Occult Hepatitis C Infection and Its Clinical Relevance in Lymphoproliferative Disorders

Salah Agha, Noha El-Mashad* and Mohamed Mofreh

Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt
*Corresponding author

A B S T R A C T

Chronic hepatitis C virus (HCV) infection remains a global health threat with 175 million carriers worldwide. Approximately 3% of the worldwide population is infected with the hepatitis C virus (HCV). Lymphoproliferative disorder (LPD) is a term that includes a wide spectrum of pathologies ranging from a minor expansion of a B-cell population (with no clinical significance) to an aggressive high-grade lymphoma. Such proliferations of B cells apparently can be triggered as a consequence of a chronic antigenic stimulation resulting from an HCV infection. A causative association between hepatotropic viruses, especially hepatitis C virus, and malignant B-cell lymphoproliferative disorders has been demonstrated utilizing epidemiologic data, biologic and molecular investigations, as well as clinical observations. These data indicate that hepatitis C virus may be responsible for the development of some malignant lymphoproliferative disorders. Occult hepatitis C virus infection (OCI) was first reported by Pham *et al.,* (2004) who examined the expression of the HCV genome in the sera, PBMC, using a highly sensitive reverse transcription (RT)-PCR-nucleic acid hybridization (RT-PCR-NAH) assay. Occult hepatitis C virus infection (OCI), defined as the presence of HCV RNA in the liver and peripheral blood mononuclear cells (PBMCs) in the absence of detectable viral RNA in serum by standard assays. It can be found in both anti-HCV positive and negative cases.

Introduction

Hepatitis C virus

Hepatitis C virus (HCV) infects over 150 million humans, and causes over 350,000 deaths per year. It is a member of the Hepacivirus genus within the Flaviviridae family. The Flaviviridae family was divided into four genera: flavivirus, pestivirus, pegivirus and hepatitis C virus (ICTV, 2014).

The hepatitis C virus genome encodes a single polyprotein precursor of approximately 3000 amino acids, which is proteolytically processed by viral and cellular proteases to produce structural (nucleocapsid, E1, and E2) and nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The virus envelope proteins consist of two heavily glycosylated proteins, E1 and E2, which act as the ligands for cellular receptors (Dibrov and Hermann, 2016).The natural targets of HCV are hepatocytes and, possibly, B lymphocytes (Okuda *et al.,* 1999).

Many of basic structural and virological characteristics shared by the members of the Flaviviridae family. Lipid bilayer envelope is present in all members, in which two or more envelope proteins (E) are anchored. The envelope surrounds the nucleocapsid, which is composed of multiple copies of core protein (C), and contains the RNA genome. The Flaviviridae genome is a positive-strand RNA molecule, with an open reading frame (ORF) encoding a polyprotein. The structural proteins are encoded in the N-terminal part of the ORF, and the nonstructural proteins are encoded in the remaining part of the (Miller and Purcell, 1990). The ORF is flanked in 5′ and 3′ by untranslated regions (UTR), which play an important role in RNA replication and polyprotein translation (Fig. 2) (Thurner *et al.,* 2004).

From a functional point of view, HCV proteins can be divided into an assembly module (core-NS2) and a replication module (NS3-NS5B, making up the replicase) (de Sanjose *et al.,* 2008).

Viral quasi species are defined as collections of closely related viral genomes subjected to a continuous process of genetic variation, competition among the variants generated, and selection of the most fit distributions in a given environment (Andino and Domingo 2015).

Human CD81 is the first identified necessary receptor for HCV cell entry, which can directly bind with HCV E2 protein. CD81 is a widely distributed cell-surface tetraspanin that participates in different molecular complexes on various cell types, including hepatocytes, B-lymphocytes, and natural killer cells.

It has been proposed that HCV exploits CD81 not only to invade hepatocytes but also to modulate the host immune responses (Ploss *et al.,* 2009).

Infection of lymphoid cell lines with HCV genotype-1a led to the selection of a quasi species with nucleotide substitutions within the 5′ UTR relative to the inoculum that conferred a 2- to 2.5-fold increase in translation efficiency in human lymphoid cell lines relative to granulocyte or monocyte cell lines Lerat *et al.,* 2000).

Furthermore, different translation efficiencies of HCV quasi species variants isolated from different cell types in the same patient were observed, suggesting cell type-specific IRES interactions with cellular factors may also modulate polyprotein translation (Dibrov and Hermann, 2016).

