Iran Red Crescent Med J. 2015 November; 17(11): e34181. doi: 10.5812/ircmj.34181

Published online 2015 November 29. **Review Article**

Occult HCV Infection: The Current State of Knowledge

Mohammad Saeid Rezaee-Zavareh,[1,2] Reza Hadi,[2] Hamidreza Karimi-Sari,[1] Mohamad Hossein Khosravi,[1] Reza Ajudani,[1] Fardin Dolatimehr,[1] Mahdi Ramezani-Binabaj,[1] Seyyed Mohammad Miri,[2,3] and Seyed Moayed Alavian[2,*]

[1]Students' Research Committee, Baqiyatallah University of Medical Sciences, Tehran, IR Iran
[2]Middle East Liver Diseases Center (MELD), Tehran, IR Iran
[3]Kowsar Medical Institute, Heerlen, The Netherlands

*Corresponding Author: Seyed Moayed Alavian, Middle East Liver Diseases Center, Tehran, IR Iran. Tel: +98-2188945186, Fax: +98-2188945188, E-mail: Alavian@thc.ir

Received 2015 October 30; **Accepted** 2015 November 2.

Abstract

Context: Occult HCV infection (OCI) is defined as the presence of HCV-RNA in hepatocytes and the absence of HCV in the serum according to usual tests. We aimed to define OCI and provide information about the currently available diagnostic methods. Then we focus on specific groups that are at high risk of OCI and finally investigate immune responses to OCI and the available treatment approaches.

Evidence Acquisition: PubMed, Scopus and Google Scholar were comprehensively searched with combination of following keywords: "occult", "hepatitis C virus" and "occult HCV infection". The definition of OCI, diagnostic methods, specific groups that are at high risk and available treatment approaches were extract from literature. An analysis of available articles on OCI also was done based on Scopus search results.

Results: OCI has been reported in several high-risk groups, especially in hemodialysis patients and subjects with cryptogenic liver disease. Furthermore, some studies have proposed a specific immune response for OCI in comparison with chronic hepatitis C (CHC).

Conclusions: With a clinical history of approximately 11 years, occult HCV infection can be considered an occult type of CHC. Evidences suggest that considering OCI in these high-risk groups seems to be necessary. We suggest that alternative diagnostic tests should be applied and that there is a need for the participation of all countries to determine the epidemiology of this type of HCV infection. Additionally, evaluating OCI in blood transfusion centers and in patients who receive large amounts of blood and clotting factors, such as patients with hemophilia, should be performed in future projects.

Keywords: Hepatitis C, Occult infection, Review, Knowledge

1. Definition and Diagnoses

HCV is a single strand RNA virus that belongs to the family of *Flaviviridae* and genus *Hepacivirus*. Diagnosis of HCV infection is based on detecting serum HCV antibodies and HCV-RNA. In 50 to 85% of cases acute infection can be changed to chronic type. About 170 million people are infected with HCV worldwide so that it can lead to about 350000 deaths per year (1-4).

In 2004, a new form of CHC has been introduced by Castillo et al. They investigated 100 patients with negative results for serum HCV antibodies and HCV-RNA regarding HCV-RNA in hepatocytes by reverse-transcription polymerase chain reaction (RT-PCR) and in-situ hybridization. Fifty seven cases were positive for HCV-RNA in liver cells getting from liver biopsy and in another word these cases had OCI (5, 6). Some studies are also available in databases that have investigated the existence of HCV-RNA in hepatocytes in patients with cryptogenic liver disease before 2004. In a cross-sectional study in 1992, it has been reported that 13 out of 22 non-a non-b hepatitis cases had HCV-RNA in their liver biopsy specimens (7). However in another study in 1996, it has been showed that none of 10 cases with cryptogenic cirrhosis had HCV-RNA in their hepatocytes. These studies have proposed nothing about occult HCV infection (8).

After all we can define OCI with two following conditions; 1) Presence of HCV-RNA in hepatocytes and 2) absence of HCV-RNA in the serum (by current clinical laboratory methods). Also a categorization based on HCV antibodies and liver function tests (LFTs) has been proposed so that OCI infected patients can be classified in two groups. One group of patients has normal LFTs with positive HCV antibodies and another group has abnormal LFTs without HCV antibodies (9).

Based on this definition, it is clear that for diagnosing OCI infected patients we need to do a liver biopsy to get hepatocyte specimens and it is considered as the gold standard method for OCI diagnosis. But we know that this procedure is invasive (10). On the other hand,

it is proposed that screening of OCI patients seems to be necessary in special high risk groups that we pointed them out later in this review (9). So introducing an alternative diagnostic method for OCI is mandatory and some project tried for this important issue and proposed some alternative methods. In an attempt to evaluate combined HCV antigen-antibody assay for diagnosing OCI, 115 OCI infected patients have been investigated with this assay. One patient has been reactive to this assay and three has been negative to weekly reactive. So it was concluded that this combined assay cannot help to OCI diagnosis (11). Evaluation of HCV-RNA presence in whole blood, ultracentrifugated serum and peripheral blood mononuclear cells (PBMCs) are other investigated OCI diagnostic method in available literature. Investigation of HCV-RNA in PBMCs (as a diagnostic method for occult infection of HCV) has been done before 2004. Oesterreicher et al. among 67 cases undergoing chronic hemodialysis reported one patient positive for OCI (12). In another study in 2004, twenty one OCI infected cases has been evaluated regarding alternative diagnostic methods. HCV-RNA has been detected in whole blood in 14% of cases and in PBMCs in 57% (13). In 2007, Bartolome et al. showed in their study that out of 106 OCI patients, 62 (58.5%) had detectable HCV-RNA in their ultracentrifuged sera (14). Finally Castillo et al. in 2010, proposed in their project that investigation of HCV-RNA in ultracentrifuged serum and in PBMCs can help to diagnosis of OCI patients in 57% and 61% of cases respectively. Also they showed that combination of these two method can improve OCI diagnosis up to 91% (10). So, investigation of HCV-RNA in PBMCs and ultracentrifuged serum is what we have until now as an alternative diagnostic method for OCI. Importance for diagnosis of OCI is related to importance of its complications. It is said that because of lower number of liver cells that are involved in OCI, this type of HCV infection is a milder disease than CHC. However it is showed that OCI can lead to necroinflammation and fibrosis in liver tissue (15). And on the other hand liver cirrhosis and hepatocellular carcinoma are other reported complications of OCI (16, 17). So there is a need for a suitable diagnostic method for OCI at least in some high risk groups that we point them below.

