REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Occult Hepatitis C Virus Infection: Is it time to get more attention!?

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Abstract

Occult hepatitis C virus infection (OCI) is broadly described as the presence of HCV-RNA in hepatocytes and/or peripheral blood mononuclear cells (PBMCs) in individuals who are HCV-RNA negative in serum by traditional diagnostic techniques. Based on the presence or absence of anti-HCV in serum, two forms of OCI are distinguished as seronegative (anti-HCV and serum HCV-RNA negative) and seropositive (anti-HCV positive and serum HCV-RNA negative). This review aims to estimate the global prevalence rate of OCI among a certain population in terms of disease epidemiology, method of transmission, infection pattern, progression, and treatment. Occult HCV infection is likely linked to liver fibrosis and disease progression. More research is needed to understand the infectivity of OCI patients and the natural course and particular clinical consequences of OCI. It will be interesting to know if direct-acting antiviral (DAA) medications efficiently eliminate HCV RNA in PBMCs or hepatocytes. Finally, some effective OCI screening strategies are advised to target people at risk of HCV infection.

Keywords: Occult HCV; peripheral blood mononuclear cells; RNA.

1. Introduction

Hepatitis C virus (HCV) is a major cause of liver disease globally, causing chronic viral hepatitis that commonly progresses to liver cirrhosis and hepatocellular carcinoma (HCC). It affects 71 million people worldwide and kills 400 000 people each year **(An et al., 2020)**. In 2015, the World Health Organization established a global health sector strategy on viral hepatitis and established some service coverage targets, such as diagnosing 90 percent of people with chronic hepatitis C and treating 80 percent of diagnosed cases to eliminate hepatitis C as a public health concern by 2030 **(Hedayati-Moghaddam et al., 2021)**.

A new form of HCV infection has been reported in which people have no detectable HCV RNA in their serum but have HCV RNA in their hepatocytes, peripheral blood mononuclear cells (PBMCs), or both **(An et al., 2020)**. This mysterious type of infection is referred to as occult hepatitis C infection (OCI) **(An et al., 2020)**. OCI was first described in 2004 by Castillo et al. in patients with cryptogenic chronic hepatitis and abnormal liver function tests who were anti-HCV negative by different commercial assays and serum HCV-RNA negative by standard PCR but had HCV-RNA in the liver and possibly viral RNA in PBMCs **(An et al., 2020)**. OCI is also defined as the presence of HCV-RNA in hepatocytes and/or PBMCs in individuals who are HCV-RNA negative in serum by traditional diagnostic techniques. Based on the presence or absence of anti-HCV in serum; two forms of OCI are distinguished as seronegative

(anti-HCV and serum HCV-RNA negative) and seropositive (anti-HCV positive and serum HCV-RNA negative) **(Wróblewska et al., 2021)**.

It is uncertain why HCV RNA is not detected in the serum of OCI patients. According to one theory, the quantity of circulating virus particles in OCI patients is too low to be identified using standard molecular methods. It is also unknown why these people do not acquire antiviral antibodies. Occult infection may be caused by mutant HCV strains that produce antibodies that aren't detectable by conventional serological testing **(Helaly et al., 2017)**. The lack of a standardized definition of OCI reflects the general confusion in classifying OCI as an illness, which may lead to OCI misdiagnosis in clinical settings.

2. Infectivity

HCV proteins coordinate membrane rearrangements inside the host cell, leading to viral replication organelles, which are largely constituted of double-membrane vesicles **(Elmasry et al., 2017)**.

HCV is a single-stranded RNA molecule with a positive-strand that belongs to the Flaviviridae family. The creation of a complementary strand of RNA, known as the negative-strand HCV RNA, is required for virus replication. The negative HCV RNA strand is thought to indicate continued viral replication **(Austria & Wu, 2018)**. According to a recent study, HCV-positive and negative RNA strands remain linked to producing double-stranded RNA (dsRNA). Semiconservative replication reproduces this dsRNA numerous times to generate multiple progenies; specifically, positive-strand viral RNA genomes are present in a 5- to 10-fold molar excess over negative-strand RNA genomes. Excess positive-strand HCV RNA promotes viral protein translation, viral genome encapsidation, and virion formation. Elmasry and her colleagues discovered comparable levels of both positive and negative HCV RNA strands **(Figure 1) (Koutsoudakis et al., 2017)**.

