving the National Guard recruits and their families and other Saudi citizens with diseases requiring highly specialized health care including patients with malignant diseases. All patients included in the study were Saudi Arab Nationals.

Patients. Study Group: A total of 56 unselected patients were included in the study. The inclusion criteria were 1) unequivocal diagnosis of B-NHL; 2) absence of history of blood component transfusion; 3) absence of past history of liver disease.

Control group (1). A total of 104 general medical patients and healthy blood donors were randomly selected and included in the study. Inclusion criteria were similar to the study group with the exception for not having B-NHL.

Control group (2). This group consisted of 41 patients with hematological malignancies other than B-NHL. Patients were excluded if they gave history of blood transfusion or liver disease.

Pathological diagnosis of B-NHL. B-cell non-Hodgkin's lymphoma was diagnosed on the basis of morphological evaluation of lymph node biopsies or extranodal tissue in the case of primarily extranodal disease. Histochemical staining and cell immunophenotyping were carried out according to the standard practice. The modified working formulation classification for lymphomas was used for grading the lymphoma.¹⁰

Assessment of HCV infection. Antibodies to HCV were detected in patients sera using Hepatitis C Encoded Antigen (recombinant c 100-3, HC-31, and HC-34) (Abbott HCV EIA 2.0, Abbott Laboratories Diagnostic Division, USA). Serum separation, handling and testing were carried out according to the standard practice and manufacturer recommendations. All positive and indeterminate results were subjected to confirmatory testing using the strip immunoblot assay for the detection of antibodies to HCV (Chiron, RIBA-HCV 2.0 SIA, Chiron Corporation, USA).

Results. Patients characteristics. Study Group. There was a total of 56 patients (40 males and 16 females) with a median age of 54 years (range 20-90 years). There were 7 patients with low grade lymphoma, 46 patients with intermediate grade lymphoma and 3 patients with high grade lymphoma.

Control group (1). There was a total of 104 patients (68 males and 36 females) with a median age of 54 years (range 20-88 years).

Control group (2). There was a total of 41 patients (28 males and 13 females) with a median age of 46 years (range 16-77 years).

Results of HCV testing. Table 1 shows the results of HCV testing in the 3 groups and in different grades of lymphoma in the study group.

Table 1 - Prevalence of hepatitis C virus infection in B-NHL patients and the control groups.

Group	No. of patients	No. HCV-positive	% HCV-positive	P-value
B-NHL	56	12	21.0	
LG	7	1	14.0	
IG	46	11	24.0	
HG	3	0	0	
Control (1)	104	3	3.0	<0.00005
Control (2)	41	2	5.0	<0.00005

No. - number; HCV - Hepatitis C Virus; B-NHL - B-cell non-Hodgkin's lymphoma; LG - low grade lymphoma; IG - intermediate grade lymphoma; HG - high grade lymphoma

Discussion. The high prevalence of HCV infection among patients with B-NHL has been described in several studies over the past 3 years. Most of these studies originated from Italy.^{8,9,11-14} Similar observations were also made in Japan and the USA.^{15,16} Data from the United Kingdom did not, however, confirm this association raising the possibility of some geographical variability.¹⁷⁻¹⁹ The current study clearly demonstrates that the prevalence of HCV infection in Saudi Arab patients with B-NHL is higher than in the 2 control groups. Although the exact prevalence of HCV infection in the Saudi Arab population at large is unknown, 1-5% prevalence has been suggested based on a limited number of community based surveys and a larger pool of healthy blood donors.²⁰ Thus, it seems that the prevalence of HCV infection in the 2 control groups falls within the estimated population rate. Available data does not indicate an absolute predominance of any particular lymphoma grade in association with HCV infection despite the fact that the low-grade category has probably been described more often than the others in some reports.^{13,21} In our group of HCV-positive lymphoma patients, the intermediate-grade lymphoma predominates but it is likely that this is secondary to the larger number of patients with this disease entity who have been included in the study.