2. Special Groups Need Special Attention

Some HCV genotypes including 1a, 2a, 2b and 3b are reported for OCI and this may indicate OCI as a worldwide issue (2, 18, 19). However more epidemiological studies should be done in the world and in different groups of patients to investigate this issue.

The literature suggests the existence of occult HCV infection (OCI) in some special populations. Data on these conditions (autoimmune hepatitis (6), lymphoproliferative disease (20, 21), glomerular nephropathies (22), antiphospholipid syndrome (23), type 2 mixed cryoglobulinemia (24-26), hemophilia (27) and hepatitis B infection (28)) are scarce, and there is thus a need for more original studies to determine the relationship of these populations with OCI. Due to length limitations, we cannot evaluate these conditions here, but we review the existence of OCI in other populations supported by currently available original research.

2.1. Patients with Cryptogenic Liver Disease

Chronic liver disease is defined as the persistent abnormality of liver enzymes for more than 6 months (2, 29, 30). However, when all standard clinical and paraclinical evaluations identify no known etiology, the patient is considered to have cryptogenic liver disease (2, 29). The prevalence of cryptogenic liver disease has been reported to vary between 5 and 20 percent in patients with chronic liver disease (2, 29, 31, 32). Occult HBV infection is now a well-known and important underlying pathology in those patients (29). With the emergence of OCI, researchers have evaluated its significance in patients with cryptogenic liver disease. These studies are presented in Table 1.

As stated previously, Castillo et al. investigated 100 patients with cryptogenic liver disease. Liver samples from 57% of the patients were positive for genomic HCV RNA by RT-PCR. Additionally, HCV RNA was detectable in PBMCs in 40 of these 57 samples. The researchers also showed the presence of anti-genomic HCV-RNA in both the liver and PBMCs, which suggested the ongoing replication of the virus. They even confirmed that OCI is associated with necroinflammatory activity and fibrosis in the liver (5). Bokharaei et al. used RT-nested PCR to evaluate the presence of HCV-RNA in the PBMCs of 69 Iranian patients with cryptogenic liver disease. OCI was present in 7 (10%) of them. They proposed OCI as a possible cause of cryptogenic liver disease (2). Keyvani et al. evaluated 45 cryptogenic cirrhosis patients, and 8.9 % of them had OCI, as shown by the presence of genomic HCV-RNA in their PBMCs. The authors recommended the detection of HCV-RNA in PBMCs of patients with cryptogenic cirrhosis prior to liver transplantation (17).

In contrast, other studies have failed to demonstrate the presence of OCI in these patients. Geller et al. investigated liver specimens of patients with cryptogenic cirrhosis using RT-PCR, and all 10 subjects were negative for HCV-RNA (8). Additionally, Halfon et al. used an ultrasensitive RT-PCR to assess the PBMCs of 22 patients with cryptogenic liver disease, and all of the samples were negative for HCV-RNA (34).

Keeping in mind that HCV infection is an important and treatable cause of liver disease and the fact that patients with cryptogenic liver disease can potentially develop cirrhosis and hepatocellular carcinoma (17), investigating the prevalence of HCV and developing more accurate techniques to diagnose OCI in these patients are critically important.

2.2. Hemodialysis and Kidney Transplant Patients

Although OCI was found first in patients on chronic hemodialysis (HD), important controversy remains con-

cerning the prevalence and clinical significance of OCI in HD and kidney transplant (KTX) patients. Due to the existence of OCI, the prevalence of HCV infection among HD patients may be underestimated (38). Studies have provided different statistics for the prevalence of OCI in HD patients, which ranges from 0 to 42.2 % (39-42). This difference may have arisen because the studies used different inclusion criteria (40, 43) and even different definitions of OCI (39, 44, 45). Additionally, they relied on the detection of HCV-RNA in serum or PBMCs, and to the best of our knowledge, liver biopsy was only performed in one study (40). However, three studies conducted on HD and/or KTX patients with no other criteria used the PBMC HCV-RNA levels to detect OCI and achieved low prevalence rates that ranged from 0.25 to 1.5 % (12, 41, 42). Table 2 shows the basic information from several studies that have evaluated OCI in HD and/or KTX patients to date. In the largest study, Baid-Agrawal et al. evaluated 417 HD patients and 417 KTX recipients and elucidated very low prevalence rates, concluding that the prevalence of OCI in those patients was not clinically relevant (42). Nicot et al. evaluated KTX recipients in whom HCV infection was eliminated during HD and showed that administration of immunosuppressive therapy during KTX did not cause a relapse, therefore concluding that OCI was not present (40). However, several other studies emphasized the prevalence and relevance of OCI in these two groups. For example, Barril et al. showed a prevalence of 45% for OCI in patients under chronic HD with persistently deranged aminotransferases and negative serum HCV-RNA and Anti-HCV; they also revealed the role of OCI in increased mortality (43). The detection of HCV-RNA in PBMCs from patients on HD who were negative for serum HCV-RNA has led some authors to suggest using this measurement to better diagnose patients with occult infections who require more attention (12, 41).

3. Immune Responses

Both cellular and humoral aspects of adaptive immune system are increased in HCV infection, and cellular responses, especially specific T cell responses, play a key role in recovery from infection (31, 59, 60). The inflammatory response to HCV may continue for a long time, even in the absence of occult viral infections (61). As a new entity, immune response to OCI is a very active field of research, and there is still much to be discovered (62).

The immune system is not only a part of an OCI patient's reaction to the virus, but it also serves as a host to the virus, thereby facilitating its replication, as indicated by antigenomic HCV-RNA strands found in these patients (55, 63-65). Evidence also shows that OCI is associated with lymphoproliferative disorders and suggests a role for it in lymphomagenesis (20).

Distinct patterns of cellular immune responses have been shown in patients with OCI, and some studies even suggest tests for the assessment of cellular immunity as a marker for OCI (66). Quiroga et al. demonstrated that OCI patients have HCV-specific cellular immune responses in their peripheral blood significantly more frequently than CHC patients. This is true for both CD4 and CD8 functional virus-specific memory T cells (59). These types of responses are maintained by fluctuating levels of viremia, which persist in OCI patients over time (67). Some studies have revealed the similarity between T-cell responses found in OCI and those in Anti-HCV positive patients following spontaneous or treatment-induced recovery (60, 68), and another study showed that sustained virological response (SVR) with OCI in PBMCs bears a lower HCV-specific T-cell response relative to SVR without OCI (69). Therefore, we believe that HCV-specific T-cell responses in OCI are more powerful than those in CHC because the former can clear the serum, restrict the presence of the virus and replication in hepatocytes and PBMCs, and lessen the clinical manifestations but are not sufficiently powerful to cause the complete eradication of the virus.

