HEPATITIS C VIRUS PREVALENCE IN HAEMODIALYSIS PATIENTS FROM THREE CENTERS IN BAGHDAD, IRAQ: A SURVEY BY POLYMERASE CHAIN REACTION AND SEROLOGICAL METHODS

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Abstract:

Hepatitis C virus infection is a major health problem among haemodialysis patients in developing countries. Nosocomial transmission of HCV infection was a considerable route, particularly during the outbreaks of infection. To compare serological and molecular methods for detection of HCV infection serum samples were screened for anti-HCV antibodies using a fourth generation enzyme-linked immunosorbent assay (ELISA) and positive samples were confirmed by immunoblot assay. All seropositive and seronegative samples were screened for the presence of HCV-RNA by using reverse transcriptase PCR (RT-PCR). The overall prevalence was (41.10%) in the three centers (range: 26.05% to 62.82%) with higher prevalence in Al-Kadhimiya Teaching Hospital. All seropositive samples were tested by reverse transcriptase PCR, and 24/92 (26.09%) of confirmed samples were found to contain HCV-RNA. Additionally, 2/5 (40%) of immunoblot-indeterminate and 1/3 (33.33%) of immunoblot-negative samples were also found to be HCV-RNA positive. Also all seronegative samples were found to be HCV-RNA by using pooling strategy and 2/136 (1.47%) of anti-HCV negative samples were found to be HCV-RNA positive. Our data emphasize the need for stricter adherence to infection control measures in haemodialysis centers and reinforce the importance of screening by both PCR and serological methods at regular intervals to identify all HCV-infected patients.

Key words: HCV, Haemodialysis, Serological test, RT-PCR.

Introduction:

repatitis C virus (HCV) is blood-borne Expathogen that appears to be endemic in most parts of the world. It is estimated that there million HCV-infected persons are 170 worldwide (Al Dhahry et al., 2003; and Senevirathna et al., 2008). The populations most affected by HCV are patients that undergo multiple blood transfusions, individuals who are intravenous and inhalant drug users. hemophiliacs. and haemodialysis patients (Alavian et al., 2011). HCV infection is a major health problem among dialysis patients in the developing countries. The higher prevalence in developing countries in comparison with developed countries reflects many factors including socioeconomic factors, bad infection control measures, use of blood transfusion before the routine use of erythropoietin to treat anemia and the higher prevalence of HCV among general population in developing countries (Chong and Zinna, 2008). Currently there are no vaccine and post exposure anti-viral prevent HCV infection. prophylaxis to Therefore, identification of HCV infected in haemodialysis (HD) centers can reduce the risk of nosocomial transmission of HCV and its clinical complication (Samimi-rad et al., 2008).

In Iraq, the prevalence of anti-HCV was reported between (40.2%) in renal dialysis unit patients in Mosul (Mohammed, 1997) to (62%) in haemodialysis patients (Khalaf et al., 1996). The prevalence rates reported in haemodialysis patients in Middle Eastern countries are 68% in Saudi Arabia with a range of 14.5% to 94.7%, 26% in Oman, and 80% in Egypt. The seroprevalence of HCV in haemodialysis centers in Jordan was 34.6% compares with 45% in Tunisia and 45% in Syria (Bdour, 2002). In Turkey, the prevalence of HCV infection among HD has been reported between 31.4% and 51% (Kaya, 2008). In Iran, several studies were done regarding HCV seroprevalence among HD patients (Ansar and Kooloobandi, 2002; Alavian et al., 2003; Amiri et al., 2005; and Hosseini-Moghaddam et al., 2006), in the largest study by Hosseini-Moghaddam and done his colleagues in 2006, 45 HD centers were enrolled in the study, including 1914 HD patients, HCV was found in 8.1% of studied population (Hosseini-Moghaddam et al., 2006). The prevalence rates reported are 1%-29% from Western Europe, 8%–36% from North America, 5.9% in Australia, and 44%-60% in Far Eastern countries (Hayat et al., 2010).