Hepatitis C virus entry

The HCV envelope is composed of two virus-encoded glycoproteins, E1 and E2. As with other enveloped viruses, the envelope glycoproteins largely define the interactions between HCV and the host cell. Moreover, HCV has been demonstrated to circulate in the blood of infected individuals in complexes with host lipoproteins and lipoprotein components which also contribute to HCV-host cell interactions (Nelson *et al.,* 2011).

The first identified entry factors, tetraspanin CD81, were discovered by their capacity to bind directly to HCV envelope glycoprotein E2 (Pileri *et al.,* 1998). Further use of screening strategies in mouse-derived cell lines identified occludin (OCLN) as a species-tropism defining entry factor, and it was determined that among the identified entry factors, CD81 and OCLN determine the tropism of HCV for human cells (Ploss *et al.,* 2009).

Epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2) are importantco-factors for HCV entry and infection. It should be noted that EGFR does not directly interact with the HCV particle, but EGFR-dependent

signaling pathways lead to the formation of CD81-CLDN1 complexes required for HCV entry (Lupberger *et al.,* 2011).

Other studies suggest that highly sulfated heparin sulfate proteoglycans (HSPG) and claudin 1 (CLDN1) as an important entry factors for HCV (Barth *et al.,* 2006), (Evans *et al.,* 2007). Interestingly, the HCV envelope glycoproteins do not directly interact with CLDN1, but CLDN1 interacts with CD81 and thereby plays an important role during post-binding steps of the HCV entry process (Krieger *et al.,* 2010).

Clinical picture of HCV

Acute infection

Hepatitis C infection causes acute symptoms in 15% of cases. Symptoms are generally mild and vague, including a decreased appetite, fatigue, nausea, muscle or joint pains, and weight loss and rarely liver failure. Most cases of acute infection are not associated with jaundice. The infection resolves spontaneously in 10–50% of cases, which occurs more frequently in individuals who are young and female (Maheshwari *et al.,* 2008)

Chronic infection

About 80% of those exposed to the virus develop a chronic infection. This is defined as the presence of detectable viral replication for at least six months. Chronic hepatitis C can be associated with fatigue and mild cognitive problems. Chronic infection after several years may cause cirrhosis or liver cancer. The liver enzymes are normal in 7–53%. Late relapses after apparent cure have been reported, but these can be difficult to distinguish from reinfection (Nelson *et al.,* 2011).

Fatty changes in the liver occur in about half of those infected and are usually present

before the development of cirrhosis. Worldwide HCV is the cause of 27% of cirrhosis cases and 25% of hepatocellular carcinoma. About 10–30% of those infected develop cirrhosis over 30 years. Excess alcohol increases the risk of developing cirrhosis 100-fold. Those who develop cirrhosis have a 20-times greater risk of hepatocellular carcinoma. Co-infection of HBV with HCV increases this risk further (Mueller *et al.,* 2009).

Extrahepatic complications

The most common problem due to HCV but not involving the liver is mixed cryoglobulinemia (usually the type II form) (Lannuzzella *et al.,* 2010). Hepatitis C is also associated with Sjögren's syndrome, a low platelet count, insulin resistance, DM, diabetic nephropathy, autoimmune thyroiditis, and B-cell lymphoproliferative disorders. In 20–30% of HCV-infected cases have rheumatoid factor. Cardiomyopathy associated with abnormal heart rhythms has also been reported. A variety of central nervous system disorders has been reported (Zignego *et al.,* 2012; Ko *et al.,* 2012).

Occult hepatitis C infection

In the last three decades, high advances in the detection, understanding life cycle, and treatment of HCV has been achieved. These advances have enabled the treatment of chronic hepatitis C infections to undergo dramatic changes since the inception of therapy with interferon α in 1991-1992 (Hajarizadeh *et al.,* 2013).

Most relapses following old treatment protocols as well as current protocols occur within 1–4 weeks after the end of treatment. However, a minority of relapses occur months to years later (Wei and Lok, 2014). Although the origin of these late relapses is uncertain,

an increasing amount of data suggests that they may represent activation of an occult hepatitis C virus infection (OCI) (Pham *et al.,* 2004; Carre˜no *et al.,* 2012).

Occult hepatitis C virus infection was first reported by Pham *et al.,*2004 who examined the expression of the HCV genome in the sera, and PBMC,using a highly sensitive reverse transcription (RT)-PCR-nucleic acid hybridization (RT-PCR-NAH) assay (Pham *et al.,* 2004).