There is also evidence of distinct profiles of antiviral and proinflammatory cytokines in OCI. In light of the role of imbalanced Th1 and Th2 responses in the persistence of viruses such as HIV, Pham et al. showed that PBMCs in CHC and OCI produce low or undetectable levels of IFN-g and IL-12 and high levels of IL-10. IL-10 is a Th2-type cytokine that is frequently associated with viral persistence. IFN-g and IL-12 are Th1-type cytokines that have a key role in T-cell-mediated immunity and viral clearance. The authors suggested that higher levels of IL-10 in OCI shift the immune response towards Th2-type cytokines, which causes the virus to persist in some compartments (60). Gad et al. focused on the difference between OCI and CHC and showed a distinct immunoregulatory cytokine profile with the predominance of Th2-type cytokines (IL-4 and IL-10) in OCI, thus allowing viral persistence in liver while the serum is clear. They contributed the less aggressive course of OCI relative to CHC to the lack of Th1-type cytokines (IL-2 and IFN-g) in OCI (70). Similar results were described by Mousa et al., who revealed that Th1-type cytokines were significantly increased in patients with CHC relative to OCI but that the serum IL-4 levels as a Th2-type cytokine were higher in OCI. The serum IL-10 levels were high in both groups, with no significant difference between CHC and occult HCV groups (71). In light of these studies, we believe that perturbation of the immune response balance toward Th2-type cytokines plays a role in the clinical scenario of OCI, causing the virus to persist with fewer manifestations than CHC.

The two types of OCI described previously can be either positive or negative for anti-HCV (55, 69, 72). After a seropositive infection, OCI can bear anti-HCV for years, but in an immunocompetent patient, primary OCI can be caused by sporadic exposure to low viral doses, resulting in undetectable antibody titers (11). However, there are distinct patterns of humoral responses in OCI patients. In one study, Quiroga et al. unveiled a 20% frequency of IgG antibody to GOR autoepitopes in anti-HCV-negative OCI. They found that both frequency and serum levels of anti-GOR IgG are lower in OCI than CHC. Although positivity for anti-GOR did

not differ in clinical background, the percentage of infected hepatocytes was significantly greater in anti-GOR-positive OCI patients, and they showed signs of necroinflammation more frequently than anti-GOR-negative patients did (73).

In another study, Quiroga et al. measured the levels of IgG antibody produced in response to a core HCV peptide in different groups. Approximately 40 % of the anti-HCV-negative OCI patients were positive, as were 99 % of CHC patients, but none of the patients with HCV-unrelated liver disease tested positive. They concluded that anti-HCV core testing is useful in identifying OCI among individuals who are negative for both anti-HCV and serum HCV-RNA (74).

There is a suggestive link between HCV infection and some autoimmune diseases (1). Rheumatoid factor, CRP, and cryoglobulins are present in 10 to 14% of OCI patients, which is less frequent than that of CHC (73). Studies have suggested the link between cryoglobulinemia and OCI (14), and Casato et al. even suggested that interferon therapy is an option in patients with apparently essential mixed cryoglobulinemia (26).

4. Treatment

Because there is still some controversy surrounding the existence of OCI, it is not surprising that data about its treatment are lacking. To the best of our knowledge, until now, only two groups have investigated the effect of HCV treatment in OCI.

Casato et al. (26) studied 3 essential mixed cryoglobulinemia patients who were repeatedly anti-HCV-negative and HCV-RNA-negative in both serum and cryoprecipitate. Two of the patients were treated with recombinant interferon and showed clinical responses. After different times elapsed from the start of treatment, they experienced relapse, and at that time, they both tested positive for HCV-RNA in cryoprecipitate while Anti-HCV was still negative, which produced a diagnosis of OCI. One of the patients received the second course of treatment and responded with clinical remission and the disappearance of HCV-RNA for a long period of time. Regarding these data, they suggested that in apparently essential mixed cryoglobulinemia, treatment with interferon is a viable option because OCI may be the underlying disease (26).

Pardo et al. (75) studied 10 OCI patients, all of whom were infected with genotype 1b, had abnormal ALT levels, were positive for HCV-RNA in their PBMCs and showed necroinflammation in their liver biopsy. They received pegylated-interferon plus Ribavirin for 24 weeks and were followed for 24 more weeks. The results at the end of treatment and follow-up showed that ALT normalization occurred in 8 and 6 patients, respectively, and the disappearance of HCV-RNA in PBMCs was seen in 8 and 7 patients, respectively. Five patients underwent liver biopsy, and despite the persistence of HCV-RNA in all of them, the viral load was lower. Interestingly, 3 of them showed histological improvement with decreases in fibrosis and necroinflammation. Ultimately, the authors demonstrated the benefits of interferon-based antiviral therapy in OCI (75).

With regard to the known complications of OCI and its prevalence in some important groups, OCI patients require special attention in treatment (76), and further studies about the indications and benefits of OCI treatment are highly warranted. Until then, we believe that individualized HCV treatment should be considered in OCI. Furthermore interferon-free based treatment and using sofosbuvir are new strategies for treatment of HCV infected patients (77-79) that can also be considered for OCI treatment.

5. Analysis of Available Articles About OCI in Scopus

As discussed previously, research on the use of HCV-RNA in liver cells for the detection of OCI dates to the year 2004. However, some articles published before 2004 applied identification methods for OCI without mentioning HCV-RNA.

We performed a simple search (July 10, 2015) in Scopus with the key word (TITLE-ABS-KEY ("Occult Hepatitis C")) and found 78 papers in the search results page. After this, we analyzed the search results. Figure 1 shows the papers that used the phrase "Occult Hepatitis C" in their title, abstract or keywords based on publication years. Two papers were published 2003 and 1994. However, most papers were published in 2014 (n = 12), 2009 (n = 10), and 2008 (n = 10). Figure 2 shows that Carreno, V (n = 21), Castillo, I (n = 21) and Bartolome, J (n = 17) have authored the most articles about this issue. As shown in Figure 3, Spain is the country with the most research on OCI, followed by France (n = 8) and Canada (5).

