Retrospective Study of Hepatitis C Virus Genotypes and its Association with Lymphoma

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Abstract: Hepatitis C virus (HCV) involves both the liver and extra hepatic organs. The aim of this study was to retrospectively evaluate the association between HCV genotypes and lymphomas.

Lymphoma cases were retrieved from our surgical pathology and hematopathology archives from January 2005 to April 2012. Patients who had positive HCV serology with subsequent viral genotyping were selected. Patients with positive Human immunodeficiency virus (HIV) serology were excluded.

We identified 17 lymphoma cases with associated HCV infection. Eleven out of 14 (79%) patients had genotype 1 HCV. Diffuse large B cell lymphoma (DLBCL) was the most common lymphoma (6 out of 17 cases) and all cases of DLBCL had genotype 1. Genotype 2 was detected in only three patients (21%) with the diagnoses of follicular lymphoma, splenic marginal zone lymphoma, and classical Hodgkin lymphoma (CHL). CHL was diagnosed in three cases and peripheral T-cell lymphoma in one case.Twelve of 17 (71%) patients were incarcerated in the Texas Department of Criminal Justice system. All 11 genotype 1 patients were male, 4 of 11 (36%) were African American, 4 of 11 (36%) were Caucasian and 3 of 11 (27%) were Hispanic.We concluded that HCV genotype 1 was more common than genotype 2 while no other genotype was detected.

Keywords: HCV genotypes, HCV associated lymphomas, HCV.

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded RNA virus belonging to the Flaviviridae family [1]. The HCV genome encodes a single polyprotein precursor of approximately 3000 aminoacids that is proteolytically processed by viral and cellular proteases to produce structural and non-structural proteins. The two HCV envelope proteins, E1 and E2, act as the ligands for cellular receptors [1]. HCV E2 protein can directly bind to the human CD81 protein [2]. CD 81 is a 26-kDa surface tetraspanins protein that is expressed on most human tissue cells, including B-cells, T-cells and hepatocytes.It is highly expressed in the germinal center B cells of lymphoid tissue [3].

There are at least six major genotypes of HCV (genotypes 1 to 6) whose prevalence varies geographically [4, 5]. These genotypes differ in about 30–35% of the nucleotides in their complete genome [6]. Genotype 1 (subtypes 1a and 1b) accounts for the majority of infections in North America, followed by genotypes 2 and 3. Because of the growing cultural diversity in United States, less common genotypes (genotypes 4-6) are being observed more [4]. HCV genotyping is part of the pretreatment evaluation in patients with HCV infection, as it influences the plan of therapy, treatment duration and likelihood of response [7].

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HCV is an agent involving both the liver and extra hepatic organs. HCV is a leading cause of cirrhosis and hepatocellular carcinoma (HCC) [8, 9]. Although the liver is the primary target of HCV, numerous studies have confirmed an association between HCV infection and B-cell non-Hodgkin lymphoma [10-15], while relatively few reports have addressed the role of HCV in T-cell lymphoma or Hodgkin lymphoma (HL) [15].

Common B-cell neoplasms associated with HCV infection include DLBCL, Small Lymphocytic Lymphoma (SLL), and Classic Hodgkin Lymphoma (CHL). DLBCL is the most common non-Hodgkin lymphoma in North America. Histological evaluation reveals sheets of enlarged neoplastic B-lymphocytes that have nuclear size more than twice the size of a lymphocyte or greater than a macrophage nucleus. The diffuse nature of DLBCL often effaces the lymphoid tissue leaving little to no residual normal lymphoid architecture. Common gene mutations in DLBCL include *BCL-6, BCL-2,* and *MYC*. DLBCL is positive for pan B-cell markers and typically has a Ki67 proliferation index of greater than 90%. SLL is primarily a disease of the elderly with most patients being above 65. Neoplastic cells are small with round nuclei and indistinct nucleoli. Degenerated lymphocytes known as smudge cells are often seen in the peripheral blood. Immunophenotypically, SLL cells are positive or dimly positive for B-cell markers CD20, CD22, and CD79a. CD5 and CD23 co-expression with FMC-7 negativity is also a characteristic finding. Overall survival of SLL can range from 2-20 years however aggressive clinical

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course is associated with CD38 and ZAP-70 immunophenotype expression. CHL is a B-cell neoplasm with four distinct subtypes: Nodular Sclerosis, Mixed Cellularity, Lymphocyte Rich, and Lymphocyte Depleted. CHL is characterized by neoplastic multinucleated Reed-Sternberg cells and mononuclear Hodgkin cells. These cells are markedly enlarged with prominent eosinophilic nucleoli and are positive for CD15, CD30 and PAX-5 while negative for CD45.

The aim of this study was to retrospectively evaluate the association between different genotypes of HCV infection and various types of lymphomas.