In the same year, Castillo *et al.,* (2004) showed that HCV-RNA is present in anti-HCV negative patients in whom the etiology of persistently abnormal results of liver function tests is unknown.

Occult hepatitis C virus infection (OCI), is defined as the presence of HCV RNA in the liver, in peripheral blood mononuclear cells (PBMCs), and in L.N; in the absence of detectable viral RNA in serum. It could be found in both positive and negative anti-HCV patients (Carre˜no *et al.,* 2012).

Currently, the gold-standard for the identification of an occult HCV infection is the detection of HCV-RNA in liver tissue or in PBMCs. The definition of an OCI has been modified by the identification of HCV-RNA in extra hepatic tissues of anti-HCV negative (Carre˜no *et al.,* 2012; Abdelrahim *et al.,* 2016).

Inspite of the studies which supporting the evidence of the presence of OCI, there is a controversy opinion by some authors who challenged the existence of OCI (Halfon *et al.,* 2008; Naga *et al.,* 2008; George *et al.,* 2009; Baid-Agrawal *et al.,* 2014).

Types of OCI

There are currently two distinct forms of OCI; the first is the persistence of HCV after resolution and second is cryptogenic OCI (Pham *et al.,* 2004).

The first type

In which OCI continuing after resolution of hepatitis C. It was first reported in 2004 in a group of 16 individuals who are followed up for 5 years (Pham *et al.,* 2004).

Despite the apparently repeated HCV-RNA negativity in serum by standard clinical assays and normal liver function tests, trace amounts of HCV-RNA were detected by PCR assays in peripheral blood mononuclear cells (PBMC) of all patients investigated. The HCV-RNA replicative strand was identified in the majority of PBMC tested. The finding was unexpected given the well-accepted notion at the time that clinical resolution of hepatitis C had reflected complete eradication of HCV infection (Pham *et al.,* 2004).

In addition, other studies also documented, the presence of small amounts of HCV-RNA in plasma or serum, PBMC and/or hepatic tissue for up to 10 years after clinical resolution of hepatitis C (Pham *et al.,* 2004; Zaghloul *et al.,* 2010; Bokharaei-Salim *et al.,* 2011).

The second type

Cryptogenic OCI was first described in 2004 by Castillo and colleagues in individuals with long-standing elevation in liver function tests of undefined causes. Unlike patients with the first type of OCI, persons with cryptogenic HCV infection are negative for antibodies against HCV (anti-HCV) (Thurner *et al.,* 2004).

In 80 % of cases, both HCV-RNA positive and negative strands are present indicating

active HCV replication. Cryptogenic HCV infection was made possible through the use of a highly sensitive RT-PCR based research assay capable of detecting minute amounts of viral genome (Castillo *et al.,* 2004).

Potential pathogenic mechanisms resulting in an OCI

The subsets of immune cells involved in the HCV infection in individuals with chronic hepatitis C (CHC) and OCI are identified. In patients with CHC, HCV-RNA was detected in all various cell subtypes, and monocytes had the greatest viral load, but in OCI, B cells having higher HCV quantities compared to monocytes (Pham *et al.,* 2008).

The mononuclear cells, including T lymphocytes, are targets for HCV and these cells are reservoirs of replicating HCV. They can be used to evaluate extra hepatic HCV replication during an active infection, and in patients during and after the course of antiviral treatment. In this context, the detection of HCV positive cells and in some cases the replicative negative viral strand in PBMCs confirms the role of these cells as HCV reservoir both during ongoing antiviral treatment and after its completion and thereby enabling the identification of cases of OCI (Chen *et al.,* 2013)

A strong and sustained HCV-specific CD4+ and CD8+ T cell responses are essential for the resolution of hepatitis C infection. Depletion of CD4+ T cells plays a major role in the persistence of hepatitis C infection while depletion of CD8+ T cells was associated with delayed clearance of HCV-RNA (Quiroga *et al.,* 2006).

The maintenance of polyfunctional HCV-specific Th1, CD4+, and CD8+ memory T cells resulted in spontaneous clearance of HCV and better outcome of treatment of the HCV infection (Flynn *et al.,* 2013).

The cytokine balance between Th1/Th2 may be an important factor in the development of OCI cases (Gad *et al.,* 2012; Mousa *et al.,* 2014).