Of the 78 papers on this issue, 51 were original articles. There were 12 letters, 6 reviews, and 5 editorials in this field. We also analyzed the search results based on the journals that published articles about OCI. The Journal of Medical Virology (n = 11) and Hepatology (n = 6) published the most articles about OCI. We believe that the 78 papers published over approximately 11 years demonstrate the importance of this topic. However, other countries should participate in research on this topic and help determine the epidemiology of OCI.

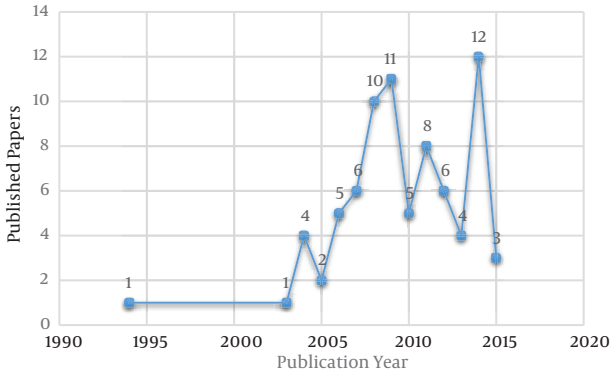


Figure 1. Number of Available Papers on OCI Based on Publication Year

Table 1. Studies Investigating the Detectability of HCV RNA in the Liver and PBMCs of Patients with Cryptogenic Liver Disease[a]

Author	Year	Country	HCV-RNA Detection (Liver)	HCV-RNA Detection (PBMCs)
Geller et al. (8)	1996	USA	0/10 (0%)	not done
Verslype et al. (33)	2003	Belgium	1/67 (1.5%) [b]	not done
Castillo et al. (5)	2004	Spain	57/100 (57%)	40/100 (40%)
Halfon et al. (34)	2008	France	not done	0/22 (0%)
Bokharaei-Salim et al. (2)	2010	Iran	not done	7/69 (10%)
Zaghloul and El-Sherbiny (35)	2010	Egypt	not done	4/40 (10%)
Idrees et al. (36)	2011	Pakistan	not done	23/31 (74.2%)
Keyvani et al. (17)	2013	Iran	not done	4/45 (8.9%)
Makvandi et al. (37)	2014	Iran	not done	17/53 (32%)

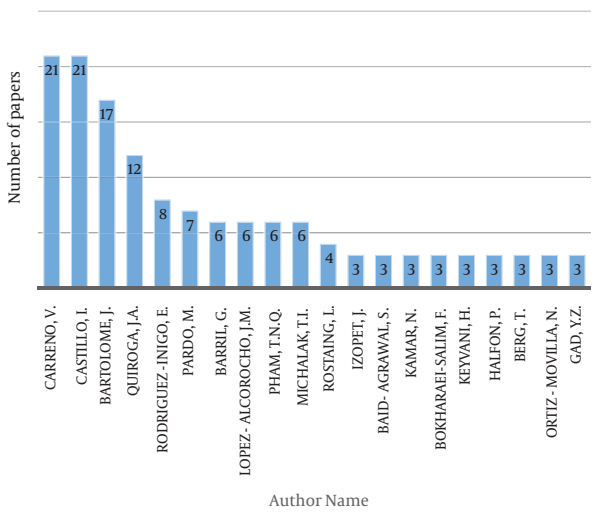
[a]Abbreviations: HCV, hepatitis C virus; OCI, Occult hepatitis C virus infection; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction.
[b]This study aimed to investigate a validated immunohistochemical staining assay. All 67 liver specimens were tested by that method. Six cases were positive. Liver HCV-RNA was tested only in these six cases.

Table 2. Occult Hepatitis C Virus in Hemodialysis Patients OR Kidney Transplant Recipients[a,b]

First Author	Year	Country	Sample Size	OCI Prevalence, %	Method of HCV-RNA Detection
Oesterreicher et al. (12)	1995	Austria	67	1.5	PBMCs HCV-RNA by PCR
Yakaryilmaz et al. (45) [c]	2006	Turkey	188	4.8	serum HCV-RNA by PCR
Barril et al. (43)	2008	Spain	109 c	45	PBMCs HCV-RNA by RT-PCR and in situ-hybridization
Thongsawat et al. (41)	2008	Thailand	231	0.86	PBMCs HCV-RNA by PCR
Jain and Nijhawan (44) [c]	2008	India	102	26.4	serum HCV-RNA by PCR
Nicot et al. (40)	2009	France	26 (KTX)	0	liver, PBMCs HCV-RNA by PCR
Hooda et al. (39) [c]	2012	India	404 (KTX)	42.2	serum HCV-RNA by PCR
Baid-Agrawal et al. (42)	2014	Germany	417 (HD) 417 (KTX)	0.25 (HD) 0.5 (KTX)	PBMCs HCV-RNA by TMA
Eslamifar et al. (46)	2015	Iran	70	0	PBMCs HCV-RNA by RT-nested PCR

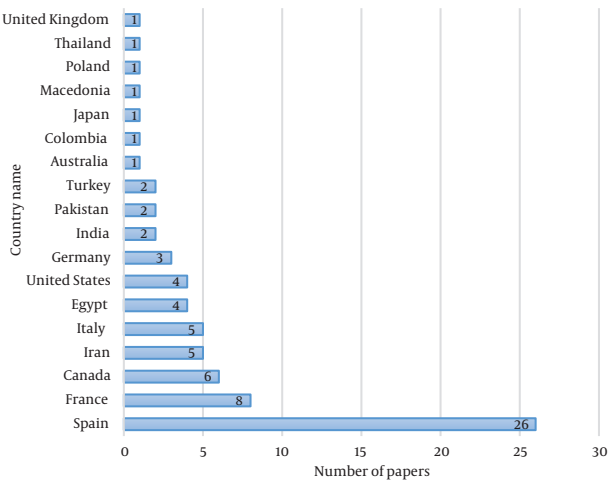
[a]Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; KTX, kidney transplantation; OCI, Occult hepatitis C virus infection; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; TMA, transcription mediated amplification.
[b]Only patients who were negative for serum HCV-RNA and anti-HCV and had elevated aminotransferases were enrolled in this study.
[c]These studies defined OCI as a positive serum HCV-RNA and a negative serum Anti-HCV.

Figure 2. Number of Available Papers on OCI Based on First Author Name



The chart only shows authors with more than 2 papers.