3. Progression

OCI was a milder form of HCV than chronic HCV, with fewer OCI patients demonstrating necroinflammation and fibrosis in the liver. This might be because OCI has fewer infected hepatocytes and less efficient HCV replication than chronic HCV **(Wróblewska et al., 2021).**

An in vitro investigation found that T cells from seronegative OCI patients produced stronger HCV-specific responses than their chronic hepatitis C counterparts. This is thought to be caused by immunosurveillance without humoral immunity. These data, taken together, suggest the possibility of a distinct host-virus interaction in OCI. Furthermore, in a limited retrospective analysis of eight patients with hepatocellular carcinoma, HCV RNA was found inside the carcinoma of two patients who tested negative for serum HCV RNA and anti-HCV, raising the likelihood of hepatocellular carcinoma as an OCI consequence **(An et al., 2020)**.

4. Epidemiology

Hedayati-Moghaddam et al. (2021) Stated that 37 studies with a total of 5200 individuals from Egypt, Iran, Pakistan, Saudi Arabia, and Turkey were examined. OCI has a pooled prevalence incidence of 10.04 percent. The pooled rate in healthy people was 4.79 percent, but it was substantially higher in patients with hematologic disorders (19.57 percent), HIV-positive individuals (12.95 percent), chronic liver disease patients (12.04 percent), patients with lymphoproliferative disorders (13.79) and multi-transfused patients (8.71 percent). The rate of OCI was not substantially associated with the nation, illness subpopulations, year of study, techniques used for HCV RNA detection, sample size, patients' HCV serostatus and gender, and no significant change in the OCI rate over time **(Hedayati-Moghaddam et al., 2021)**.

HCV genotyping was also investigated, and OCI was identified in genotypes 1a, 2b, 3b, 4, and 6a, implying that OCI may be a global problem given the different geographical predominance of each of these genotypes **(Lin et al., 2016; Nasimzadeh et al., 2021)**.

5. Populations at risk

5.1. Dialysis patients

Hepatitis C is a leading cause of chronic liver disease in people with end-stage renal disease (ESRD). Hepatitis C is more common among dialysis patients, with several studies indicating rates ranging from less than 5% to 60%, depending on geographical area. The prevalence of HCV has reduced due to the deployment of routine screening as well as efforts designed to prevent its spread. However, because existing screening methods do not discover OCI, it is possible that it is under-