On comparing the cytokines responses between OCI and CHC, authers found that Th1 cytokines (IL-2 and IFN-γ) are significantly greater in cases of CHC patients than in those with OCI or control non-infected individuals. On the other hand, individuals with an OCI had higher serum IL-4 levels than in CHC and the healthy controls. Serum levels of IL-10 were higher in both OCI and CHC groups compared with control (Mousa *et al.,* 2014).

Several investigators have shown that HCV-infected subjects can harbor HCV quasispecies in their PBMCs that are not detectable in plasma (Inokuchi *et al.,* 2012; Flynn *et al.,* 2013). A potential additional explanation for the distribution differences of viral quasispecies may be related to the occurrence of viral mutations that confer a unique cellular tropism for PBMCs (Feld *et al.,* 2013; Fujiwara *et al.,* 2013).

The IL28B gene locus encodes for IFN-κ3, a member of type III IFN family (Chandra *et al.,* 2014). Many studies have demonstrated that the presence of a single nucleotide polymorphism (SNP) at the IL28B locus is associated with a reduced response to Peg IFN/RBV therapy and also an increased prevalence of PBMC infection (Amanzada *et al.,* 2011; Youssef *et al.,* 2013).
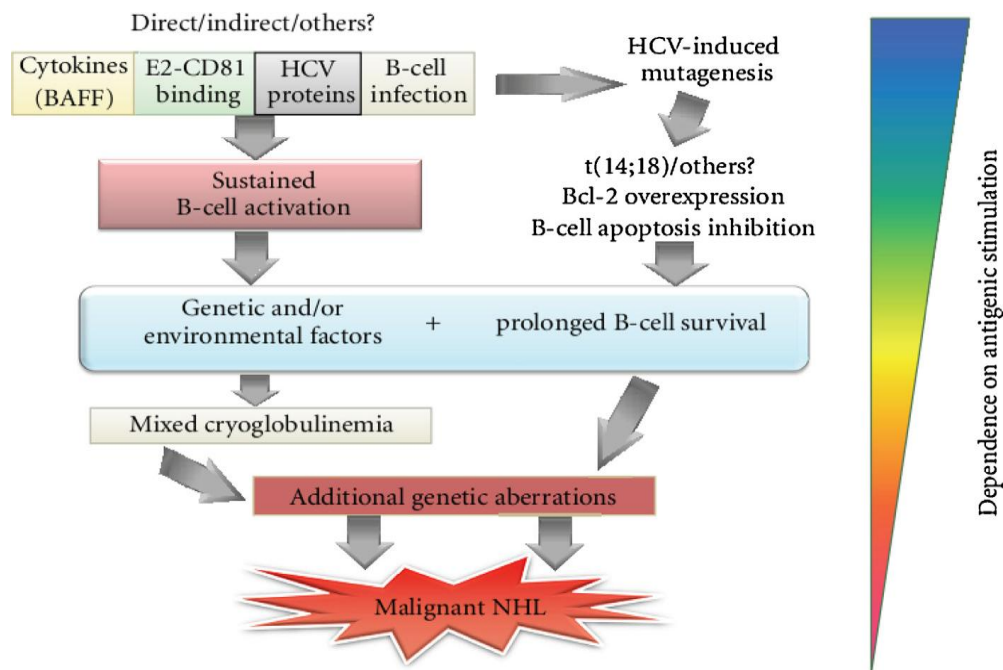
Fig.1 The structural organization of HCV genome
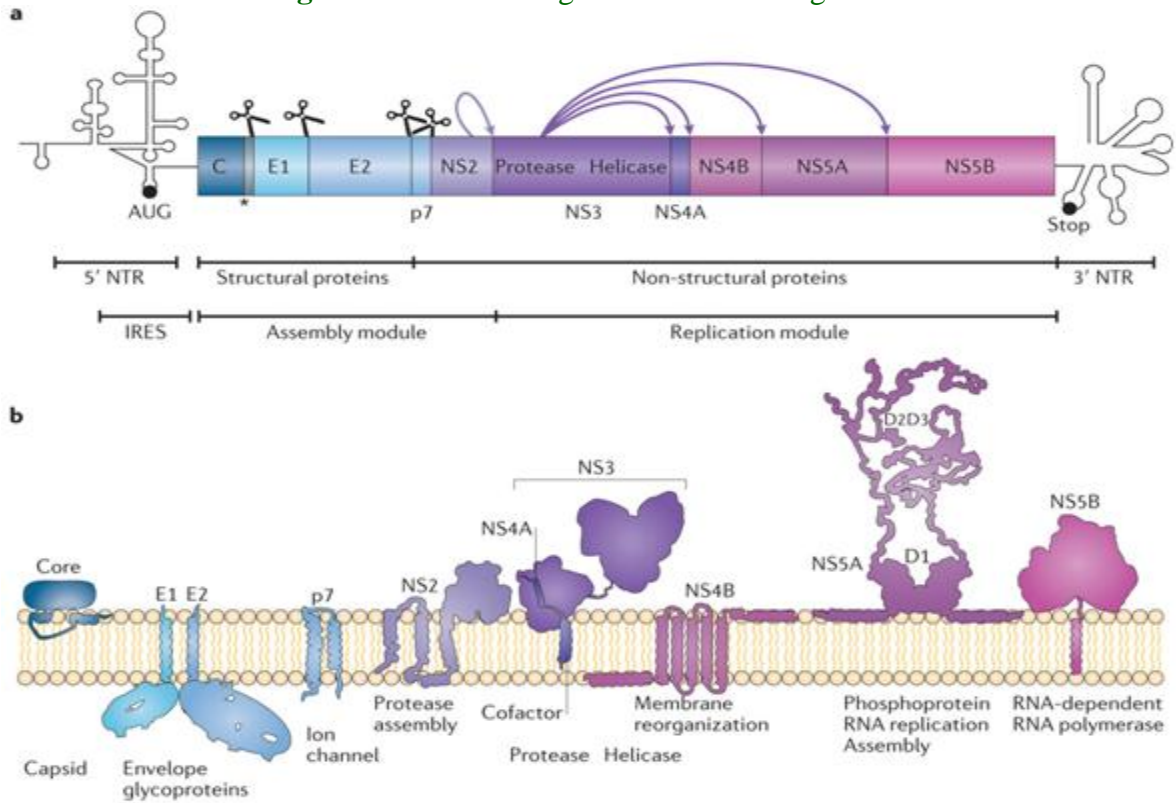
Fig.2 The possible mechanisms that may be integrated and cooperate in a pathogenetic model of HCV-associated B-cell lymphoproliferation
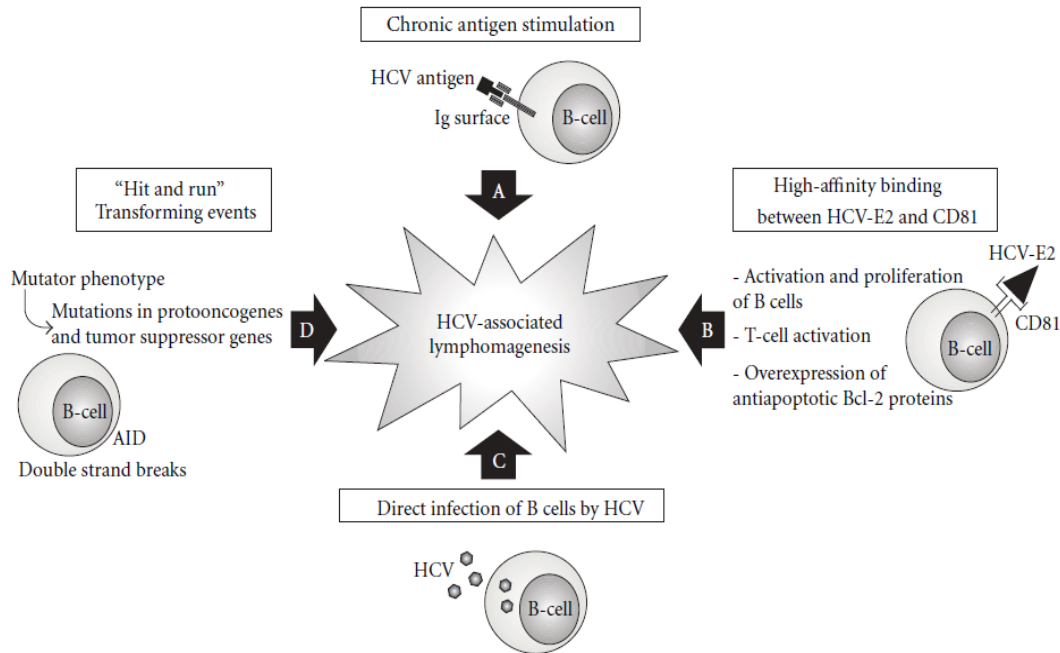
Fig.3 Pathogenesis of HCV related Lymphoproliferative disorders

Another potential explanation for the failure to clear HCV-RNA from PBMC is a host-based resistance to the therapeutic actions of ribavirin (RBV). It has been shown that cellular uptake of RBV into PBMCs decreases over time and may explain at least in part why mononuclear cells become a reservoir of HCV and potentially contributes to the development of treatment failure, disease recurrence, and in some cases the development of an OCI (Ibarra *et al.,* 2011).

