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Global prevalence of occult hepatitis C virus, a systematic review and meta-analysis

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Abstract

BACKGROUND

Occult hepatitis C infection (OCI) is characterized by the presence of hepatitis C virus (HCV) RNA in liver, peripheral blood mononuclear cells (PBMC) and/or ultracentrifugated serum in the absence of detectable HCV-RNA in serum. OCI have been described in several categories of populations including hemodialysis patients, patients with a sustained virological response, immunocompromised individuals, patients with abnormal hepatic function, and apparently healthy subjects.

AIM

To highlight the global prevalence of OCI in the World.

METHODS

We performed a systematic and comprehensive literature search in 4 electronic databases PubMed, Embase, Global Index Medicus, and Web of Science until 6th May 2021 to retrieve relevant studies published in the field. Included studies were unrestricted population categories with known RNA status in serum, PBMC, liver tissue and/or ultracentrifuged serum. Data were extracted independently by each author and the Hoy *et al* tool was used to assess the quality of the included studies. We used random-effect meta-analysis model to estimate proportions of OCI and their 95% confidence intervals (95%CI). The Cochran's Q-test and the I^2 test statistics were used to assess heterogeneity between studies. Funnel plot and Egger test were used to examine Publication bias. R software version 4.1.0 was used to perform all analyses.

RESULTS

The electronic search gave 3950 articles. We obtained 102 prevalence data from 85 included studies. The pooled prevalence of seronegative OCI was estimated to be 9.61% [95%CI = 6.84-12.73] with substantial heterogeneity (I^2= 94.7% [95%CI = 93.8%-95.4%], p<0.0001). Seropositive OCI prevalence was estimated to be 13.39% [95%CI = 7.85-19.99] with substantial heterogeneity (I^2 = 93.0% [90.8%-94.7%]). Higher seronegative OCI prevalence was in Southern Europe and Northern Africa and in patients with abnormal liver function, hematological disorders, and kidney diseases. Higher seropositive OCI prevalence was in Southern Europe, Northern America, and Northern Africa.

CONCLUSION

In conclusion, in the present study, it appears that the burden of OCI is high and variable across the different regions and population categories. Further studies on OCI are needed to assess their transmissibility, clinical significance, long term outcome, and need for treatment.
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Key Words: Occult hepatitis C virus infection; Prevalence; Worldwide

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Core Tip: Overall, this study showed that the burden of seropositive and seronegative OCIs are high and variable in different regions and population categories. Patients with hematological disorders, kidney diseases, and abnormal liver function showed the highest OCI prevalence.

INTRODUCTION

In 2019, the World Health Organization (WHO) estimated that 58 million people are living with hepatitis C virus (HCV) [1], making HCV infection a major global public health problem [2, 3]. Each year, more than 1.5 million people around the world are newly infected with HCV [4] and more than 290,000 people die from it [5]. HCV infection is increasingly affecting healthcare and particularly in highly endemic areas [3]. The prevalence of HCV varies greatly between regions with a range from 0.2 to 20% in the general population [6]. HCV infection can lead to liver cirrhosis (10-20% of cases) and hepatocellular carcinoma (HCC) (1-5% of cases) [7].

The principal multiplication site for HCV is hepatocytes, but evidence of HCV replication has been reported in peripheral blood mononuclear cells (PBMC) and other extrahepatic organs [8, 9].

In 2004, occult hepatitis C infection (OCI) was first described by Castillo *et al* [10]. This new form of hepatitis is defined as the absence of RNA in serum and its presence in hepatocytes, peripheral blood mononuclear cells (PBMC) or ultracentrifuged serum [3, 11-

13]. The OCI is further classified as seronegative OCI in subjects who are anti-HCV negative and seropositive OCI for those who are anti-HCV positive [14]. Seropositive OCI individuals represent those chronically infected with HCV who have recovered (absence of RNA in serum) either spontaneously or through treatment. There are asymptomatic carriers of OCI with normal liver enzyme levels and some with abnormal liver function [10, 15-23]. The OCI can also lead to hepatic attacks including cases of liver cirrhosis and even HCC in high-risk groups [24]. The first syntheses performed at the global level and in the Middle East and the Eastern Mediterranean showed highly variable OCI prevalence (ranging from 0-89%) according to the population groups including apparently healthy individuals, patients with hematological disorders, chronic liver disease, HIV, patients who have achieved a sustained virological response (SVR), and transplant recipients [20, 25, 26]. The review conducted in the Middle East and Eastern Mediterranean revealed that high frequencies of OCI were recorded in patients with chronic liver disease, HIV, and injecting drug users [20]. In the review by Hedayati-Moghaddam *et al*, no statistically significant difference was observed in the variability of OCI prevalence across countries, patient anti-HCV status, and HCV detection method [20]. The OCI highlight multiple concerns including the potential for transmission of this form of infection through blood transfusion or hemodialysis [27]. To date, there is no global data synthesis on the prevalence of OCI in different population categories. To hope for eradication of HCV infection by 2030 as recommended by WHO, making data available on the burden of OCI is crucial [28, 29]. The objective of this systematic review and meta-analysis is to determine the global prevalence of OCI and evaluating the potential factors resulting in heterogeneity between the population groups and regions. Findings from this review may help prioritize population groups and regions most at risk for OCI screening and managing programs.