MATERIALS AND METHODS

Eight hundred and thirty seven cases with the diagnosis of lymphoma were retrieved from surgical pathology and hematopathology archives of the University of Texas Medical Branch (UTMB) from January 2005 to April 2012. The diagnosis of lymphoma was established according to the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues. Selected among these lymphoma cases were patients who had HCV serology tested and HCV genotype analyzed in the molecular diagnostic laboratory in the department of pathology at University of Texas Medical Branch before the lymphoma was diagnosed. Patients who were human immunodeficiency virus (HIV) positive were excluded from our study.

HCV Sequencing and Genotyping

HCV RNA was extracted using QIAGEN MagAttract virus mini M48 kit (Qiagen, Valencia, CA, USA). RT-PCR of extracted HCV RNA were performed using Versant HCV Amplification 2.0 kit (Siemens Medical Solution Diagnostics, Tarrytown, NY, USA) and PCR was performed using GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Genotype analysis was performed by Versant HCV Genotype Line Probe 2.0 kit (Siemens Medical Solution Diagnostics, Tarrytown, NY) in the molecular diagnostic laboratory in the department of pathology at UTMB.

RESULTS

A total of 17 lymphoma cases with positive HCV antibodies and documented HCV viral genotypes were identified in this study. In three cases, the genotype was indeterminate due to low viral load (less than 615 IU/ML). These cases were DLBCL, CHL and small lymphocytic lymphoma.

Eight of 17 (47%) patients in our study with HCV infection and lymphoma were Caucasian. Five of 17 (29%) were Hispanic and 4 of 17 (24%) were African American. Twelve of 17 (71%) patients were prisoners in the Texas Department of Criminal Justice system. Sixteen of 17 (94%) of patients were male. The average age of the patients in this study was 50 and all patients resided in the state of Texas. Medical records of all patients were examined and failed to show any co-existing non-hematopoietic malignancies.

Overall, genotype 1 was the predominant genotype in our study. Eleven out of 14 (79%) patients with a known genotype had genotype 1 HCV infection. All 11 genotype 1 patients were male, 4 of 11 (36%) were African American, 4 of 11 (36%) were Caucasian and 3 of 11 (27%)were Hispanic. Eight of 11 (73%) genotype 1 patients were prisoners. The genotype 2 HCV infection was detected only in three patients (21%). Two of the patients were Caucasian and the other was Hispanic. All 3 genotype 2 patients were prisoners. The genotype 2 HCV infection was detected in three patients with three different diagnoses; one case of follicular lymphoma, splenic marginal zone lymphoma, and CHL respectively.

Among the HCV associated lymphomas (Table **1**), DLBCL was the most common type of lymphoma (6 out of 17 cases, 35%) and all DLBCLs had genotype 1 HCV. CHL was diagnosed in three cases (18%) with genotype 1, 2 and indeterminate genotype. Follicular lymphoma and splenic marginal zone lymphoma were diagnosed in two cases (12%) with infection of genotype 1 and genotype 2, respectively. SLL was diagnosed in two cases (12%), one case was infected with genotype 1 HCV and the other had indeterminate genotype of HCV infection. Peripheral T-cell lymphoma and marginal B-cell lymphoma were diagnosed in one case (5%) each; both cases were infected with genotype 1 HCV. The peripheral T-cell lymphoma was diagnosed in the patient with long standing HCV infection. This case had an unusual and rare presentation with new onset ascites. Also, the patient was seropositive for HTLV-II virus.

DISCUSSION

HCV infection is a major public health problem and is a leading cause of chronic liver disease. More than 180 million people are estimated to be infected by HCV

Table 1: HCV Genotypes in HCV Associated Lymphomas

	HCV genotype	Diagnosis	Race	Incarcerated
1	1	DLBCL	African American	Yes
2	1	DLBCL	Hispanic	No
3	1	DLBCL	Caucasian	No
4	1	DLBCL	Caucasian	Yes
5	1	DLBCL	Hispanic	Yes
6	1	SLL	African American	Yes
7	1	Marginal zone B-cell lymphoma	Caucasian	No
8	1	FL, grade 1	Caucasian	Yes
9	1	SMZL	African American	Yes
10	1	Peripheral T-cell lymphoma	African American	Yes
11	1	CHL	Hispanic	Yes
12	2	SMZL	Caucasian	Yes
13	2	CHL	Hispanic	Yes
14	2	FL, grade 1	Caucasian	Yes
15	Indeterminate	CHL	Hispanic	No
16	Indeterminate	DLBCL	Caucasian	Yes
17	Indeterminate	SLL	Caucasian	No

DLBCL: Diffuse large B cell lymphoma, SLL: Small lymphocytic lymphoma, FL: Follicular lymphoma, SMZL: Splenic marginal zone lymphoma, CHL: Classical Hodgkin lymphoma.

worldwide [4]. It is estimated that approximately 1.3 % of the population in the United States is infected with HCV [7].