Subjects, Materials and Methods:

Subjects: A total of 236 dialysis patients, 150 (63.6%) male and 86 (36.4%) female, their age ranged from 15 to 78 (44.39 \pm 15.06 S.D.) years. They were attendants the three HD centers in Baghdad; Al-Yarmouk Teaching Hospital, Al-Kadhimiya Teaching Hospital and Al-Karama Hospital. Samples were taken between May and October 2010. The mean duration of HD treatment was (33.91 ± 25.75) months. All patients were dialyzed 2 or 3 times per week and each HD treatment took three to four hours, the patients were distributed into two shifts depending on their haemodialysis centers. Dialyzer membranes were disposable and single use. The clinical diagnosis was obtained from patient records and interview and ethical approval for use of all specimens was obtained. Our exclusion criteria were peritoneal dialysis or history of receiving antiviral and/or interferon therapy for HCV (+) subjects.

Samples: Blood was obtained by vein puncture immediately before HD sessions. Sera were separated from whole blood under optimal conditions for RNA extraction. For this purpose, the blood samples were allowed to clot in the room temperature for 20 minutes and then centrifuged at 2,000 rpm for 10 minutes (-4°C). All samples were divided into three aliquots then immediately frozen and stored at (-20°C) and (-80°C), for serological and molecular assays respectively to minimize degradation of viral nucleic acid, prevent cross contamination and unnecessary thawing and freezing.

<u>Materials and Methods</u>: For anti-HCV antibodies detection two commercial kits were utilized in this part of study. The initial screening for anti-HCV IgG antibody was determined by fourth generation enzyme-linked immunoassay (ELISA) (Bioelisa HCV 4.0 ELISA, Biokit, Spain). The results were interpreted according to manufacturers' instructions. All ELISA positive samples were subjected to confirmatory test using immunoblot assay (EIBA) (Bioblot HCV, Biokit Spain). All seropositive samples were tested individually for the presence of HCV RNA by qualitative RT-PCR (Sacace Biotechnologies, REF V-1-100R, Italy). To permit the molecular analysis of the large number of seronegative samples, a pooling strategy was developed, similar to the method described by Schneeberger et al. (1998). This involved the pooling of four seronegative serum samples and the analysis of the mixture for the presence of HCV RNA. Twenty-five µl of each of the four samples were mixed together, and then 100µl pool was used for the assay. RT-PCR based on four major processes: isolation of HCV RNA from specimens, reverse transcription of the RNA, nucleic acid amplification and detection of the amplified products on agarose gel. To avoid possible contamination with exogenous sequences during extraction or amplification. all nucleic acid extraction, amplification, and detection steps were performed in separate laboratories. Negative and positive controls were extracted, reverse transcribed, and amplified in each batch of samples tested by PCR. All amplified product were analyzed using electrophoresis.

Statistical analysis: Descriptive analysis was done using the statistical package for social studies (SPSS) program for windows software package release 15.

<u>Results:</u>

Seroprevalence of HCV in haemodialysis patients:

The collected sera were subjected to serological screening and confirmation analysis to determine the presence of HCV. The results are summarized in Table (1). Sera from 100/236 (42.37%) patients were found to be anti-HCV positive by ELISA and 97 (41.10%) were subsequently confirmed as being positive by bioblot; 92 (38.98%) of these sera were antibioblot positive HCV giving the true seroprevalence of anti-HCV antibody, five sera gave indeterminate results on immunoblotting and three were negative.

Table (1): Anti-HCV seropositivity by ELISA and bioblot in 236 HD patients.

No.	%
236	100
136	57.63
100	42.37
92	38.98
5	
3	
	236 136 100 92 5

Variation in the prevalence of anti-HCV antibody was observed between the haemodialysis centers. The distribution of HCV-infected patients among the three dialysis centers is shown in Figure (1). Anti-HCV antibody was found in 31/119 (26.05%), 49/78 (62.82%), 17/39 (43.58%) in Al-Yarmouk Teaching Hospital, Al-Kadhimiya Teaching Hospital and Al-Karama hospital, respectively. Thus, the prevalence of HCV infection ranged from 26.05% to 62.82%.

