Prevalence and Risk Factors of Hepatitis C Virus Infection in Haemodialysis Patients: Testing Antibodies, RNA and Genotypes

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Abstract

Hepatitis C virus (HCV) infection is an important global public health problem affecting approximately 180 million people. Multiple risk factors are associated with HCV transmission among haemodialysis (HD) patients leading to an increased risk for liver-related mortality. Patients undergoing HD may show a decreased humoral and cellular immunity, which lowers the sensitivity of the HCV antibodies (Abs) test resulting in false negative antibody test, thus requiring HCV RNA testing. Our study is to determine the prevalence of HCV markers (antibody RNA and genotype) and risk factors of HCV infection among patients in HD unit in Baghdad. A sample of 54 patients were interviewed. HCV Abs (anti-HCV) was tested using third generation enzyme immunoassay (EIA-3) and immunoblot assay (Lia-Tek III) as screening and confirmatory test respectively. Sera of 46 patients (irrespective to anti-HCV results) were subjected to molecular analysis, using the most developed RT-PCR and DNA Enzyme immunoassay (DEIA) method. Seropositive rate of anti-HCV and HCV-RNA were (66.6%) and (60.9%) respectively. Anti-HCV seropositive rate was significantly higher in males (77.1%), and history of blood transfusion (85%). Blood transfusion acts as a significant risk for acquiring HCV (OR 44.2, 95% CI 7.6-256.9). Genotype 4 was the most prevalent (33.3%), followed by genotype 1a (25.9%) and genotype 1b (22.2%). We concluded that, the prevalence of HCV among the haemodialysis patients is high. It is significantly related to gender, duration of dialysis and number of blood transfusion. Blood transfusion acts as a significant risk factor. Molecular test for detection for HCV RNA is necessary and proper nosocomial prevention program should be implemented to prevent HCV transmission.

Keywords: Hemodialysis, HCV infection, prevalence, risk factors, genotypes

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1.0 INTRODUCTION

Hepatitis C virus (HCV) infection is a very important global public health problem; with approximately 180 million people affected currently [1]. The global prevalence rate is approximately 3% with a heterogeneous pattern of HCV [2]. The epidemiology of HCV infection has undergone substantial changes over the past two decades with progressive decrease in incidence and a shift in risk factors especially in developed countries [3, 4]. Clinically it is common to see HCV patients with pre-existing renal disease. Thus the prevalence of HCV infection is apparently increasing in patients with end-stage renal disease (ESRD) on hemodialysis (HD) compared to the general population [5]. The prevalence rates differ between regions of the world, ranging from 1% to more than 90% [6]. Several studies have demonstrated that HCV infection has a significant adverse effect on the health of persons with ESRD on dialysis [7]. It is associated with more rapid liver disease progression leading to an
increased risk for all-cause and liver-related mortality such as cirrhosis and hepatocellular carcinoma [7, 8].

It is still unclear how HD patients become HCV-infected. Nevertheless, both intra dialysis (number of blood transfusions, duration and prevalence of HCV in the hemodialysis unit, standard infection control practices) and extra dialysis (high risk of lifestyle behaviour) variables have been identified [6]. Rates of HCV infection in HD patients vary markedly among different countries and hospitals [9]. Despite the elimination of post transfusion HCV infection, incidence of HCV infection among HD patients ranged between 0.2%-15% per year of dialysis. Thus, it was highly possible that nosocomial factor continues to be a cause of concern [4] especially in regions like Iraq with poor socioeconomic conditions, where the qualified medical staff and equipments available to treat HD patients were very limited [4].

The Enzyme-Linked Immunosorbent Assay (ELISA) method is highly sensitive and specific and is used for the diagnosis and antibody screening of HCV in hemodialysis patients. However, patients undergoing dialysis treatments may show a decrease in humoral and cellular immunity, which may lower the sensitivity of the test and give a false-negative result. For this reason, RNA-HCV detection using the RT-PCR technique (reverse transcription polymerase chain reaction) is necessary, as it will overcome any false-negative results and confirmed the HCV diagnosis in these patients [9]. This is the gold standard technique for detecting and identifying the genotypes and subtypes of HCV. Determining the genotype of HCV is an important tool for diagnosis and epidemiological purposes that may allow tracing the source of infection and will shed light on the route of HCV transmission [10]. Management of HCV in patients with renal disease present unique challenges, in terms of duration, dose and types of antivirals especially in ESRD patients [8].

