



# Occult Hepatitis C Infection Among Hemodialysis Patients: A Prevalence Study

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## ABSTRACT

**Introduction and aim.** Occult hepatitis C infection (OHCI) is the presence of HCV-RNA in the liver or peripheral blood mononuclear cells (PBMC) accompanying with negative serologic results. The aim of this study was to evaluate the prevalence of OHCI among Iranian chronic hemodialysis (HD) patients. **Material and methods.** In this cross sectional study 200 chronic HD patients with negative HCV antibody enrolled the study. Blood sample of patients were obtained, followed by Polymerase Chain reaction (PCR) testing for detection of HCV RNA. Patients with positive serum HCV RNA were considered as manifest hepatitis C infection (MHCI). However, patients with negative serum HCV RNA underwent further tests on PBMCs for detection of OHCI. **Results.** Serum HCV RNA was positive in 2 (1%) patients whom considered as MHCI, and 6 (3.03%) patients had positive PBMC HCV RNA. **Conclusion.** In conclusion, chronic HD patients have been considered as a high risk group for hepatitis C infection. The results of this study suggest that these patients are also at risk for OHCI. Furthermore, evaluating PBMCs to detect HCV RNA would be a sensitive diagnostic method to find OHCI patients.

**Key words.** ESRD. Hepatitis C virus. Kidney transplant. Liver disease. Peripheral Blood Mononuclear Cell (PBMC).

## INTRODUCTION

Hepatitis C Virus (HCV) is an enveloped RNA virus from Flaviridae family and Hepacivirus genus. It has a single positive-strand RNA. HCV is the most common cause of chronic liver diseases.<sup>1</sup> Chronic hepatitis C infection (HCI) is a major health problem that affects 2-3% of global population.<sup>2,3</sup> While hepatitis B infection has brought under control over the past 20 years, HCI is still considered the major culprit of chronic liver diseases in hemodialysis patients.<sup>4</sup> Although all blood products are carefully screened for HCV contamination, HCI is demonstrated to be a major issue in end-stage renal disease (ESRD) patients receiving maintenance hemodialysis (HD)<sup>5</sup> and its prevalence is more than general healthy population.<sup>6</sup>

HCV infection has been identified as the main cause of liver disease in the patients undergoing kidney transplantation.<sup>2</sup> These complications vary from chronic hepatitis, the common form of liver disease in these patients, to an uncommon and severe complication known as fibrosing cholestatic hepatitis (FCH) leading to rapidly progressive liver failure.<sup>2,7</sup> Unfortunately, Interferon-alpha (INF-alpha) -one of the common anti-HCV therapies- increases the risk of severe acute allograft rejection.<sup>2</sup> New treatment approaches such as IFN-free treatment options have given hope; however, inadequate data are available with respect to their use in HD patients with HCI.<sup>8</sup> Baid-Agrawal, *et al.* believe that although some studies have shown that kidney transplantation is the most appropriate option in the ESRD patients with HCI who are on the waiting list, and

therefore, HCI by itself should not delay kidney transplantation,<sup>2</sup> it is recommended to treat all kidney transplant candidates who are already infected by HCV before transplantation. In fact, commencing antiviral medication before transplantation prevents post-transplant hepatic diseases as well as post-transplant HCV-related renal disease and new onset post-transplant diabetes.<sup>2</sup>

While evaluating PBMCs to identify occult hepatitis C infection was described for the first time in 1995,<sup>9</sup> apparently, occult hepatitis C infection as an entity was introduced in 2004 and is defined as “the presence of HCV-RNA in the liver or peripheral blood mononuclear cells (PBMC) and negative results for both serum HCV-RNA PCR and anti-HCV antibodies”.<sup>10</sup> The prevalence of HCI varies worldwide among the HD patients and it ranges from 2% to 60% depending on geographic location.<sup>11</sup> Epidemiologic studies in Iran have demonstrated that the prevalence of MHCI is from 0.05% to 6.25% in general population and varies from 3% to 55.9% in HD patients.<sup>12</sup>

Jain, *et al.* showed that prevalence of OHCI is considerably higher than MHCI in ESRD patients.<sup>13</sup> Moreover, in ESRD patients with impaired liver function tests precise investigation to explore OHCI might not be performed, readily leading to HCV spread among these patients.<sup>5</sup> Considering this high prevalence and the importance of treating these patients, more extensive survey is required to investigate the precise prevalence of OHCI. Therefore, we performed an in-depth epidemiologic investigation in chronic HD patients in Iran to provide more precise estimate.