MATERIALS AND METHODS

Study design

We used the preferred reporting items for systematic reviews and meta–analyses (PRISMA) checklist to pile up the design of this systematic review (Supplementary Table 1) [30]. The systematic review was declared in the PROSPEOR international database under the number CRD42021252763.

Inclusion criteria

We included all studies without time restriction, published in peer-reviewed journal in English or in French and which fulfilled the following criteria: having a cross-sectional or case-control study design and for cohorts and clinical trials, only the baseline data were considered. We considered studies with patients of all ages tested for seropositive OCI (anti-HCV positive) and for seronegative OCI (anti-HCV negative). One study could contribute to several prevalence data that we called effect ratings. We included studies that detected HCV RNA by molecular methods in PBMCs, hepatocytes or ultracentrifuged serum [11, 15, 20, 31]. To strengthen the robustness of our estimates, we considered only studies with at least 10 participants.

Exclusion criteria

We excluded all studies that did not provide an opportunity to extract data on OCI prevalence, studies with no baseline data for longitudinal study. Case reports, studies selecting participants with an already known OCI result, comments on an article, reviews, editorials, duplicates and studies for which the full article or abstract could not be found were also excluded.

Search strategy

We performed a systematic and comprehensive literature search in 4 electronic databases: PubMed, Embase, Global Index Medicus, and Web of Science from inception until 6th May 2021 to retrieve relevant studies published in the field. The electronic search strategy conducted in PubMed covered the key words of OCI (Occult Hepatitis C OR Occult Viral hepatitis C OR Occult Hepatitis C Virus OR Occult HCV) and was adapted to other databases. We also searched manually all included studies and previous systematic reviews on the topic to identify additional references.

Study selection

The duplicate articles found in the databases were removed by using EndNote software. Two investigators (JTEB and SK) independently selected articles on the basis of title and abstract using Rayyan review platform. Then full texts of selected articles were read by 22 authors on the basis of the eligibility criteria. Disagreements were resolved through discussion and consensus.

Data extraction

Data were extracted independently by each author *via* the Google Forms for articles that met the inclusion criteria. The data extracted were; the name of the first author, the date of publication, the period of recruitment of the participants, the design of the study, the sampling method, the number of study sites, the time of collection of the data, country, UNSD region, type of population studied, patient demographic details such as gender, age, and location of recruitment, OCI type (seronegative or seropositive), risk of bias assessment, detection test, target detected, type of sample used, number of samples tested, and number of samples positive for OCI. All disagreements regarding eligibility and data collected were resolved by discussion and consensus.

Appraisal of the methodological quality of included studies and risk of bias

We used the Hoy *et al* tool to assess the quality of the included studies (Supplementary Table 2) [32]. This tool takes into account 10 elements to assess the internal and external validity of prevalence studies. For each item, a score of 1 is assigned to a "yes" response and a score of 0 is assigned to the other responses ("no", "not clear", "not applicable"). Basically, a study was considered to be low risk, moderate risk, or high risk of bias if the total score was respectively 0-3, 4-6, and 7-10.

Data synthesis and analysis

To estimate proportions of OCI and their 95% confidence intervals (95%CI), we choose random-effect meta-analysis model due to the heterogeneity expected for observational studies. The I^2 statistics and Cochran's Q-test were used to assess heterogeneity between studies [33]. The I^2 cut-offs > 50% indicates substantial heterogeneity. Potential sources of heterogeneity were explored by subgroup analyses and metaregression including covariates: study design, sampling, setting, timing of samples collection, countries,

WHO Region, United Nations Statistics Division (UNSD) region, country income level, age range, population categories, OCI diagnostic method, sample types. We used Funnel plot and Egger test to examine Publication bias [34]. R software version 4.1.0 was used to perform analyses [35, 36].

RESULTS

Study selection and characteristics

The electronic search gave 3950 articles (Embase (2025), Web of Science (1183), PubMed (706), and Global Index Medicus (36) (Figure 1). The eligibility review of 179 articles resulted in the exclusion of 94 and the inclusion of 85. The excluded articles and the individual reasons for exclusion are presented in Supplementary Table 3 while the included articles are indicated in Supplementary Text 1.