Globally, genotype 1, especially the type 1a virus, causes approximately 90% of infections, while types 2, 3 and 4 are less represented [10]. HCV genotypes 1 and 2 are universally distributed, whereas HCV genotypes 4, 5, and 6 are confined to more specific geographical areas such as in the Middle East and Central Africa where genotype 4 is more predominant[11].

The data on epidemiology of HCV among patients with ESRD on dialysis in developing countries including Iraq are less abundant and more heterogeneous, but the overall prevalence and incidence rates seem to be higher than developed countries. Therefore, we designed this study to determine the prevalence and risk factors of HCV infection in patients treated in dialysis unit in AL-Kadhymia Teaching Hospital in Baghdad.

The aim of our study was to determine the prevalence of hepatitis C measured serologically by HCV antibodies RNA and genotype among participants. The study also aimed to identify the risk factors for the transmission of hepatitis C virus among hemodialysis patients.

### 2.0 MATERIALS & METHOD

A cross-sectional study was conducted among all patients in haemodialysis (HD) unit at AL-Kadhymia Teaching Hospital in Baghdad. The study protocol was approved by the ethics committee of the Medical Faculty of the Al-Nahrain University, and Ministry of Health (MOH), Iraq. Only 54 out of 57 (94.7%) patients gave informed consent and participated in this study. All participants were interviewed to obtain socio-demographic data and risk factors associated with HCV infection (sex and age, duration on HD, and number of blood transfusions). Blood sample (4-5 ml) was obtained from each patient before HD was started. Blood was centrifuged and serum sample of each participant was dispensed into two frozen small tubes, stored at -20°C- and -70°C. The former was utilized to assess Hepatitis C antibodies, using a third generation enzyme immunoassay (UBI HCV EIA 4.0 NY USA). All reactive sera were tested twice. Positive results were further confirmed by a third generation immunoblot assay (Lia-Tek-III) Organon Amsterdam. This test yield to 3 results interpretation (Positive, Indeterminate or Negative). A specimen was considered seropositive for HCV antibodies only if it was reactive by Lia-Tek III.

Forty six serum samples (stored al-70°C C) comprising of 36 positive, 4 negative and 6 indeterminate determined by Lia-Tek III were transferred in an ice card to the laboratories of Sorin Diagnostica (Sallugia, Italy). These samples were then subjected to molecular analysis for evaluating the HCV-RNA positivity and subsequently HCV-genotype, using an advanced molecular (DEIA) method. This method is based on combination of two well-established techniques: the polymerase chain reaction (PCR) and DNA enzyme immunoassay (DEIA). In the first step, viral RNA was extracted from 140 μL serum of HD patient, and subjected to reverse transcription using primer specific kit. The newly developed cDNA was amplified at 5’UTR region by single step PCR. The amplified cDNA was then hybridized to probe-specific oligonucleotide, fixed to solid-phase through an avidin–biotin bridge, using avidin coated plate from GEN-ETI-K, DEIA (Sorin Diagnostica,Sallugia, Italy). Hybridization reaction was detected by the use of anti-double stranded DN consisting of 10% FCS of monoclonal antibody from GEN-ETI-K DEIA supplied by SORIN Biomedica Saluggia-Italy.

Finally, the result was obtained by spectrophotometer at wavelength 450 nm and 630 nm. The detection of genotyping variation of HCV was carried-out by the same method (DEIA) based on six deferent oligonucleotide probes corresponding to the six HCV genotypes as well as their different subtypes. Classification of HCV genotypes/subtypes was performed according to Simmonds nomenclature for HCV genotypic classification as proposed by international HCV collaboration group (1994). All of the laboratory
procedures were done according to the manufacturer’s instructions.

The statistical analysis was carried out using SPSS 21.0. Percentages were used for the categorical variables, while mean and standard deviation were used for quantitative variables. Univariate and bivariate analyses were performed. Chi-squared ($\chi^2$) and the t-test were used. Odds ratios (ORs) and 95% CIs were calculated, considering $p<0.05$ as significant.