Six distinct HCV genotypes and multiple subtypes have been identified [4]. In the United States, genotypes 1a and 1b are the most common types, followed by genotypes 2 and 3 [4, 5]. Other genotypes are rare in the US and Europe, but common in other areas, such as Egypt (genotype 4), South Africa (genotype 5), and Southeast Asia (genotype 6). Knowing the HCV genotype is important because it has been shown that it has a predictive value in terms of response to antiviral therapy in HCV-positive patients with chronic hepatitis [7, 8]. In this study HCV genotype 1 was more common than genotype 2; while genotypes 3-6 were not identified.

Several studies have confirmed differences between various HCV genotypes infection [16-18]. In genotype 1 HCV infection, serum HCV RNA titer was significantly higher compared to those with genotype 2, while patients with genotype 2 had a longer duration of HCV infection as compared to genotype 1. Progression to chronic hepatitis in genotype 1 appears to have a more rapid and severe course compared to genotype 2 [17].

Occasionally (<5%), tested samples cannot be genotyped. This usually results from low viral levels, problem with the PCR amplification, or extreme nucleotide variability within the HCV genome [19]. In our study, there were three cases where the genotyping could not be ascertained due to low level of HCV virus (<615 IU/ML).

Although the mechanisms that HCV infection causes lymphoma remains unclear, working hypotheses propose that either persistent HCV antigen stimulation results in clonal expansion of B cells that finally leads to the development of lymphoma, similar to induction of gastric MALT lymphoma by Helicobacter pylori chronic infection, or HCV infection itself is directly oncogenic in B-cells [20, 21].

In a multicenter case-control study by Nieters *et al*. [22] carried out in 7 European countries, chronic HCV infection with genotypes 1b or 2 was associated with a 2.6-fold and 4.2-fold elevated risk of lymphoma, respectively. In that study, DLBCL was the subtype of lymphoma that was most clearly associated with HCV infection, while no evidence for a role of HCV infection in the T-cell lymphoma, follicular lymphoma, and Hodgkin's lymphoma was reported; whereas large pooled analysis in the study by de Sanjose *et al*. [23]

combined data from 7 case-control studies conducted in the United States, Europe, and Australia demonstrated that presence of HCV infection was linked to indolent lymphoma such as marginal zone lymphoma and lymphoplasmacytic lymphoma, as well as aggressive lymphoma like DLBCL. A meta-analysis study of Dal Maso *et al.* [15] found significant associations between HCV and all major subtypes of NHL and T-cell lymphoma. Also, weaker associations were found with Hodgkin lymphoma and multiple myeloma. The broad spectrum of NHL subtypes that have been associated with HCV infection suggested an involvement of the majority of germinal and post germinal center B-NHLs [15]. These important observations suggest that these tumors cannot arise as a consequence of chronic antigenic stimulation of B cells solely. In this study, HCV was linked to the various indolent and aggressive B-cell lymphomas, as well as T-cell lymphoma and Hodgkin lymphomas similar to what has been reported by Dal Maso *et al*. [15].

Interestingly, we observed that patients with DLBCL had a higher prevalence of HCV genotype 1 compared to patients with indolent B-NHL who had a higher prevalence of genotype 2 HCV infections. These findings were also reported elsewhere [16].

Epidemiologically, 12 of 17 (71%) patients analyzed in this study were prisoners in the Texas Department of Criminal Justice system. There is a strong, documented prevalence of HCV infection in this specific prison population, especially in Hispanics, African Americans, and Caucasians [27, 28]. All patients were residents of the state of Texas. This information along with the previously mentioned genotype data correlates with previous studies showing HCV genotype 1 is more common in North America than other genotypes.

Several trials showed that antiviral therapy resulted in complete or partial remission of lymphoma in HCV positive but not HCV-negative NHL patients [24-26].

Although, the role of HCV in rare NHL subtypes, such as T-cell lymphoma, and Hodgkin lymphoma is not clear; Takahashi *et al*. [24] reported a case of HCV-associated nodular lymphocyte predominate Hodgkin lymphoma (NLPHL) with marked regression following interferon-based antiviral treatment that supports the etiologic link between NLPHL and HCV infection. In our study, a few cases of CHL in HCV infected patients were present. These findings provide further evidence of the HCV association with various lymphomas, including Hodgkin lymphomas.

In conclusion, our results suggest that the lymphoma risk in patients with chronic HCV may be stratified according to the HCV genotype. We also report epidemiological associations such as race, residency and incarceration status. The genotype difference may be related to the pathogenesis of HCV associated lymphoma as well as epidemiologyand prompts further investigation.

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