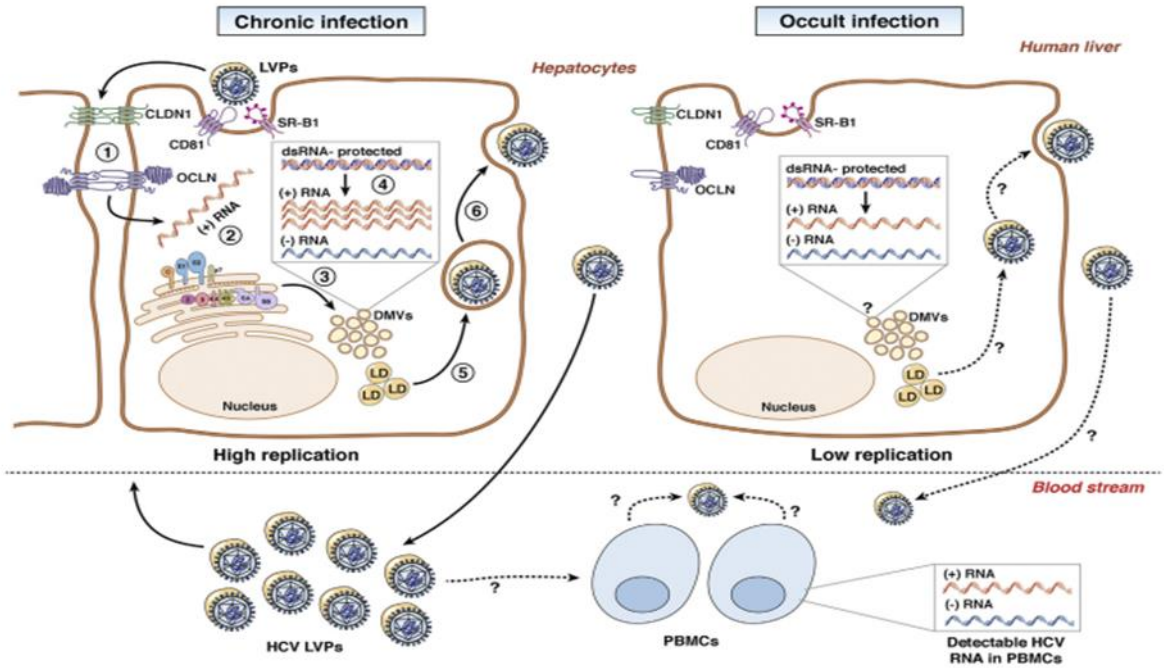

Figure 1. Chronic versus occult HCV infection model **(Koutsoudakis et al., 2017)**. HCV virions circulate in the bloodstream in combination with lipoproteins (LVPs) during chronic infection. HCV RNA is translated in the rough endoplasmic reticulum after entrance (step 1) and uncoating to produce the viral polyprotein, which is then cleaved into mature proteins (step 2). In cooperation with host factors, viral proteins enhance the development of intracellular membrane alterations that comprise the membranous web (step 3). The major elements of the membranous web in which HCV RNA replication proceeds are double-membrane vesicles (DMVs). The (þ) RNA acts as a template for the production of a negative-sense copy of the genome [(-) RNA], and these molecules will most likely form a double strand RNA (dsRNA). DMVs may shield dsRNA from detection by dsRNA sensors (TLR3, MDA5, and RIG-I). The dsRNA then acts as a template for the generation of progeny positive- and negative-sense copies of the genome (step 4). Excess positive-strand genomes ensure that these molecules egress to encapsidation sites (lipid droplets), where new virions are likely to be assembled (step 5). Finally, the newly produced virions bind to lipoproteins and are released via the constitutive secretory channel (step 6), where they can infect more hepatocytes and perhaps PBMCs. During occult infection (right). Inadequate IFN-mediated responses may explain some of the low-level HCV RNA persistence. Alternatively, HCV RNA may stay protected inside hepatocyte DMVs or in an undiscovered cell compartment, or inside PMBCs; the lack of particle formation may be explained by an altered ratio of (þ) vs (-) strand HCV RNA. MDA5, melanoma differentiation-associated gene 5; OCLN, occludin; PBMCs, peripheral blood mononuclear cells; RIG-I, retinoic acid-inducible gene-I; SR-B1, scavenger receptor class B type 1; TLR3, Toll-like receptor 3.

recognized and is to blame for the continuous spread of HCV **(Austria & Wu, 2018)**.

5.2. Glomerular nephropathies

HCV is linked to a wide range of renal disorders, and there is evidence that occult HCV may be involved in the pathogenesis of immune-mediated glomerulonephritis. In 2014, Castillo et al. discovered that 39% of patients with immune-mediated glomerulonephritis tested positive for OCI. Furthermore, individuals with occult HCV had a considerably lower renal function and were more likely to advance to ESRD than those who did not have occult HCV. Classical HCV-associated glomerular illness is assumed to be caused by the deposition of HCV immune complexes in glomeruli, which is not necessarily the pathogenic process in OCI. On the other hand, Occult HCV may play a direct pathogenic role **(Austria & Wu, 2018)**.

5.3. Post sustained Virologic Response (SVR) patients

Many findings showed that OCI might be present in many people who had essentially "clean" the virus from their serum, either naturally or after antiviral therapy. Identification of indications of persistent infection, such as HCV RNA in PBMCs (especially the negative-sense strand) following apparent sustained virologic response (SVR) after direct acting antiviral (DAA) therapy, maybe a significant diagnostic tool of non-cure that elevates the possibility of subsequent recurrence **(Mekky et al., 2019)**.

5.4. Patients with hematological disorders

In 2017, **Helaly et al. (2017)** observed that seronegative OCI in patients with hematological disorders was 36% among studied patients. As a result, seronegative OCI represents significant clinical problems in patients with hematological disorders, justifying the wider use of molecular tests for diagnostic purposes. This method should be paired with regular evaluations of liver-function tests in order to reduce the incidence of transmission of this virus **(Helaly et al., 2017)**.

5.5. Other high-risk categories

Three surveys among 417 HCV-seronegative and seropositive samples demonstrated OCI prevalence in HIV-positive persons. All investigations were done in Iran after 2014 to assess HCV RNA in PBMCs. The pooled mean prevalence of OCI among this cohort was estimated to be 12.95 percent **(Bokharaei-Salim et al., 2016; Donyavi et al., 2019; Jamshidi et al., 2020)**.

In terms of occult hepatitis C in injection drug users (IDUs), one study recently discovered HCV RNA in PBMCs in 18.18 percent of 77 Iranian HIV-positive IDUs (Donyavi et al., 2019). Furthermore, another Iranian study found an OCI incidence of 9.57 percent among 115 HBV- and HIV-negative IDUs **(Sheikh et al., 2019)**. Both surveys found HCV genomes in HCV seronegative and seropositive samples.