Characteristics of included studies

Overall, we obtained 102 prevalence data from the 85 included studies (75 seronegative OCI, 24 seropositive OCI, and 3 seropositive OCI and/or seronegative OCI (Supplementary Table 4 and 5). The prevalence data were published from 1995 to 2021 and for studies with data reported, the participants were recruited from 2002 to 2019. The majority of the prevalence data were cross-sectional design (94 out of 102) with non-random sampling (97 out of 102) and consecutive sampling methods (95 out of 102). The setting of study was hospital-based (98 out of 102) and monocentric (83 out of 102). Prevalence data were reported predominantly in the Eastern Mediterranean (51 out of 102) and in Europe (41 out of 102) WHO regions. The highest number of prevalence data were from high-income countries (40 out of 102) and Upper-middle-income countries (35 out of 102). Prevalence data predominantly involved adults (33 out of 102), patients on hemodialysis (25 out of 102), and patients who achieved SVR (15 out of 102). The most used sample type was PBMC (86 out of 102). The OCI diagnosis was performed using classical RT-PCR (49 out of 102) or real-time RT-PCR (44 out of 102). In most prevalence data, the risk of bias was moderate (64 out of 102) (Supplementary Table 6).

The prevalence of seronegative occult C infection

A total of 75 prevalence data reporting seronegative OCI were conducted across 4 WHO regions: America, Eastern Mediterranean, Europe, and Western Pacific (Figure 2). The pooled OCI prevalence was estimated to be 9.6% [95%CI = 6.8-12.7] in samples of 8535 participants with high heterogeneity (I^2= 94.7% [95%CI = 93.8%-95.4%], p<0.0001) (Figure 3, Table 1, and Supplementary Figure 1). There was a significant publication bias (Supplementary Figure 2, P = 0.006). Trim-and-fill adjusted analysis indicated a lower prevalence of 5.3% [95%CI = 2.9-8.2] with an addition of 10 studies.

The prevalence of seropositive occult C infection

Prevalence data reporting seropositive OCI were conducted in 4 WHO regions including America, Eastern Mediterranean, Europe, and Western Pacific (Figure 2). Overall, seropositive OCI prevalence was estimated to be 13.3% [95%CI = 7.8-19.9] with a total of 2642 participants from 24 prevalence data (Figure 4). High heterogeneity was observed in the overall estimate of the prevalence of seropositive OCI (I^2 = 93.0% [90.8%-94.7%], p<0.0001). There was a significant publication bias (Supplementary Figure 3, P = 0.017). Trim-and-fill adjusted analysis indicated a lower prevalence of 5.3% [95%CI = 1.4-10.7] with an addition of 8 studies.

Seronegative and/or seropositive occult C infection

Prevalence data reporting seronegative and/or seropositive OCI were conducted in 2 WHO regions (Eastern Mediterranean and Europe). Overall, seronegative and/or seropositive OCI prevalence was estimated to be 12.6% [95%CI = 1.2-32.2] with a total of 285 participants from 3 prevalence data. High heterogeneity was observed in the overall estimate of the prevalence of seronegative and/or seropositive OCI (I^2 = 93.0% [83.0%-97.1%], p<0.0001).

Subgroup analyses and metaregression

Seronegative occult hepatitis C infection

Higher proportions of seronegative OCI were estimated for studies selected participants by non-probabilistic sampling (P = 0.001), conducted in Spain and Egypt (p<0.001), in

Southern Europe and Northern Africa (p<0.001), or in countries with lower-middle income economies (P = 0.045), investigated children (P = 0.01) or patients with abnormal liver function, hematological disorders, and kidney diseases (p<0.001), and detected OCI cases by Real-time RT-PCR (p<0.001) or by examining liver tissue (p<0.001) (Supplementary Table 7). The heterogeneity of the prevalence of seronegative OCI was explained at 84.0% (R2=84.0%) (Supplementary Table 8).

Seropositive occult hepatitis C infection

Higher proportions of seropositive OCI were estimated for studies performed as case controls (p<0.001), conducted in Italy, United States of America, and Egypt (p<0.001), in Southern Europe, Northern America, and Northern Africa (P = 0.001), or by examining liver tissue and PBMC (P = 0.023). The heterogeneity of the prevalence of seropositive OCI was explained at 46.2% (R2=46.2%).

DISCUSSION

This systematic review came up with a summary of the prevalence of seronegative and seropositive OCIs obtained in relevant articles published between 1995 and 2021 in 17 countries across 4 WHO regions: America, Europe, Eastern Mediterranean, and Western Pacific. Overall, we found a high prevalence of seronegative OCI (9.61%) and seropositive OCI (13.39%) respectively. Higher seronegative OCI prevalence was in Southern Europe and Northern Africa and in patients with abnormal liver function, hematological disorders, and kidney diseases. Higher seropositive OCI prevalence was in Southern Europe, Northern America, and Northern Africa.