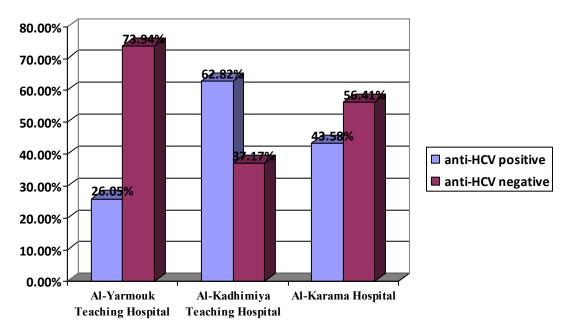


Figure (1): Seroprevalence of HCV infection in the three hemodialysis centers.

HCV RNA prevalence in haemodialysis patients:

HCV-RNA was detected in 29/236 (12.29%) of haemodialysis patients sera. In 92 of 100 (92%) confirmed anti-HCV antibody by bioblot; 24 of these sera were HCV RNA-positive. Five sera gave indeterminate results on immunoblotting; two were HCV RNA-positive and three were negative on immunoblotting; one was HCV RNA-positive), as shown in Table (2) and Figure (2).

All 136 anti-HCV antibody-negative sera were tested by RT-PCR divided among 34 pools. Among the 34 pools of seronegative samples, 2 yielded positive signals for HCV RNA. Then these two positive pools were retested individually by RT-PCR and resulted in two of 136 (1.47%) seronegative sera were confirmed to be HCV-RNA positive. The combination of serological and molecular methods resulted in the most accurate estimation of the number of HCV infections among haemodialysis patients. Using only antibody assays in this population, three HCV RNA-positive patients would have been missed.

Table (2): Comparison	of ELISA,	bioblot and	a RT-PCR	results:	data	on	anti-	HCV	positive	and
negative HD patients with	n HCV RNA	A detection.								

Tes	t results	No. of patients (%)			
EIA	Bioblot	Total	%	PCR positive	%
Positive	Positive	92/100	92%	24/92	26.09%
Positive	Indeterminate	5/100	5%	2/5	40%
Positive	Negative	3/100	3%	1/3	33.33%
Negative		136/236	57.63%	2/136	1.47%
Total		236	100%	29/236	12.29%

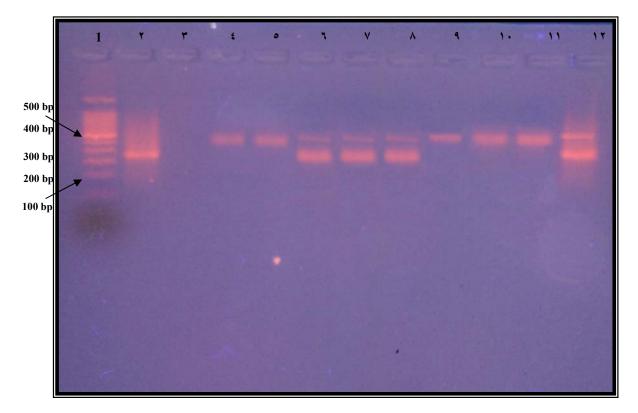


Figure (2): PCR products from haemodialysis patients. Lane 1, DNA marker (100-bp ladder).The 500bp band was present at triple the intensity of the other fragments and serves as a reference indicator, while all other fragments appear with equal intensity on the gel; lane 2, HCV cDNA (C+) serves as positive control for amplification; lane 3, DNA-buffer (C-) serves as negative control for amplification; lane 3, DNA-buffer (C-) serves as negative control for amplification; lane 6 to 8, DNA from haemodialysis patient positive for HCV antibodies; lane 9 to 11, DNA from haemodialysis patients negative for HCV antibodies; lane 12, HCVC+ Rec Fag serves as positive control for RNA isolation.

Discussion:

Prevalence of anti-HCV antibody:

Several prevalence studies of HCV infection have been undertaken in HD patients. The prevalence of anti-HCV antibody in HD subjects ranged between 10% and 55% (Horoz *et al.*, 2006). Epidemiological studies about HCV infection among HD patients in Iraq have reported a prevalence of 7.1%-62% in different cities (Khalaf *et al*, 1996; and Kattab; 2008). While the reported prevalence in the general population in Iraq is ranges from (0.2%) to (0.5%) (Fayadh and Jureidini, 2001).