Figure 3. Number of Available Papers on OCI Based on Country



6. Conclusions

With a clinical history of approximately 11 years, occult HCV infection can be considered an occult type of CHC. OCI is milder than CHC generally, but some reports have shown a link between OCI and liver cirrhosis and hepatocellular carcinoma. Also OCI has been reported in several high-risk groups, especially in hemodialysis patients and subjects with cryptogenic liver disease. Furthermore, some studies have proposed a specific immune response for OCI in comparison with CHC. All of these points suggest that considering OCI in these high-risk groups seems to be necessary. We suggest that alternative diagnostic tests should be applied and that there is a need for the participation of all countries to determine the epidemiology of this type of HCV infection. Additionally, evaluating OCI in blood transfusion centers and in patients who receive large amounts of blood and clotting factors, such as patients with hemophilia, should be performed in future projects.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med.* 2001;**345**(1):41–52. doi: 10.1056/NEJM200107053450107. [PubMed: 11439948]
2. Bokharaei-Salim F, Keyvani H, Monavari SH, Alavian SM, Madjd Z, Toosi MN, et al. Occult hepatitis C virus infection in Iranian patients with cryptogenic liver disease. *J Med Virol.* 2011;**83**(6):989–95. doi: 10.1002/jmv.22044. [PubMed: 21503911]
3. Zaltron S, Spinetti A, Biasi L, Baiguera C, Castelli F. Chronic HCV infection: epidemiological and clinical relevance. *BMC Infect Dis.* 2012;**12 Suppl 2**:S2. doi: 10.1186/1471-2334-12-S2-S2. [PubMed: 23173556]
4. Jamali R. Epidemiologic Studies on Viral Hepatitis: A Short Review. *Thrita.* 2014;**3**(1):e15376.
5. Castillo I, Pardo M, Bartolome J, Ortiz-Movilla N, Rodriguez-Inigo E, de Lucas S, et al. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis.* 2004;**189**(1):7–14. doi: 10.1086/380202. [PubMed: 14702147]
6. Rezaee Zavareh MS, Alavian SM, Karimisari H, Shafiei M, Saiedi Hosseini SY. Occult hepatitis C virus infection in patients with autoimmune hepatitis. *Hepat Mon.* 2014;**14**(8):e16089. doi: 10.5812/hepatmon.16089. [PubMed: 25337141]
7. Bresters D, Cuypers HT, Reesink HW, Chamuleau RA, Schipper ME, Boeser-Nunnink BD, et al. Detection of hepatitis C viral RNA sequences in fresh and paraffin-embedded liver biopsy specimens of non-A, non-B hepatitis patients. *J Hepatol.* 1992;**15**(3):391–5. [PubMed: 1280289]
8. Geller SA, Nichols WS, Rojter SE, Chan RCK, Petrovic LM, Vierling JM, et al. Hepatitis C virus is not recoverable from liver tissue in cryptogenic cirrhosis: Failure to identify hepatitis C virus-RNA using reverse transcription-mediated polymerase chain reaction. *Hum Pathology.* 1996;**27**(11):1161–5. doi: 10.1016/S0046-8177(96)90309-3.
9. Rezaee-Zavareh MS, Ramezani-Binabaj M, Moayed Alavian S. Screening for occult hepatitis C virus infection: Does it need special attention? *Hepatology.* 2015;**62**(1):321–2. doi: 10.1002/hep.27626. [PubMed: 25476196]
10. Castillo I, Bartolome J, Quiroga JA, Barril G, Carreno V. Diagnosis of occult hepatitis C without the need for a liver biopsy. *J Med Virol.* 2010;**82**(9):1554–9. doi: 10.1002/jmv.21866. [PubMed: 20648609]
11. Quiroga JA, Castillo I, Pardo M, Rodriguez-Inigo E, Carreno V. Combined hepatitis C virus (HCV) antigen-antibody detection assay does not improve diagnosis for seronegative individuals with occult HCV infection. *J Clin Microbiol.* 2006;**44**(12):4559–60. doi: 10.1128/JCM.01440-06. [PubMed: 17021056]
12. Oesterreicher C, Hammer J, Koch U, Pfeffel F, Sunder-Plassmann G, Petermann D, et al. HBV and HCV genome in peripheral blood mononuclear cells in patients undergoing chronic hemodialysis. *Kidney Int.* 1995;**48**(6):1967–71. [PubMed: 8587259]
13. Carreno V, Castillo I, Bartolome J, Rodriguez-Inigo E, Ortiz-Movilla N, de Lucas S, et al. Comparison of hepatitis C virus RNA detection in plasma, whole blood and peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *J Clin Virol.* 2004;**31**(4):312–3. doi: 10.1016/j.jcv.2004.08.005. [PubMed: 15494276]
14. Bartolome J, Lopez-Alcorocho JM, Castillo I, Rodriguez-Inigo E, Quiroga JA, Palacios R, et al. Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. *J Virol.* 2007;**81**(14):7710–5. doi: 10.1128/JVI.02750-06. [PubMed: 17475654]
15. Pardo M, Lopez-Alcorocho JM, Rodriguez-Inigo E, Castillo I, Carreno V. Comparative study between occult hepatitis C virus infection and chronic hepatitis C. *J Viral Hepat.* 2007;**14**(1):36–40. doi: 10.1111/j.1365-2893.2006.00783.x. [PubMed: 17212642]
16. Comar M, Dal Molin G, D'Agaro P, Croce SL, Tiribelli C, Campello C. HBV, HCV, and TTV detection by in situ polymerase chain reaction could reveal occult infection in hepatocellular carcinoma: comparison with blood markers. *J Clin Pathol.* 2006;**59**(5):526–9. doi: 10.1136/jcp.2005.033050. [PubMed: 16537674]
17. Keyvani H, Bokharaei-Salim F, Monavari SH, Esghaei M, Nassiri Toosi M, Fakhim S, et al. Occult hepatitis C virus infection in candidates for liver transplant with cryptogenic cirrhosis. *Hepat Mon.* 2013;**13**(8):e11290. doi: 10.5812/hepatmon.11290. [PubMed: 24082889]
18. De Marco L, Gillio-Tos A, Fiano V, Ronco G, Krogh V, Palli D, et al. Occult HCV infection: an unexpected finding in a population unselected for hepatic disease. *PLoS One.* 2009;**4**(12):e8128. doi: 10.1371/journal.pone.0008128. [PubMed: 19956542]
19. Cortes-Mancera FM, Restrepo JC, Osorio G, Hoyos S, Correa G, Navas MC. Occult hepatitis C virus infection in a re-transplanted patient with liver failure of unknown etiology. *Colomb J Gastroenterol.* 2010;**25**(1):76–85.
20. Youssef SS, Nasr AS, El Zanaty T, El Rawi RS, Mattar MM. Prevalence of occult hepatitis C virus in egyptian patients with chronic lymphoproliferative disorders. *Hepat Res Treat.* 2012;**2012**:429784. doi: 10.1155/2012/429784. [PubMed: 23304473]
21. Farahani M, Bokharaei-Salim F, Ghane M, Basi A, Meysami P, Keyvani H. Prevalence of occult hepatitis C virus infection in Iranian patients with lymphoproliferative disorders. *J Med Virol.* 2013;**85**(2):235–40. doi: 10.1002/jmv.23460. [PubMed: 23168913]
22. Castillo I, Martinez-Ara J, Olea T, Bartolome J, Madero R, Hernandez E, et al. High prevalence of occult hepatitis C virus infection in patients with primary and secondary glomerular nephropathies. *Kidney Int.* 2014;**86**(3):619–24. doi: 10.1038/ki.2014.68. [PubMed: 24646855]
23. Prieto J, Yuste JR, Beloqui O, Civeira MP, Riezu JI, Aguirre B, et al. Anticardiolipin antibodies in chronic hepatitis C: implication of hepatitis C virus as the cause of the antiphospholipid syndrome. *Hepatology.* 1996;**23**(2):199–204. doi: 10.1002/hep.510230201. [PubMed: 8591841]
24. Karlsberg PL, Lee WM, Casey DL, Cockerell CJ, Cruz PJ. Cutaneous vasculitis and rheumatoid factor positivity as presenting signs of hepatitis C virus-induced mixed cryoglobulinemia. *Arch Dermatol.* 1995;**131**(10):1119–23. [PubMed: 7574826]
25. Walker NJ, Zurier RB. Liver abnormalities in rheumatic diseases. *Clin Liver Dis.* 2002;**6**(4):933–46. [PubMed: 12516200]
26. Casato M, Lilli D, Donato G, Granata M, Conti V, Del Giudice G, et al. Occult hepatitis C virus infection in type II mixed cryoglobulinaemia. *J Viral Hepat.* 2003;**10**(6):455–9. [PubMed: 14633180]
27. Parodi C, Culasso A, Aloisi N, Garcia G, Baston M, Corti M, et al. Evidence of occult HCV genotypes in haemophilic individuals with unapparent HCV mixed infections. *Haemophilia.* 2008;**14**(4):816–22. doi: 10.1111/j.1365-2516.2008.01773.x. [PubMed: 18505462]
28. Castillo I, Bartolome J, Quiroga JA, Carreno V. High prevalence of occult hepatitis C virus infection in patients with chronic hepatitis B virus infection. *J Med Microbiol.* 2013;**62**(Pt 8):1235–8. doi: 10.1099/jmm.0.058297-0. [PubMed: 23639983]