Many studies have previously shown that multiple transfused subjects are at high risk of HCV infection [20, 25, 37-39]. Seronegative OCIs aligned well with classical HCVs and are very predominant in subjects with hematological disorders and renal diseases in this study. It is therefore important to implement screening measures for OCI in blood transfusion banks, dialysis and/or transplant units [40]. As in the present review, it has also been shown previously that patients with abnormal liver functions are at high risk of OCI [26]. There is, however, a significant residual of heterogeneity in our estimates

that could be related to the different types of chronic liver disease that we did not take into account. North Africa and particularly Egypt is the country with the highest prevalence of HCV in the world [41, 42]. The findings of the present study corroborate this fact and show higher seronegative and seropositive OCI prevalence in North Africa (Egypt). It should however be noted that Southern Europe and North America also showed high prevalence of OCI in this study while most other regions were absent or poorly represented in the estimates. HIV patients, people who inject drugs and men who have sex with men are groups known to be at high risk for HCV infections were poorly represented in this review. Additional studies characterizing the epidemiology of HCV in these groups are awaited to fully explain the global epidemiology of OCI and more specifically in the WHO regions of Africa and South-East Asia. In a first global review without meta-analysis conducted in 2017, Dolatimehr *et al* reported OCI prevalence of 0-45% and 0-2% in hemodialysis patients (10 studies) and kidney transplant recipients (2 studies) respectively [25]. In a conference abstract, Fu *et al* reported highly variable prevalence of OCI in different population groups ranging from 0% in patients with autoimmune hepatitis, 9% in patients with cryptogenic liver disease, 22% in patients with chronic liver disease who have achieved SVR, 33% in patients with long-standing abnormal liver-enzyme levels to 89% in patients with abnormal level of serum aminotransferases [26]. More recently, Hedayati-Moghaddam *et al* reported the prevalence of OCI in the Middle East and Eastern Mediterranean in several population categories [20]. This last systematic review also revealed a significant variability in the OCI prevalence according to the category of the population with 19% for patients with haematological disorders, 12% for HIV-infected patients, 12% for patients with chronic liver diseases, 9% for hemodialysis patients, 8% for multitransfused patients, and 4% for apparently healthy populations. Like the reviews mentioned above, our study also noted a statistically significant difference in the prevalence of seronegative OCI according to population categories. It should be mentioned, however, that the above reviews only included participants tested for PBMCs unlike our work which additionally considered liver biopsies and

ultracentrifuged serum. The strong heterogeneity recorded in our work could also be explained by the differences in HCV prevalence according to the regions with the areas of high HCV endemicity which should also be the areas of high prevalence of OCI [11]. As the different risk factors and the different approaches to controlling HCV infection also vary widely between studies, regions and populations, this should also potentially represent a considerable source of the variability observed in our work. We can cite the example of history of accidental exposure to infected needle sticks, history of blood transfusion, history of surgery, history of endoscopy, history of unsafe sexual intercourse, history of liver disease, the length and frequency of dialysis sessions, immunodepression, injecting drugs, tattoos or imprisonment. Other potential sources of heterogeneity in our estimates may also include gender of participants, sample size, and year of participant recruitment. Our study reveals that although PBMC is an excellent non-invasive sampling approach for diagnosing OCI, liver tissue exhibits superior sensitivity for seronegative OCI. It also emerges from this study that with the ultracentrifuged serum we obtain a not insignificant fraction of additional patients positive for OCI. These results suggest that it is potentially insufficient to test OCI in a single type of sample. We also observed that real-time RT-PCR was significantly more sensitive for the detection of seronegative OCI. This suggests a further improvement in the sensitivity of molecular techniques for OCI detection.

Our study is limited by the representativeness of the included studies where the WHO Africa and South-East Asia regions are not represented. Our OCI prevalence could therefore be over or underestimated. Substantial residual statistical heterogeneity in prevalence measures was identified in all aggregate and subgroup meta-analyses. Despite these limitations, the main strength of our study is that we identified a very large number of studies, which covered multiple categories of symptomatic, apparently healthy populations and at high risk for HCV infection. We also took into account in our estimates the variability of the prevalence according to the anti-HCV serostatus.

Our results suggest that reflections should be initiated on the implementation of screening programs for OCI in high-risk populations, especially patients with

hematologic complications, hemodialysis patients, and patients with chronic liver disease. More studies are needed to assess the transmissibility, clinical significance, long-term outcome, and need for OCI treatment.

CONCLUSION

In conclusion, in the present study, it appears that the burden of seronegative and seropositive OCIs is high and very variable according to regions and categories of populations.

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