The prevalence of anti-HCV antibody in our study was found to be (41.10%) and ranged from (26.05%) to (62.82%) according to the dialysis center (Table: 1) and (Figure: 1), these results in accordance with that reported by others (Khalaf et al, 1996; Mohammed, 1997; Othman and Monem, 2001; and Al-Shohaib et al., 2003). HCV prevalence in haemodialysis patients is highly variable between different countries and between different centers in the same locality (Devesa et al., 1997). This difference can be attributed to several influencing factors. First, these studies were performed in different regions, with various HCV prevalence among their normal population. Second, adherence of haemodialysis centers to precautionary measures might be different between centers of various parts of the same country (Alavian et al., 2011). Technical reasons are likely to account for this difference since a different generation EIA and confirmatory immunoblot assay were used for evaluating HCV-antibody seropositivity.

In this study, different seroprevalence of HCV had detected in the three HD centers in Baghdad. A much higher prevalence (62.82%) of anti-HCV was documented among Al-Kadhimiya Teaching Hospital patients and this strongly points to the magnitude of HCV problem among this selected group of patients. The second high prevalence was found in Al-Karama hospital (43.58%), followed by Al-Yarmouk Teaching hospital which has the lowest prevalence (26.05%) (Figure: 1). Nosocomial transmission of HCV infection has been reported to be a considerable route in modern hospital dialysis centers, particularly during the outbreaks of infection (Sabry et al., 2007; and Arrais et al., 2008).

HCV RNA prevalence in haemodialysis patients:

Detection of HCV-PCR is currently the most sensitive and specific method for detecting active infection and to overcome two other problems: that the serological test can not differentiate between acute and chronic infection and cannot detect evidence of infection during window period (Al-Kubaisy et al., 2003). In the present study RT-PCR was used to screen for the presence of HCV RNA in all 236 serum samples. HCV RNA was detected in 29 (12.29%) samples, Table (2). Of these 236 serum samples, 97 were seropositive and HCV-RNA was detected in 26 (26.80%). We conclude that prevalence of hepatitis C infection is high in HD patients in our region, but not associated with active HCV infection. Similarly, Arababadi et al. (2009) from Kerman, Iran found that 30 out of 90 (33.3%) HD patients were RT-PCR positive for HCV-RNA. Also Bdour (2002) from Jordan, found that 30 out of 98 (30.6%) anti-HCV positive sera was harbor HCV-RNA by RT-PCR. In Brazil, HCV viremia was present in 63.5% of the anti HCV positive patients in one study (Carneiro et al., 2001) and (7.6%) in another study (De Albuquergue et al., 2005).

Possible explanations for this low percentage of viremic patients in our population include: the level of viremia may be low and below detectable level of PCR assay at time of sampling, the pattern of fluctuating viremia or intermittent viremia, patients might be cured from HCV infection at time of sampling (Bdour, 2002; Al-Kubaisy *et al.*, 2003; and Wang *et al.*, 2004). PCR-based methods reliability may further be compromised if viral RNA is lost in the serum or plasma through storage or improper laboratory handling or if it is absent from the circulation during sample collection (Zein, 2000).

However, it has also been reported that 7– 68% of haemodialysis patients have intermittent viremia with periods of undetectable HCV RNA for up to 4 weeks. The viral load is relatively low in this group of patients and long-term maintenance haemodialysis decreases the HCV RNA level but does not produce clearance of viremia (Bdour, 2002; and Horoz *et al.*, 2006). Barril *et al.* (2008) detected the presence of antigenomic HCV-RNA in 53% of HD patients with occult HCV (presence of HCV- RNA in liver in absence of anti-HCV and serum HCV-RNA), showing that HCV is replicating in peripheral blood mononuclear cells (PBMC) and suggesting that these patients could be potentially infectious; therefore, occult HCV infection could play a role in HCV spread within HD centers which is a very serious problem.