29. Honarkar Z, Alavian SM, Samiee S, Saeedfar K, Zali MR. Occult hepatitis B among chronic liver disease patients. *Saudi Med J.* 2005;**26**(4):601–6. [PubMed: 15900369]
30. Seeff LB, Hollinger FB, Alter HJ, Wright EC, Cain CM, Buskell ZJ, et al. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: A National Heart, Lung, and Blood Institute collaborative study. *Hepatology.* 2001;**33**(2):455–63. doi: 10.1053/jhep.2001.21905. [PubMed: 11172349]
31. Welker MW, Zeuzem S. Occult hepatitis C: how convincing are the current data? *Hepatology.* 2009;**49**(2):665–75. doi: 10.1002/hep.22706. [PubMed: 19105211]
32. Kodali VP, Gordon SC, Silverman AL, McCray DG. Cryptogenic liver disease in the United States: further evidence for non-A, non-B, and non-C hepatitis. *Am J Gastroenterol.* 1994;**89**(10):1836–9. [PubMed: 7942678]
33. Verslype C, Nevens F, Sinelli N, Clarysse C, Pirenne J, Depla E, et al. Hepatic immunohistochemical staining with a monoclonal antibody against HCV-E2 to evaluate antiviral therapy and reinfection of liver grafts in hepatitis C viral infection. *J Hepatol.* 2003;**38**(2):208–14. [PubMed: 12547410]
34. Halfon P, Bourliere M, Ouzan D, Sene D, Saadoun D, Khiri H, et al. Occult hepatitis C virus infection revisited with ultrasensitive real-time PCR assay. *J Clin Microbiol.* 2008;**46**(6):2106–8. doi: 10.1128/JCM.00345-08. [PubMed: 18448695]
35. Zaghloul H, El-Sherbiny W. Detection of occult hepatitis C and hepatitis B virus infections from peripheral blood mononuclear cells. *Immunol Invest.* 2010;**39**(3):284–91. doi: 10.3109/08820131003605820. [PubMed: 20380524]
36. Idrees M, Lal A, Malik FA, Hussain A, Rehman I, Akbar H, et al. Occult hepatitis C virus infection and associated predictive factors: the Pakistan experience. *Infect Genet Evol.* 2011;**11**(2):442–5. doi: 10.1016/j.meegid.2010.12.004. [PubMed: 21184846]
37. Makvandi M, Khalafkhany D, Rasti M, Neisi N, Omidvarinia A, Mirghaed AT, et al. Detection of Hepatitis C virus RNA in peripheral blood mononuclear cells of patients with abnormal alanine transaminase in Ahvaz. *Indian J Med Microbiol.* 2014;**32**(3):251–5. doi: 10.4103/0255-0857.136553. [PubMed: 25008816]
38. Rostami Z, Lessan Pezeshki M, Soleimani Najaf Abadi A, Einollahi B. Health Related Quality of Life in Iranian Hemodialysis Patients With Viral Hepatitis: Changing Epidemiology. *Hepat Mon.* 2013;**13**(6):e9611. doi: 10.5812/hepatmon.9611. [PubMed: 24032050]
39. Hooda A, Varma P, Chopra G, Kaur J. High prevalence of hepatitis C virus-ribonucleic acid positivity in anti-hepatitis C virus negative renal transplant patients. *Med J Armed Forces India.* 2012;**68**(2):123–8. doi: 10.1016/s0377-1237(12)60023-9. [PubMed: 24669050]
40. Nicot F, Kamar N, Mariame B, Rostaing L, Pasquier C, Izopet J. No evidence of occult hepatitis C virus (HCV) infection in serum of HCV antibody-positive HCV RNA-negative kidney-transplant patients. *Transpl Int.* 2010;**23**(6):594–601. doi: 10.1111/j.1432-2277.2009.01025.x. [PubMed: 20002658]
41. Thongsawat S, Maneekarn N, Kuniholm MH, Pantip C, Thungsuputi A, Lumlertkul D, et al. Occult hepatitis C virus infection during an outbreak in a hemodialysis unit in Thailand. *J Med Virol.* 2008;**80**(5):808–15. doi: 10.1002/jmv.21126. [PubMed: 18360894]
42. Baid-Agrawal S, Schindler R, Reinke P, Staedtler A, Rimpler S, Malik B, et al. Prevalence of occult hepatitis C infection in chronic hemodialysis and kidney transplant patients. *J Hepatol.* 2014;**60**(5):928–33. doi: 10.1016/j.jhep.2014.01.012. [PubMed: 24447875]
43. Barril G, Castillo I, Arenas MD, Espinosa M, Garcia-Valdecasas J, Garcia-Fernandez N, et al. Occult hepatitis C virus infection among hemodialysis patients. *J Am Soc Nephrol.* 2008;**19**(12):2288–92. doi: 10.1681/ASN.2008030293. [PubMed: 18684893]
44. Jain P, Nijhawan S. Occult hepatitis C virus infection is more common than hepatitis B infection in maintenance hemodialysis patients. *World J Gastroenterol.* 2008;**14**(14):2288–9. [PubMed: 18407613]
45. Yakaryilmaz F, Gurbuz OA, Guliter S, Mert A, Songur Y, Karakan T, et al. Prevalence of occult hepatitis B and hepatitis C virus infections in Turkish hemodialysis patients. *Ren Fail.* 2006;**28**(8):729–35. doi: 10.1080/08860220600925602. [PubMed: 17162434]