Clinical relevance of OCI

About 8% of patients, who achieved a SVR developed a late recurrence. The late relapses were more frequent in patients with cirrhosis [5/28 (18%) versus 3/72 (4%) without cirrhosis]. The data demonstrate that while a SVR is variable in most patients, some individuals particularly those with cirrhosis experience late relapses. The late relapses and the frequency of OCI in cirrhotic which developed earlier than in non-cirrhotics still remained to be determined (Sood *et al.,* 2010).

Patients treated with peg interferon-α2a/ribavirin in combination with a direct acting antiviral agent were investigated for the SVR. One hundred and three patients with chronic hepatitis C who achieved a SVR to triple therapy were followed. Two cases of a late relapse were observed. One of these two patients was cirrhotic. The relapses occurred 8 and 12 months after cessation of their antiviral therapy. Subsequent cloning sequence studies identified the genomic sequence in both patients as being identical to that of their original virus (Rutter *et al.,* 2013). Giannini (2010) followed up 231 chronic HCV patients who had at least 48 weeks after achieving a SVR to PEG-IFN and ribavirin. The original SVR was maintained in 211 out of 231 patients (91%). HCV-PCR became positive in 18 patients (8%), during the first six months after the end of treatment, and two patients (<1%) within one year after the SVR.

Although the relevance of HCV-RNA detection in PBMCs alone, or in the liver in the absence of serum viremia, as well as other

tissues is poorly understood, the ability of the virus to replicate in these extra hepatic cells, raises questions about the potential transmission risk to the liver from these sites and to other individuals as a result of blood exposure (Quiroga *et al.,* 2009).

HCV and lymphoproliferative disorders

Evans and Mueller (1990) proposed that either virologic or epidemiologic guidelines need to be fulfilled to support an etiologic role for a virus in a given human cancer. The suggested epidemiologic guidelines included the following: (a) the geographic distribution of viral infection should coincide with that of the tumor; (b) the presence of viral markers should be higher in case subjects than in matched control subjects; (c) viral markers should precede the tumor, with a higher incidence of tumors in persons with the marker than in those without; (d) prevention of viral infection should decrease tumor incidence. The suggested virologic guidelines included the following: (a) the virus should be able to transform human cells *in vitro*; (b) the viral genome should be demonstrated in tumor cells and not in normal cells; (c) the virus should be able to induce the tumor in an experimental animal (Evans and Mueller, 1990).

The association between HCV infection and occurrence of B-NHL is concerned, most of the epidemiologic guidelines for causality from Evans and Mueller are met. Hepatitis C virus is associated with certain B-NHL types, especially in geographic areas with HCV endemicity, like Italy, Japan, and Egypt, where prevalence rates range from 20% to 40% (Talamini *et al.,* 2004) ; (Marcucci *et al.,* 2011). While in non endemicareas, like Northern Europe, North America and the United Kingdom, the prevalence of HCV infection in B-NHL is far less than 5% (Sy and Jamal 2006) ; (Tsukiyama-Kohara 2011); (Nicolosi *et al.,* 2012).

Several epidemiological studies have been performed to investigate prevalence of HCV RNA in various types of lymphoma and described that HCV is a risk factor for lymphomas in Egypt (Goldman *et al.,* 2009); (Farawela *et al.,* 2o12); (Khorshied *et al.,* 2014).

The International Lymphoma Epidemiology Consortium (Inter Lymph) study reported the results of HCV related B-NHL from a large international multicenter data source. The study included 11,053 participants, 4,784 cases, and 6,269 controls from seven case-control studies conducted in the United States, Europe, and Australia with information on HCV infection. HCV infection was detected in 172 NHL cases (3.6%) and in 169 (2.7%) controls (de Sanjose *et al.,* 2008). Another meta-analysis reviewed data from 23 studies and found a stronger association (Matsuo *et al.,* 2004).