### 3.0 RESULTS AND DISCUSSION

The study population consisted of 35 (64.80%) males and 19 (35.89%) females. The mean age of patients was 59.26 ± 14.86 years. Moreover, the mean dialysis onset of patients was 24.75 ± 20.82 months. Using the third generation immunoblot assay (LiaTek III), 36 sera confirmed positive anti-HCV antibodies, while eight and ten sera showed as indeterminate and negative respectively. The overall prevalence for HCV among HD patients was 66.60% in this study. This finding is almost ten times higher than that (7.1%) of the general population in Iraq [12]. This is much higher than surveys done in Brazil (8.0%), Turkey (19.0%), Tunisia (20.0%), and Saudi Arabia (43.0%). However, our finding is lower than Kuwait (71%), Morocco (76%) and Egypt (80%) [7]. These variations may be related to patients’ behavioral and cultural differences, geographic location, and socioeconomic factors.

Indeed, the prevalence of HCV among the HD patients in any developing country such as Iraq which surpasses that in the developed countries who follow the strict regulations of HCV prevention [12]. Dialysis centers are usually overloaded with patients, with a shortage of material support (filters), leading to multiple use of the filter without proper sterilization in some cases. In addition, there is an insufficient number of dialysis machines in which the number of patients dialyzed per machine is twice than that in developed countries causing large outbreaks in such centers via patient-to-patient transmission. Interestingly, Abdulkarim et al. (1998) stated that hepatitis C virus in plasma remains viable and detectable after drying and environmental exposure to room temperature for at least 16 hours. Therefore, blood-contaminated surfaces and objects can serve as sources for HCV transmission. These can also be a source for nosocomial infection [13]. Hinrichsen et al. (2002) reported that the duration of more than 10 years on haemodialysis is an independent risk factor for developing Hepatitis C infection [14]. In another study by Sanjaya et al. (2014), the duration was less whereby the haemodialysis duration of HCV-infected was 101.6 ± 80.6 months [15]. However, in this study, the mean duration of dialysis in our patients was much less (24.75 months), but anti-HCV seropositive rate was significantly related to the duration of HD which is in concurrence with the report by Yue-Cheng et al. (2014) which states that the risk of HCV infection increases with the number and length of hemodialysis exposure [6].

A significant difference in anti-HCV positive rate between the two genders was detected. Anti-HCV seropositive rate was significantly higher (77.14%) among males compared to (47.37%) females ($X^2=4.913$, $p=0.026$). Interestingly, using univariate analysis, males had been significantly exposed to risk of HCV infection almost four times than females, OR = 3.75, 95% CI 1.13-12.4 (Table 1). This is similar to the findings of Sidney et al. (2014) but not to the findings of Sanjaya et al. (2014) and Hinrichsen et al. (2002) [14, 16]. The higher rate among males could be related to occupation, culture and behaviour related risks exposure (e.g: smoking or alcohol consumption).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HD Patient No. (%)</th>
<th>Anti-HCV antibodies N=54</th>
<th>Test of significance</th>
<th>P value</th>
<th>OR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>35 (64.8)</td>
<td>27 (77.1)</td>
<td>8 (22.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>19 (35.8)</td>
<td>9 (47.37)</td>
<td>10 (52.6)</td>
<td>$X^2=4.913$, 0.026</td>
<td>3.75</td>
<td>1.13-12.4</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40</td>
<td>34 (85)</td>
<td>6 (15)</td>
<td>$X^2=26.4$, 0.001</td>
<td>44.2</td>
<td>7.6-256</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>2 (14.3)</td>
<td>12 (85.7)</td>
<td></td>
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</tr>
</tbody>
</table>