46. Eslamifar A, Ramezani A, Ehteram H, Razeghi E, Ahmadi F, Amini M, et al. Occult hepatitis C virus infection in Iranian hemodialysis patients. *J Nephropathol.* 2015;**4**(4):116–20. doi: 10.12860/jnp.2015.22. [PubMed: 26457258]
47. Sugden PB, Pham TN, Ratnarajah S, Cameron B, Bull R, White PA, et al. Rare occurrence of occult hepatitis C virus in apparently uninfected injecting drug users: a two-centre, masked, case-control study. *J Viral Hepat.* 2013;**20**(10):725–8. doi: 10.1111/jvh.12098. [PubMed: 24010647]
48. Sugden PB, Cameron B, Bull R, White PA, Lloyd AR. Occult infection with hepatitis C virus: friend or foe? *Immunol Cell Biol.* 2012;**90**(8):763–73. doi: 10.1038/icb.2012.20. [PubMed: 22546735]
49. Post JJ, Pan Y, Freeman AJ, Harvey CE, White PA, Palladinetti P, et al. Clearance of hepatitis C viremia associated with cellular immunity in the absence of seroconversion in the hepatitis C incidence and transmission in prisons study cohort. *J Infect Dis.* 2004;**189**(10):1846–55. doi: 10.1086/383279. [PubMed: 15122521]
50. Rai RR, Mathur A, Mathur D, Udawat HP, Nepalia S, Nijhawan S. Prevalence of occult hepatitis B & C in HIV patients infected through sexual transmission. *Trop gastroenterol (official journal of the Digestive Diseases Foundation).* 2006;**28**(1):19–23.
51. Pisaturo M, Guastafierro S, Filippini P, Tonziello G, Sica A, Di Martino F, et al. Absence of occult HCV infection in patients experiencing an immunodepression condition. *Infez Med.* 2013;**21**(4):296–301. [PubMed: 24335460]
52. Gatserelia L, Sharvadze L, Karchava M, Dolmazashvili E, Tsertsvadze T. Occurrence of occult HCV infection among Hiv infected patients in Georgia. *Georgian Med News.* 2014;(226):37–41. [PubMed: 24523330]
53. El Shazly Y, Hemida K, Rafik M, Al Swaff R, Ali-Eldin ZA, GadAllah S. Detection of occult hepatitis C virus among healthy spouses of patients with HCV infection. *J Med Virol.* 2015;**87**(3):424–7. doi: 10.1002/jmv.24074. [PubMed: 25185940]
54. Castillo I, Bartolome J, Quiroga JA, Barril G, Carreno V. Hepatitis C virus infection in the family setting of patients with occult hepatitis C. *J Med Virol.* 2009;**81**(7):1198–203. doi: 10.1002/jmv.21483. [PubMed: 19475603]
55. De Marco L, Manzini P, Trevisan M, Gillio-Tos A, Danielle F, Balloco C, et al. Prevalence and follow-up of occult HCV infection in an Italian population free of clinically detectable infectious liver disease. *PLoS One.* 2012;**7**(8):e43541. doi: 10.1371/journal.pone.0043541. [PubMed: 22457823]
56. Ashrafi Hafez A, Baharlou R, Mousavi Nasab SD, Ahmadi Vasmehjani A, Shayestehpour M, Joharinia N, et al. Molecular epidemiology of different hepatitis C genotypes in serum and peripheral blood mononuclear cells in jahrom city of iran. *Hepat Mon.* 2014;**14**(5):e16391. doi: 10.5812/hepatmon.16391. [PubMed: 24976833]
57. Hartleb M, Gutkowski K, Zejda JE, Chudek J, Wiecek A. Serological prevalence of hepatitis B virus and hepatitis C virus infection in the elderly population: Polish nationwide survey–PolSenior. *Euro j gastroenterol hepatol.* 2012;**24**(11):1288–95. doi: 10.1097/meg.0b013e328357632a.
58. Nagao Y, Kawahigashi Y, Sata M. Association of Periodontal Diseases and Liver Fibrosis in Patients With HCV and/or HBV infection. *Hepat Mon.* 2014;**14**(12):e23264. doi: 10.5812/hepatmon.23264. [PubMed: 25737729]
59. Quiroga JA, Llorente S, Castillo I, Rodriguez-Inigo E, Pardo M, Carreno V. Cellular immune responses associated with occult hepatitis C virus infection of the liver. *J Virol.* 2006;**80**(22):10972–9. doi: 10.1128/JVI.00852-06. [PubMed: 17071928]
60. Pham TN, Mercer SE, Michalak TI. Chronic hepatitis C and persistent occult hepatitis C virus infection are characterized by distinct immune cell cytokine expression profiles. *J Viral Hepat.* 2009;**16**(8):547–56. doi: 10.1111/j.1365-2893.2009.01092.x. [PubMed: 19215578]
61. Tsuda N, Yuki N, Mochizuki K, Nagaoka T, Yamashiro M, Omura M, et al. Long-term clinical and virological outcomes of chronic hepatitis C after successful interferon therapy. *J Med Virol.* 2004;**74**(3):406–13. doi: 10.1002/jmv.20190. [PubMed: 15368524]
62. Besharat S, Katoonizadeh A, Moradi A. Potential mutations associated with occult hepatitis B virus status. *Hepat Mon.* 2014;**14**(5):e15275. doi: 10.5812/hepatmon.15275. [PubMed: 24829588]