Regarding the prevalence of OCI in patients with lymphoproliferative disorders, one study occurs in Iran discovered the presence of HCV RNA in PBMCs in 1.92 percent of 104 Iranian patients **(Farahani et al., 2013)**, and another study performed in Egypt found the OCI existence of 13.79 percent among studied patients, and all confirmed OCI were Non-Hodgkin lymphoma (NHL) type **(Youssef et al., 2012)**.

6. Transmission

The route and extent of OCI transmission remain limited. A study of intrafamilial transmission showed that OCI and chronic Hepatitis C patients could transmit HCV to family members, which signified the possibility of OCI transmission via close proximal contact. Specifically, 9.8 percent of family members of seronegative OCI patients were found to be plasma HCV RNA-positive and/or anti-HCV positive compared to the 3.4 percent transmission from the chronic hepatitis C patients **(An et al., 2020)**.

7. Diagnosis

Identifying HCV RNA in biopsied liver hepatocytes by nucleic acid amplification testing (NAT) is the gold standard for OCI diagnosis. However, this procedure is invasive, has several risks, and is not widely available; alternate diagnostic methods for OCI detection have been developed. A NAT test that detects HCV RNA in PBMCs or serum is the most often used approach. Compared to liver biopsy, these OCI diagnostic procedures had 57 percent sensitivity for ultra-centrifuged serum, 61 percent for PBMCs, and 85 percent when combined with the two methods **(An et al., 2020)**.

8. Treatment

In contrast to chronic HCV, the best therapy for OCI has yet to be determined. Early investigations looked into OCI therapy using an interferon and ribavirin regimen, which reduced detectable HCV RNA in PBMCs and adjusted abnormal alanine aminotransferase (ALT) levels **(An et al., 2020)**.

There is a lack of research on OCI treatment combined with DAA therapy (direct-acting antiviral). In post-SVR patients with prior chronic hepatitis C, HCV RNA can persist for years, and the presence of HCV RNA in PBMCs is a predictor of serological relapse (Abd Alla et al., 2018). As a result, it will be interesting to see if DAA effectively eradicates HCV RNA in PBMCs or hepatocytes in cases of OCI and if this impacts reduce serologically and, more importantly clinical relapse **(An et al., 2020)**.

9. Conclusion

Data from the past two decades show a new worldwide phenomenon of OCI that differs and is comparable to HCV infection. With breakthroughs in HCV therapy, it is critical to know whether more patients are entering an OCI condition with a high risk of future relapse. Future research should focus on improving identification in at-risk individuals or people with undiagnosed liver disease and investigating the danger of OCI transmission by blood transfusion, particularly in endemic regions.

10. Recommendations

More suitable OCI screening programs target those at high risk of HCV infection, particularly dialysis

patients are required. Additionally, further research is needed on OCI in other risk populations, including HIV- and HBV-infected individuals, IDUs, and thalassemia and hemophilia patients.

11. Conflict of interest

None of the authors have any conflicts of interest.

References:

Abd Alla, M. D. A., El Awady, M. K., Dawood, R. M., Elhawary, M. A., Al-Azhari, S. S., & Galal, A.-S. G. M. (2018). Hepatitis C virus serologic relapse after treatment with direct-acting antivirals is dependent on viral RNA levels in peripheral blood mononuclear cells and the grade of liver cirrhosis. Archives of Virology 2018 163:10, 163(10), 2765–2774. https://doi.org/10.1007/S00705-018-3922-7

An, T., Dean, M., Flower, R., Tatzenko, T., Chan, H. T., Kiely, P., & Faddy, H. M. (2020). Understanding occult hepatitis C infection. Transfusion, 60(9), 2144–2152. https://doi.org/10.1111/TRF.16006

Austria, A., & Wu, G. Y. (2018). Occult hepatitis C virus infection: A review. Journal of Clinical and Translational Hepatology, 6(2), 155–160. https://doi.org/10.14218/JCTH.2017.00053

Bokharaei-Salim, F., Keyvani, H., Esghaei, M., Zare-Karizi, S., Dermenaki-Farahani, S. S., Hesami-Zadeh, K., & Fakhim, S. (2016). Prevalence of occult hepatitis C virus infection in the Iranian patients with human immunodeficiency virus infection. Journal of Medical Virology, 88(11), 1960–1966. https://doi.org/10.1002/JMV.24474

Donyavi, T., Bokharaei-Salim, F., Khanaliha, K., Sheikh, M., Bastani, M. N., Moradi, N., Babaei, R., Habib, Z., Fakhim, A., & Esghaei, M. (2019). High prevalence of occult hepatitis C virus infection in injection drug users with HIV infection. Archives of Virology, 164(10), 2493–2504. https://doi.org/10.1007/S00705-019-04353-3/TABLES/4