Mechanisms of HCV-Induced lymphoproliferation

The biological rational for investigating a causal link between HCV infection and B-NHL depend on clinical and epidemiological perceptions. There are limited information available about the biological mechanisms of HCV-induced lymphoproliferation. Evidences from experimental studies suggest that several different mechanisms may be involved in HCV-mediated B-cell transformation (Hartridge-Lambert *et al.,* 2012).

Chronic antigen stimulation

The concept of chronic stimulation by antigen leading to a monoclonal proliferation may also be applied to HCV as the association of Helicobacter pylori infection and gastric MALT lymphoma (Stathis *et al.,* 2010). Further evidence comes from the antibody response and immunoglobulin variable (Ig

VH) gene usage in patients with chronic HCV infection and HCV-associated B-NHL (Marasca *et al.,* 2001). The VH1-69 immunoglobulin segment is expressed in the restricted repertoire of fetal liver B lymphocytes and is thought to be involved in natural immunity. A productive VH1-69 rearrangement is present in 1.6% of normal B lymphocytes in adults. However, VH1-69 is rearranged in 10% to 20% of B-cell chronic lymphocytic (Perotti *et al.,* 2008).

HCV-E2 protein is the primary target of antibody responses against HCV. Quinn *et al.,* obtained the cloning of the B-cell receptor from one HCV-positive DLBCL and its expression as a soluble immunoglobulin. Suggesting that some HCV-associated BNHL may originate from B-cells that were initially activated by HCV-E2 protein (Quinn *et al.,* 2001). Other studies suggest an indirect, antigen-driven lymphomagenetic role of HCV, by HCV-E2 protein recognized as one of the most important antigens involved in chronic B-lymphocyte (Marcucci and Mele, 2011); (Hartridge-Lambert *et al.,* 2012).

High-affinity binding between HCV-E2 and CD81

A second mechanism, potentially involved in HCV associated lymphomagenesis, derives from the high-affinity binding between HCV-E2 and one of its receptors, the tetraspanin CD81, expressed on B-cellslymphocyte (Marcucci and Mele, 2011). CD81 is known to form B-cell costimulatory complex with CD19, CD21, and CD225 proteins. This complex decreases the threshold for B-cell activation via the B-cell receptor by bridg in antigen specific recognition and CD21-mediated complement recognition. It was reported that engagement of CD81 on human B-cells by a combination of HCV E2 protein and anti-CD81 mAb leads to the proliferation of naive B-cells, and E2- CD81 interaction induces protein tyrosine phosphorylation and

hypermutation of the immunoglobulin genes in B cell lines (Rosa *et al.,* 2005).

In addition to direct effects on B-cells, engagement of CD81 on T-cells lowered the threshold for interleukin-2 production, resulting in strongly increased T cell proliferation. This could lead to T-cell activation in response to suboptimal stimuli and bystander activation of B-cells. Taken together, these results suggest that CD81 engagement on B- and T-cells may lead to direct or indirect activation (Marcucci and Mele, 2011).

Chronic B-cell proliferation, in response to antigenic stimulation or polyclonal activation, may predispose to genetic lesions such as translocation and/or overexpression of the antiapoptotic protein Bcl-2. In a study, human Burkitt's lymphoma cell line (Raji cells) and primary human B lymphocytes (PHB) were subjected to HCV-E2 protein and HCV particles produced by cell culture (HCVcc).The results showed that both E2 and HCVcc triggered phosphorylation of IkBα, with a subsequent increased expression of NF-kB and NF-kB target genes, such as antiapoptotic Bcl-2 family proteins (Bcl-2 and Bcl-xL). In addition, both E2 protein and HCVcc increased the expression of costimulatory molecules CD80, CD86, and CD81 itself, and decreased the expression of complement receptor CD21. Hence, E2-CD81 engagement plays a role in activating B-cells, protecting B-cells from activation induced cell death, and regulating immunological function. These latter mechanisms may contribute to the pathogenesis of HCV-associated B-cell lymphoproliferative disorders (Chen *et al.,* 2013).

Direct infection of B-cells by HCV

Another oncogenetic mechanism that has been proposed is the direct infection of B-cells by HCV. In the early 1990s, the

presence of HCV RNA was demonstrated by PCR not only in serum/plasma and liver tissues but also in peripheral blood mononuclear cells (PBMCs), especially in B-cells, of patients infected with (Houghton *et al.,* 1991; Ferri *et al.,* 1993). Nevertheless, although HCV has been detected in lymphocytes from HCV infected patients and patients with mixed cryoglobulinemia, only in a minority of cases RNA-negative strands, the HCV replicative intermediates suggestive of viral replication, were also detected in the cells (Inokuchi *et al.,* 2009). Stamataki *et al.,* (2009), have provided experimental evidence that HCV might infect B-cells, but B-cells were not able to support active viral replication. Overall, these results should indicate that PBMC may not be permissive to HCV replication (Marcucci and Mele, 2011).