63. Pham TN, Coffin CS, Churchill ND, Urbanski SJ, Lee SS, Michalak TI. Hepatitis C virus persistence after sustained virological response to antiviral therapy in patients with or without past exposure to hepatitis B virus. *J Viral Hepat.* 2012;**19**(2):103–11. doi: 10.1111/j.1365-2893.2011.01442.x. [PubMed: 21699630]
64. Pham TN, King D, Macparland SA, McGrath JS, Reddy SB, Bursey FR, et al. Hepatitis C virus replicates in the same immune cell subsets in chronic hepatitis C and occult infection. *Gastroenterology.* 2008;**134**(3):812–22. doi: 10.1053/j.gastro.2007.12.011. [PubMed: 18243182]
65. Castillo I, Rodriguez-Inigo E, Bartolome J, de Lucas S, Ortiz-Movilla N, Lopez-Alcorocho JM, et al. Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *Gut.* 2005;**54**(5):682–5. doi: 10.1136/gut.2004.057281. [PubMed: 15831916]
66. Carreno V, Bartolome J, Castillo I, Quiroga JA. New perspectives in occult hepatitis C virus infection. *World J Gastroenterol.* 2012;**18**(23):2887–94. doi: 10.3748/wjg.v18.i23.2887. [PubMed: 22736911]
67. Castillo I, Bartolome J, Quiroga JA, Barril G, Carreno V. Long-term virological follow up of patients with occult hepatitis C virus infection. *Liver Int.* 2011;**31**(10):1519–24. doi: 10.1111/j.1478-3231.2011.02613.x. [PubMed: 22093326]
68. Pham TN, Coffin CS, Michalak TI. Occult hepatitis C virus infection: what does it mean? *Liver Int.* 2010;**30**(4):502–11. doi: 10.1111/j.1478-3231.2009.02193.x. [PubMed: 20070513]
69. Roque-Cuellar MC, Sanchez B, García-Lozano JR, Praena-Fernandez JM, Márquez-Galan JL, Núñez-Roldan A, et al. Hepatitis C virus-specific cellular immune responses in sustained virological responders with viral persistence in peripheral blood mononuclear cells. *Liver Int.* 2014;**34**(6):e80–8. [PubMed: 24127783]
70. Gad YZ, Ahmad NA, Mousa N, Farag RE, Abdel-Aziz AA, Abousmra NM, et al. Occult hepatitis C infection: the prevalence and profile of its immunoregulatory cytokines. *Egypt Liver J.* 2012;**2**(4):108–12. doi: 10.1097/01.ELX.0000419586.77734.0d.
71. Mousa N, Eldars W, Eldegla H, Fouda O, Gad Y, Abousamra N, et al. Cytokine profiles and hepatic injury in occult hepatitis C versus chronic hepatitis C virus infection. *Int J Immunopathol Pharmacol.* 2014;**27**(1):87–96. [PubMed: 24674682]
72. Carreno V. Occult hepatitis C virus infection: a new form of hepatitis C. *World J Gastroenterol.* 2006;**12**(43):6922–5. [PubMed: 17109511]
73. Quiroga JA, Castillo I, Bartolome J, Carreno V. Serum immunoglobulin G antibodies to the GOR autoepitope are present in patients with occult hepatitis C virus (HCV) infection despite lack of HCV-specific antibodies. *Clin Vaccine Immunol.* 2007;**14**(10):1302–6. doi: 10.1128/CVI.00128-07. [PubMed: 17699833]
74. Quiroga JA, Castillo I, Llorente S, Bartolome J, Barril G, Carreno V. Identification of serologically silent occult hepatitis C virus infection by detecting immunoglobulin G antibody to a dominant HCV core peptide epitope. *J Hepatol.* 2009;**50**(2):256–63. doi: 10.1016/j.jhep.2008.08.021. [PubMed: 19070391]
75. Pardo M, Lopez-Alcorocho JM, Castillo I, Rodriguez-Inigo E, Perez-Mota A, Carreno V. Effect of anti-viral therapy for occult hepatitis C virus infection. *Aliment Pharmacol Ther.* 2006;**23**(8):1153–9. doi: 10.1111/j.1365-2036.2006.02886.x. [PubMed: 16611276]
76. Rezaee-Zavareh MS, Einollahi B. Treatment of occult hepatitis C virus infection: does it need special attention? *Hepat Mon.* 2014;**14**(7):e16665. doi: 10.5812/hepatmon.16665. [PubMed: 25067939]
77. Alavian SM. Sofosbuvir has come out of the magic box. *Hepat Mon.* 2013;**13**(12):e16916. doi: 10.5812/hepatmon.16916. [PubMed: 24358046]
78. Pourhoseingholi MA, Ashtari S, Alavian SM. Sofosbuvir vs. Combination of Pegylated Interferon and Ribavirin; How Much Shall Pay for Iranian Patients? *Hepat Mon.* 2014;**14**(11):e25540. doi: 10.5812/hepatmon.25540. [PubMed: 25598793]
79. Gentile I, Buonomo AR, Zappulo E, Borgia G. Interferon-free therapies for chronic hepatitis C: toward a hepatitis C virus-free world? *Expert Rev Anti Infect Ther.* 2014;**12**(7):763–73. doi: 10.1586/14787210.2014.929497. [PubMed: 24918116]