Elmasry, S., Wadhwa, S., Bang, B. R., Cook, L., Chopra, S., Kanel, G., Kim, B., Harper, T., Feng, Z., Jerome, K. R., Kahn, J. A., & Saito, T. (2017). Detection of Occult Hepatitis C Virus Infection in Patients Who Achieved a Sustained Virologic Response to Direct-Acting Antiviral Agents for Recurrent Infection After Liver Transplantation. Gastroenterology, 152(3), 550-553.e8. https://doi.org/10.1053/J.GASTRO.2016.11.002

Farahani, M., Bokharaei-Salim, F., Ghane, M., Basi, A., Meysami, P., & Keyvani, H. (2013). Prevalence of occult hepatitis C virus infection in Iranian patients with lymphoproliferative disorders. Journal of Medical Virology, 85(2), 235–240. https://doi.org/10.1002/JMV.23460

Hedayati-Moghaddam, M. R., Soltanian, H., & Ahmadi-Ghezeldasht, S. (2021). Occult hepatitis C virus infection in the Middle East and Eastern Mediterranean countries: A systematic review and meta-analysis. World Journal of Hepatology, 13(2), 242–260. https://doi.org/10.4254/WJH.V13.I2.242

Helaly, G. F., Elsheredy, A. G., El Basset Mousa, A. A., Ahmed, H. K. F., & Oluyemi, A. E. G. S. (2017). Seronegative and occult hepatitis C virus infections in patients with hematological disorders. Archives of Virology, 162(1), 63–69. https://doi.org/10.1007/S00705-016-3049-7

Jamshidi, S., Bokharaei-Salim, F., Esghaei, M., Bastani, M. N., Garshasbi, S., Chavoshpour, S., Dehghani-Dehej, F., Fakhim, S., & Khanaliha, K. (2020). Occult HCV and occult HBV coinfection in Iranian human immunodeficiency virus-infected individuals. Journal of Medical Virology, 92(12), 3354–3364. https://doi.org/10.1002/JMV.25808

Koutsoudakis, G., Pérez-del-Pulgar, S., & Forns, X. (2017). Occult Hepatitis C Virus Infection: Are We Digging Too Deep? Gastroenterology, 152(3), 472–474. https://doi.org/10.1053/J.GASTRO.2016.12.015

Lin, H., Chen, X., Zhu, S., Mao, P., Zhu, S., Liu, Y., Huang, C., Sun, J., & Zhu, J. (2016). Prevalence of Occult Hepatitis C Virus Infection among Blood Donors in Jiangsu, China. Intervirology, 59(4), 204–210. https://doi.org/10.1159/000455854

Mekky, M. A., Sayed, H. I., Abdelmalek, M. O., Saleh, M. A., Osman, O. A., Osman, H. A., Morsy, K. H., & Hetta, H. F. (2019). Prevalence and predictors of occult hepatitis C virus infection among Egyptian patients who achieved sustained virologic response to sofosbuvir/daclatasvir therapy: A multi-center study. Infection and Drug Resistance, 12, 273–279. https://doi.org/10.2147/IDR.S181638

Nasimzadeh, S., Azaran, A., Jalilian, S., Makvandi, M., Seyedian, S. S., keikhaei, B., & Mehr, F. J. (2021). Prevalence of occult hepatitis C virus infection in beta-thalassemia major patients in Ahvaz, Iran. Archives of Virology, 166(10), 2703–2710. https://doi.org/10.1007/S00705-021-05126-7/FIGURES/2

Sheikh, M., Bokharaei-Salim, F., Monavari, S. H., Ataei-Pirkooh, A., Esghaei, M., Moradi, N., Babaei, R., Fakhim, A., & Keyvani, H. (2019). Molecular diagnosis of occult hepatitis C virus infection in Iranian injection drug users. Archives of Virology, 164(2), 349–357. https://doi.org/10.1007/S00705-018-4066-5

Wróblewska, A., Bielawski, K. P., Sikorska, K., Cruz-Martins, N., & Rodrigues, C. F. (2021). Occult Infection with Hepatitis C Virus: Looking for Clear-Cut Boundaries and Methodological Consensus. Journal of Clinical Medicine 2021, Vol. 10, Page 5874, 10(24), 5874. https://doi.org/10.3390/JCM10245874

Youssef, S. S., Nasr, A. S., El Zanaty, T., El Rawi, R. S., & Mattar, M. M. (2012). Prevalence of Occult Hepatitis C Virus in Egyptian Patients with Chronic Lymphoproliferative Disorders. Hepatitis Research and Treatment, 2012, 1–6. https://doi.org/10.1155/2012/429784