It has been reported that HCV may infect and replicate only in a relatively rare subset of B-cells, such as CD5+ B-cells. These cells have been shown to express high levels of CD81 and to expand in HCV-infected liver (Curry *et al.,* 2003). A Japanese group established HCV transgenic mice that expressed the full HCV genome in B-cell (RzCD19Cre mice) (Tsukiyama-Kohara *et al.,* 2011). Interestingly, RzCD19Cre mice with substantially elevated serum-soluble interleukin-2 receptor α-subunit (sIL-2Rα) levels developed B-NHL. Another mouse model of lymphoproliferative disorder was established by persistent expression of HCV structural proteins through disruption of interferon regulatory factor-1 (irf-1 −/−/CN2 mice). Irf-1 −/−/CN2 mice showed extremely high incidences of lymphomas and lymphoproliferative disorders (Tsukiyama-Kohara *et al.,* 2011).

"Hit and run" transforming events

HCV has been found to induce high mutation frequency of cellular genes (immunoglobulin heavy chain, Bcl-6, p53 and beta-catenin genes), in B-cell lines and PBMCs *in vitro*, by inducing double strand breaks and by activating error-prone-polymerases and activation-induced cytidinedeaminase (AID). These mutations of cellular genes are amplified in HCV-associated B-NHL in vivo, suggesting that HCV-induced mutations in proto-oncogenes and tumor suppressor genes may lead to oncogenetic transformation of the infected B-cells. Mutations induced by HCV acute and chronic infection in B-cells may be considered a "hit and run" mechanism of cell transformation (Machida *et al.,* 2004).

HCV genotypes and lymphoproliferative disorders

The possible association between specific viral genotypes and malignant lymphoproliferative disorders remains a controversial issue. There are at least six major HCV genotypes whose prevalence varies geographically. Genotype 1 accounts for the majority of infections in North America, South America, and Europe (Rosen, 2011). It has been documented an unexpectedly lower prevalence of HCV genotype 1b in patients with B-NHL. Conversely, the prevalence of genotypes 2a and 2b was higher in patients with B-NHL, thus suggesting that different HCV variants may show greater lymphotropism (Fujiwara *et al.,* 2013).

Epidemiologic evidence from a multicenter retrospective study also suggested that genotype 2 may be more prevalent and carcinogenic in lymphoma patient. Genotype 1 predominated (84%) in immunocompetent as compared to patients with HCC (74%) or lymphoma (59%). By contrast, genotype 2 was more prevalent in patients with lymphoma (24%), compared to immunocompetent (8%), yielding a 3-fold increase in cancer risk among HCV-infected patients than other genotypes (Torres *et al.,* 2012).

Interestingly, it has been observed that DLBCL patients had a high association with genotype 1 and a shorter duration of HCV infection, as compared to patients with indolent, low-grade B-NHL, who showed a high association with genotype 2 and longer duration of HCV infection. Because HCV genotype 2 is associated with a longer duration of viral infection, it has been speculated that over time it may induce a persistent chronic immune stimulation of B cells (Pellicelli *et al.,* 2011).

In Egypt, some authors studied the association of LPD with HCV genotype 4, the predominant genotype in Egypt. Diffuse large-cell histology was the dominant subtype followed by the follicular subtype in serum positive patients (72% and 18%) (Gouda *et al.,* 2010).

In conclusion, Hepatitis C virus (HCV) is a major health problem in the world. HCV have ability to infect not only the liver cells but also the lymphocytes and other cells. This is due to liver cells and lymphocytes express the same HCV receptor (CD81). The lymphotropism might be the cause of extra hepatic manifestations.

Lymphoproliferative disorders (LPDs) are neoplastic diseases of the lymphoid tissue. Several etiological factors have been reported including immunodeficiency, exposure to some toxic substances (pesticides) or radiation, smoking, and, recently, the infection by EBV and HCV.

Several epidemiological studies have been performed to investigate the prevalence of HCV RNA in various types of LPDs and they showed that HCV is a risk factor for development of LPDs. Furthermore, eradication of the chronic HCV infection has been correlated with regression of certain LPDs.

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