The association of HCV and B-NHL raises the possibility that the virus plays a pathogenic role through an unknown mechanism. As the viral ribonucleic acid (RNA) genomic sequence can not be integrated into the host genome, an indirect mechanism of malignant B-cell transformation has to be considered. Hepatitis C virus is known to be hepatotropic and lymphotropic.^{22,23} The persistence of HCV and its replicative intermediates in the peripheral blood mononuclear cells, namely B and T lymphocytes and monocytes, may result in chronic

stimulation and expansion of the B-cells,²⁴ which may lead to polyclonal and later monoclonal expansion of these cells. In support of this concept is that, although HCV is not known to be an oncogenic virus, it has recently been shown that both HCV non-structural protein NS3 and HCV core protein are able to induce cells to transform into tumorigenic phenotype in nude mice and that HCV core protein can regulate cellular protooncogenes at the transcriptional level.^{25,26} A simpler alternative mechanism by which B-NHL can develop in HCV-infected subjects could be the existence of a common factor such as an immune defect that predisposes the subject independently to both HCV infection and B-NHL. Evidence for the latter hypothesis is, however, lacking. Little data is also available on HCV genotype in patients with lymphoproliferative disorders. Mazzaro and coworkers¹¹ reported a high prevalence of the genotype 1b while Silvestri and associates²⁷ reported a high prevalence of the genotype 2ac in their HCV-positive B-NHL patients. Others could not demonstrate any specific genotype predominance.^{12,21} We have not tested for HCV genotypes in our study.

The impact of HCV-infection on the clinical behavior of the lymphoma is still not well-defined. Silvestri and associates found that HCV-positive NHL patients tend to be more symptomatic, have more organ involvement, and frequent cryoglobulinemia.²⁸ In addition, the disease was more likely to evolve into a high-grade lymphoma. The quality of life was described to be inferior in patients with HCV infection than in noninfected individuals despite similar duration of survival. When extranodal involvement by the lymphoma occurred in HCV-infected patients, the liver was not usually involved.¹⁶

Although the prognostic and therapeutic implications of the association between HCV infection and B-NHL is still not certain, it might be reasonable, however, to assume that the prevention of virus transmission, or curing the infection may invoke a new treatment strategy for patients with HCV-associated hematological malignancies in a manner similar to the regression of gastric B-NHL in some patients following the eradication of *Helicobacter pylori* by the use of appropriate antimicrobial agents.²⁹ This concept is supported by the observation that a limited number of patients with HCV-infection related B-NHL and cryoglobulinemia had shown disease regression following interferon treatment.^{27,30} Interferon could have produced this effect through its cellular antiproliferative and viral antireplicative activities. Although these effects seem to have been related to certain genotypes of the virus and observed in a small number of patients, the therapeutic potential for interferon seems appealing in this setting and deserves further exploration.