In the present study, PCR was used to screen for the presence of HCV RNA in all 136 seronegative serum samples. HCV RNA was detected in 2 of 136 (1.47%) of the seronegative samples using pooling strategy, retesting positive pools individually confirmed these result. Also HCV RNA was detected in 1 of 3 (33.33%) of the bioblot negative samples which might be bioblot false negative. The presence of HCV anti-HCV viremia in negative haemodialysis patients has been frequently reported by others researchers (Schneeberger et al., 1998; and Carneiro et al., 2001). In contrast, none of the anti-HCV negative patients were shown to be viremic by the PCR as mention by De Albuquergue et al. (2005). The detection of HCV RNA in non-hepatitis patients could be explained by the fact that the patients might be in the early stage of acute hepatitis, and the antibody had not been produced yet (Wang et al., 2004). On the other hand, one cannot exclude the existence of impaired immune responses some of these patients in (Schneeberger et al., 1998).

Conclude that the detection of HCV RNA by PCR technique permits direct detection of the presence of the virus and also permits detection of infectivity during the seronegative window which reinforces the importance of screening by both serological and PCR methods at regular intervals to identify all HCV-infected patients.

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انتشار عدوى التهاب الكبد الفايروسي نمط "ج" في مرضى غسيل الكلى من ثلاثة مراكز في بغداد، العراق: دراسة استقصائية بواسطة تفاعل البلمرة والطرق المصلية

الخلاصة:

تعتبر العدوى بالتهاب الكبد الوبائي نمط ج مشكلة صحية كبيرة بين مرضى غسيل الكلي في البلدان النامية. وتعتبر عدوى المستشفيات من اهم طرق انتقال الفايروس، ولا سيما خلال تفشى المرض. للمقارنة بين الطرق المصلية والجزيئية في الكشف عن فيروس التهاب الكبد الوبائي تم فحص العينات المصلية لوجود الاجسام المضادة لفايروس التهاب الكبد باستخدام الجيل الرابع لفحص الامتزاز المناعى المقترن بالأنزيم (ELISA) وتم تأكيد الايجابية باستخدام مقايسة اللطخة المناعية (immunoblot (assay. كما وتم اختبار جميع العينات لوجود الحامض النووي الرايبوزي للفايروس (HCV-RNA) بطريقة سلسلة تفاعل البلمرة العكسي (RT-PCR). لقد تم تقييم مدى انتشار العدوى بفايروس التهاب الكبد نمط ج لدى ٢٣٦ من مرضى الغسيل الكلوي في ثلاث من وحدات غسيل الكلي في مدينة بغداد (مستشفى اليرموك التعليمي، مستشفى الكاظمية التعليمي، ومستشفى الكرامة). وكان معدل الانتشار العام (41,10%) في الوحدات الثلاثة (ضمن المجال:٢٦،٠٥ % إلى ٦٢،٨٢%) مع أعلى معدل انتشارسجل في مستشفى الكاظمية التعليمي. وتم اختبار جميع العينات إيجابية المصل بطريقة سلسلة تفاعل البلمره العكسي، وتم العثور على ٩٢/٢٤ (٢٦،٠٩) من العينات الايجابية باللطخة المناعية أكدت أحتواءها على الحامض النووي الرايبوزي. بالإضافة إلى ذلك تم العثور أيضا على ٢/٥ (٤٠%) من العينات غير محددة باللطخة المناعية ، و ٣/١ (٣٣،٣٣) من العينات السلبية باللطخة المناعية لتكون ايضاً إيجابية للحامض النووي الرايبوزي. كما تم فحص جميع العينات المصلية السالبة لوجود الاجسام المضادة للفايروس باستخدام استراتيجية التجميع لوجود الحامض النووي الرايبوزي وكانت النتيجة أيجابية في ٢٣٦/2 (١،٤٧%). أكدت هذه الدراسة على ضرورة الالتزام الصارم بأجراءات السيطرة على العدوى في مراكز غسيل الكلي وتعزيز أهمية الفحص بواسطة كل من الطرق المصلية و تفاعل البلمره العكسي على فترات منتظمة لتحديد جميع المرضي المصابين بالتهاب الكبد الفايروسي نمط ج.

الكلمات المفتاحية: فايروس التهاب الكبد نمط ج، الغسيل الكلوي، الاختبارات المصلية ، تفاعل البلمره العكسي.