## MATERIAL AND METHODS

### Study population

The subjects of this study were selected from three HD centers of Iran University of Medical Sciences by multi-stage random sampling. Two hundreds of patients on maintenance HD (at least 6 months history of dialysis) and negative anti-HCV antibodies at the initiation of HD were included in this cross sectional study. A questionnaire containing of the age, gender, duration of kidney disease, duration of ESRD, etiology of ESRD, duration of HD, history of previous kidney transplantation and HCV antibody at the initiation of HD was completed for each patient.

### Collection and Preparation of the Specimens

Blood samples were obtained in the morning between 8-10 a.m. in an EDTA-containing sterile tube and were sent to referral laboratory. After separation of plasma, it

was stored at  $-80^{\circ}\text{C}$  for later detection. The PBMCs of the specimens were isolated by Ficoll Hypaque gradient centrifugation (Lymphoprep, Oslo, Norway), and PBMC pellets were then washed three times with phosphate-buffered saline ( $\text{pH} = 7.3 \pm 0.1$ ). The PBMCs were counted and resuspended in 200  $\mu\text{L}$  RNALater solution (Ambion Inc., Austin, TX, USA), and afterwards stored at  $-80^{\circ}\text{C}$  until use.

### Isolation of RNA and Detection of HCV-RNA Using Reverse Transcriptase -Nested Polymerase Chain Reaction (RT-nested PCR) Method

RNA was extracted from 140 mL of plasma and a pellet of about  $3-5 \times 10^6$  PBMC samples using High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instruction. The cDNA synthesis from extracted RNA and Nested-PCR was performed as previously described in detail.

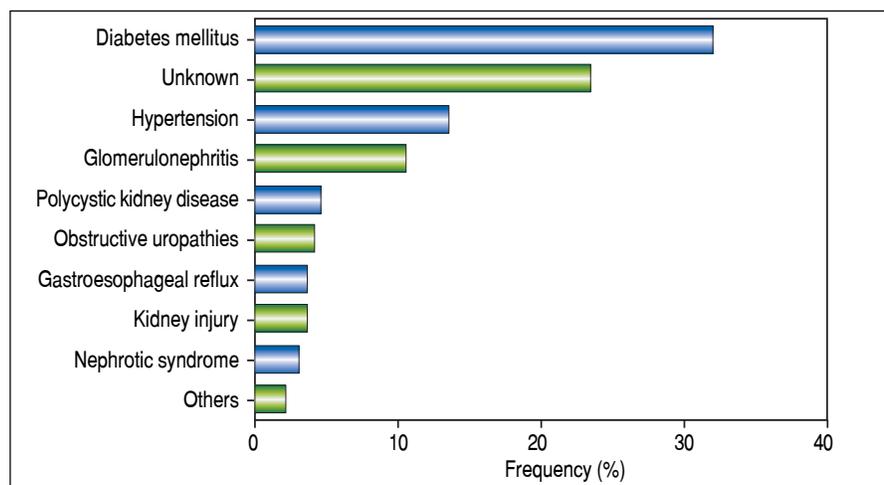
### Statistical analysis and ethics

Data are expressed as means  $\pm$  SD and percentages. Statistical analyses was conducted by SPSS 18 (SPSS, Chicago, IL, USA). This study was approved by the National Research Council of the Islamic Republic of Iran and was performed with the approval of the ethics human research review committee of the Iran University of Medical Sciences. All individuals in this study had signed their informed consent prior to enrollment into the study.

## RESULTS

The study population included 109 (54.5%) male and 91 (45.5%) female participants with the average ( $\pm$  SD) age of  $56.09 \pm 15.58$  years ranging from 22 to 88 years. The duration of HD was  $51.28 \pm 60.96$  months ranging between 7 to 365 months, so that 152 patients (76%) had history of HD from 6 months to 4 years while the remaining 48 patients (24%) had the HD history longer than 4 years. The background diseases of patients are portrayed in *figure 1*.

While HCV antibodies were negative for all patients, HCV RNA was detected by RT-PCR in serum of 2 patients (1%) revealing that these two patients have MHCI. One of these patients has had a history of HD for more than 4 years and the other one had HD duration between 6 months to 4 years. Moreover, one of the patients with positive serum HCV RNA had hypertension and the other one had glomerulonephritis. Six patients out of remaining 198 patients (3.03%) were diagnosed with OHCI by positive HCV RNA in PBMCs. Two of the OHCI patients aged between 40 and 60 and the remaining 4 patients were older than 60 years. Three individuals of



**Figure 1.** Frequency of background diseases of ESRD patients in this study.

OHCI patients were female. Regarding duration of HD, three occult hepatitis C infected had undergone HD for more than 4 years and the other 3 patients had HD duration between 6 months to 4 years. Two occult hepatitis C infected patients had diabetes mellitus, two patients had glomerulonephritis, and the remaining patients had hypertension.