References

1. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH. An Assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989; 244: 362-364.
2. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M, et al. Isolation of a c-DNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; 244: 359-362.
3. Misiani R, Bellavita P, Fenili D, Borelli G, Marchesi D, Massazza M. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. *Ann Intern Med* 1992; 117: 573-577.
4. Angello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; 327: 1490-1495.
5. Pascual M, Perrin L, Giostra E, Schifferli JA. Hepatitis C virus in patients with cryoglobulinemia type II. *J Infectious Dis* 1990; 162: 569-575.
6. Monteverde A, Rivano MT, Allegra GC, Monteverde AI, Zingrossi P, Baglioni P. Essential mixed cryoglobulinemia, Type II : a manifestation of a low-grade malignant lymphoma? *Acta Hematologica* 1988; 79: 20-25.
7. Ferri C, Monti M, La Civita L, Careccia G, Mazzaro C, Longombardo G. Hepatitis C virus infection in non-Hodgkin's B-cell lymphoma complicating mixed cryoglobulinemia. *European J Clin Investigation* 1994; 24: 781-784.
8. Pozzato G, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. *Blood* 1994; 84: 3047-3053.
9. Ferri C, Caracciolo F, Zignego AL, Civita LL, Monti M, Longombardo G. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Br J Haematol* 1994; 88: 392-394.
10. National Cancer Institute Study of Classification of non-Hodgkin's lymphoma: Summary and description of a working formulation for clinical usage. The non-Hodgkin's Lymphoma Pathologic Classification Project. *Cancer* 1982; 49: 2112-2135.
11. Mazzaro C, Zagonel V, Monfardini S, Tulissi P, Pussini E, Fanni M, et al. Hepatitis C virus and non-Hodgkin's lymphoma. *Br J Haematol* 1996; 94: 544-550.
12. De Rosa G, Gobbo ML, De Renzo A, Notaro R, Garofalo S, Grimaldi M, et al. High prevalence of hepatitis C virus infection in patients with B-cell lymphoproliferative disorders in Italy. *Am J Hematol* 1997; 55: 77-82.
13. Silvestri F, Pipan C, Barillari G, Zaja F, Fanin R, Infanti L, et al. Prevalence of hepatitis C infection in patients with lymphoproliferative disorders. *Blood* 1996; 87: 4296-4301.
14. Luppi M, Longo G, Ferrari MG, Ferrara L, Marasca R, Barozzi P, et al. Additional neoplasms and HCV infection in low-grade lymphoma of MALT type. *Br J Haematol* 1996; 94: 373-375.
15. Izumi T, Sasaki R, Miura Y. Primary hepatosplenic lymphoma: association with hepatitis C virus infection. *Blood* 1996; 87: 5380-5381.
16. Zuckerman E, Zuckerman T, Levine AM, Douer D, Gutekunst K, Mizokami M, et al. Hepatitis C virus infection in patients with B-cell non-Hodgkin's lymphoma. *Ann Intern Med* 1997; 127: 423-428.
17. Brind AM, Watson JP, Burt A, Kestevan P, Wallis J, Proctor SJ, et al. Non-Hodgkin's lymphoma and hepatitis C virus infection. *Leukemia and Lymphoma* 1996; 21: 127-130.
18. Hanley J, Jarvis L, Simmonds P, Parker A, Ludlam C. HCV and non-Hodgkin's lymphoma. *Lancet* 1996; 347: (letter) 1339.

19. McColl MD, Tait RC. Hepatitis C virus infection in patients with lymphoproliferative disorders. *Br J Haematol* 1996; 92: 766-773.
20. Al-Faleh FZ, Rania S. Hepatitis C virus (HCV) infection in Saudi Arabia: A review. *Annals of Saudi Medicine* 1997; 17: 77-82.
21. Luppi M, Ferrari MG, Bonaccorsi G, Longo G, Narni F, Barozzi P, et al. Hepatitis C virus infection in subsets of neoplastic lymphoproliferation not associated with cryoglobulinemia. *Leukemia* 1996; 10: 351-355.
22. Zignego AL, Macchia D, Monti M, Thiers V, Mazzetti M, Fosch M, et al. Infection of peripheral blood mononuclear cells by hepatitis C virus. *J Hepatol* 1992; 15: 382-386 .
23. Ferri C, Monti M, La Civita L, Longombardo S, Greco F, Pasero G, et al. Infection of peripheral blood mononuclear cells by hepatitis C virus in mixed cryoglobulinemia. *Blood* 1993; 82: 3701-3704.
24. Franzin F, Efremov DG, Pozzato G, Tulissi P, Batista F, Burrone OR. Clonal B-cell expansion in peripheral blood of HCV-infected patients. *Br J Haematol* 1995; 95: 548-552.
25. Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS 3 transforms NIH 3T3 cells. *J Virology* 1995; 69: 3893-3896.
26. Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis c virus core protein cooperates with ras and transforms primary rat embryo fibroblast to tumorigenic phenotype. *J Virology* 1996; 70: 4438-4443.
27. Silvestri F, Barillari G, Fanin R, Ripan C, Falasca E, Salmaso F, et al. The genotype of hepatitis C virus in patients with HCV-related B-cell non-Hodgkin's lymphoma. *Leukemia* 1997; 11: 2157-2161.
28. Silvestri F, Barillari G, Fanin R, Zaia F, Infanti L, Salmaso F, et al. Lymphoplasmacytoid lymphoma/immunocytoma: impact of hepatitis C virus infection on clinical features, quality of life and survival. *Blood* 1996; 88 (suppl 1): 223a.
29. Watherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 1993; 342: 575-577.
30. Mazzaro C, Franzin F, Tulissi P, Pussini E, Crovatto M, Carniello GS, et al. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. *Cancer* 1996; 77: 2604-2613.