In the developing countries, blood and blood products remain a major cause of spread of HCV [12]. Denniston et al. (2014) stated that one of the factors that were significantly associated with chronic HCV is recipient of blood transfusion before 1992 [17]. Therefore, In terms of blood transfusion, 40 (74.1%) patients said they had received blood at least once in their lifetime. Of these 40 patients, 34 of the sera demonstrated positive anti-HCV antibodies. Significantly higher anti-HCV seropositive rate was (85.0%) detected among patients with history of blood transfusion.
compared to patients (14.3%) with no such a history ($X^2=26.4$, $p=0.0001$). Moreover, we found that blood transfusion acts as a risk for acquiring HCV (OR= 44.2, 95% CI=7.6-256.9). In addition, it was found that the mean number of blood units transfused was significantly higher (3.89±0.39) in anti-HCV-positive patients than (3.25± 0.43) of anti-HCV-negative patients ($t=5.1477$, $p < 0.0001$). This is less than Hinrichsen et al. (2002) who found that more than five blood transfusions was an independent risk factor for hepatitis C infection [14]. However, Mohammad Hossein et al. (2014) found that there is no significant relation between blood transfusion and HCV infection [18].

Among the 46 samples sent for molecular analysis, 28 (60.9%) were identified as positive HCV-RNA. Out of the 36 positive by Lia-Tek III, 26 (72.2%) showed positive HCV- RNA, which is higher than (64.9%) the findings of Hinrichsen et al. (2002). This is probably due to the use of Lia-Tek III which is more sensitive than ELISA [14]. None of the Anti-HCV negative sera exhibited positive HCV- RNA. On the other hand, 27.7% positive Lia-Tek III demonstrated negative HCV-RNA in concurrence with Saito & Ueno (2013) who mentioned that Hepatitis C virus will clear spontaneously in about 15% of HCV infected patients [19]. Several studies have suggested that differences in host immune response determine viral clearance. Indeed the intensity of immune response can be dictated by host genetic factor [20]. Certain HLA alleles have been associated with specific outcome of HCV infection [21]. Interestingly, two of six (33.3%) indeterminate sera showed positive HCV-RNA.

Therefore the prevalence of viremia in 54 HD patients was 51.6% which is in agreement with Hinrichsen et al. (2002) and Mohamed, et al. (2014) who stated that even when HCV screening test is performed the risk of becoming infected is still visible. This however makes it necessary to add HCV-RNA detection [12, 14].

Determining the genotype of HCV is an important tool for diagnosis and epidemiological purposes [10] and HCV management particularly in patients with renal disease presents unique challenges [8, 6]. All the 28 positive HCV-RNA samples were genotyped except one. Five genotypes and subtypes (1, 1a, 1b, 4, and 3a) were detected in this study, with single infection (1, 1a, 1b, and 4) while genotypes 3a was mixed with genotype 4. In disagreement with Hinrichsen et al. (2002), Sanjaya K et al (2014) and Andriulli et al.(2015), [14, 22, 15], our study detected that the most predominant HCV-genotypes among Iraqi HD patients was HCV genotype 4 which is the most prevalent 9/27 (33.3%), followed by HCV-1a 7/27 (25.9%); HCV- 1b 6/27 (22.2%) and genotypes 1, 4/27(14.8%), [Table 2]. This finding is in agreement with other studies [10, 16] stating that HCV-4 was the most predominant genotype in the Middle East particularly among Egyptian population [10, 18]. This high prevalence of HCV-4 in Iraq may have been contributed via blood donation where high proportion of Egyptians resided in Iraq until 1990.

<table>
<thead>
<tr>
<th>Anti-HCV Antibody Status</th>
<th>HCV-RNA</th>
<th>HCV-Genotype/sub genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive N=36</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Indeterminate N=6</td>
<td>4</td>
<td>2*</td>
</tr>
<tr>
<td>Negative N=4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*One untested

The limitation of this study is that only patients from one center were included and the study design did not permit inferences of the causal nature of the associations and a distinction between the patients infected before and after initiation of dialysis.

4.0 CONCLUSION

HCV prevalence among the HD patients was high. It is significantly related to the duration of dialysis and the number of blood transfusion. Males on haemodialysis were more prone to HCV infection than females. HCV-4 genotype was the most predominant genotype which is more related to blood transfusion. Therefore, universal precautions especially stringent adherence to all necessary bio-safety measures during haemodialysis will be the keystones to minimize HCV transmission during HD. Erythropoietin should be used to reduce the need of blood transfusion in HD patients. It is also necessary to add molecular tests for HCV-RNA detection as
indeterminate Lia-Tek III showed positive HCV-RNA.

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References