## DISCUSSION

HCI is a major global health issue. The prevalence of HCI has been reported to be significantly higher in HD patients as well as kidney transplanted patients compared to general population.<sup>8</sup> In addition, this type of infection has a disappointing impact on the survival of HD patients and graft of those undergone kidney transplantation.<sup>2</sup>

The current guidelines have recommended combination therapy with PEG-IFN-alpha and low dose ribavirin in HD patients because it has been demonstrated to be the most effective approach of treatment in these patients as well as patients who are not on dialysis. However, treatment with INF-alpha increases the risk of severe acute allograft rejection.<sup>2</sup> Therefore, the management of these patients is a challenging issue and it demands early diagnosis, and antiviral treatment needs to be considered before transplantation. Furthermore, close monitoring and follow-up of these patients should be taken into account.<sup>2</sup>

Diagnosis of HCI in HD patients through common serologic tests is neither optimal nor reliable in that 12.5 % of the findings have been reported to be false negative<sup>14</sup> that might be explained by the impaired immune response of chronic HD patients. Therefore, the combination of serologic testing and molecular diagnostic methods is beneficial.<sup>14</sup>

By definition, occult hepatitis C infection is “the presence of HCV-RNA in the liver or peripheral blood mono-

nuclear cells (PBMC) in the absence of both serum HCV-RNA and anti-HCV antibodies”.<sup>10</sup> Seemingly, this terminology firstly was introduced by Oesterreicher, *et al.* in 1995<sup>9</sup> and then was used by Castillo, *et al.* in 2004.<sup>10</sup> Unfortunately, sufficient knowledge is lacking in terms of the prevalence of OHCI, natural history, potential transmission risk, and its impact both in general population and in patients on hemodialysis.<sup>15</sup> While, Castillo, *et al.* showed that presence of HCV-RNA in PBMC corresponds to the existence of virus RNA in liver.<sup>10</sup> Moreover, despite the absence of HCV genome and antibody in serum of these patients, they can transfer this infection via their peripheral blood mononuclear cells.<sup>16</sup>

This study was conducted as a cross sectional study in order to estimate the prevalence of OHCI in HD patients, regardless of their liver function tests. The survey has been carried out in a large group of HD patients and this could be a great advantage of the study. Eight patients were found with positive molecular results (2 positives in serum and 6 positives in PBMCs) while their serologic results were already negative. Six patients were detected with positive HCV RNA in PBMCs, demonstrating 3.03% prevalence for OHCI among Iranian HD patients. Our study for the first time showed presence of OHCI among Iranian HD patients. In a former study, Eslamifar, *et al.* did not find any OHCI among 70 HD patients of 3 dialysis centers in Iran.<sup>17</sup>

By comparison, Oesterreicher, *et al.* found 1 patient with OHCI in 54 patients of a HD center without evidence of MHCI.<sup>9</sup> Baid-Agrawal, *et al.* found 1 patient with positive OHCI in 407 HD patients of six HD centers who were confirmed to have negative serum HCV RNA.<sup>18</sup> In another study, 15.1% of the 53 patients of 1 dialysis center were reported to have positive OHCI.<sup>15</sup> Abdelrahim, *et al.* observed that 3 out of 81 HD patients (with negative anti HCV antibodies and negative PCR for serum HCV RNA)

in two Egyptian dialysis centers were positive for OHCI.<sup>19</sup> Of note, in the study conducted by Barril, *et al.* there was a positive correlation between the duration of dialysis and probability of detecting OHCI;<sup>5</sup> however, no significant correlation was found in our study.

In conclusion, considering the importance of OHCI in HD patients our study showed prevalence of 3.03% in Iranian HD patients. Therefore, more precise investigation including exploring HCV RNA in the PBMC seems crucial. With regard to the importance of this health issue, more studies are required to obtain a better estimate of occult HCV infection in HD patients. Furthermore, performing specific studies focusing on the genotypes of HCV as well as association of occult hepatitis C infection with the route of transmission would be pretty advantageous.

## ABBREVIATIONS

- **ESRD**: end-stage renal disease.
- **FCH**: fibrosing cholestatic hepatitis.
- **HCI**: chronic hepatitis C infection.
- **HCV**: hepatitis C virus.
- **HD**: hemodialysis.
- **INF**: interferon.
- **MHCI**: manifest hepatitis C infection.
- **OHCI**: occult hepatitis C infection.
- **PBMC**: peripheral blood mononuclear cell.
- **PCR**: polymerase chain reaction.

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