

Prevalence of Hepatitis B Virus among Egyptian Hemodialysis Patients with or Without Hepatitis C Viral Infection

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Abstract

Hemodialysis (HD) patients are at increased risk for both hepatitis C virus (HCV) and hepatitis B virus (HBV) infections associated with contaminated blood and blood product transfusion and exposure to contaminated HD equipment during treatment. Thus, the present study aimed to assess the prevalence of HBV, HCV and HBV/HCV co-infection among HD-patients in the most common main three dialysis units in Ismailia governorate, Egypt. Furthermore, to find out the impact of HCV on the HBV infection in those HD-patient. This was done using both serological ELISA and real time PCR molecular techniques. The results showed that HCV infection was the most prevalent one representing about 26%, the HBV infection was less prevalent than HCV 9.3%, and the dual infection was rare representing only 2% of the studied HD-patients in Ismailia governorate. In addition, there was a non-significant difference of both HBV incidence and viral load (copies/mL) between the studied HD-patients with and without HCV infection [P-value=0.36].

Keywords: Hemodialysis, Hepatitis C virus, Hepatitis B virus, Egypt.

Introduction

Hemodialysis patients (HD) are at high risk for acquiring Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections due to the high number of blood transfusion sessions and the potential for exposure to infected patients and contaminated HD machines and equipments (*Telaku et al., 2009*) as well as interpersonal horizontal

transmission in the dialysis units. It was known that higher rates of mortality and morbidity in HD patients than in the general population are caused with HBV and HCV nosocomial infections (*Chu and Lee, 2008*). Moreover, patients with HBV/HCV co-infection have a higher risk of progression to cirrhosis and

hepatocellular cancer (HCC) (*Perz et al., 2006*).

About 170 million people all over the world are infected with HCV (*WHO, 2011*). HCV is an enveloped, positive-sense, single-stranded RNA virus from the family Flaviviridae and belongs to genus Hepacivirus (*Afridi et al., 2014*). Whereas, HBV is one of the most common infectious viruses worldwide. It is estimated that more than two billion people are infected. Approximately 360 million of these are chronically infected (*Dienstag, 2008*). It is a member of *hepadnaviridae* family and the *Orthohepadnavirus* genus. The HBV genome is a partial, double-stranded DNA with four overlapping open reading frames (*Tiollais et al., 1985*).

Theoretically, HCV infection suppresses the replication of HBV and also the expression of HBV surface proteins *in vitro* and *in vivo* (*Chu et al., 1998*). *In vitro* studies performed since the early 90s had clearly demonstrated that the HCV “core” protein strongly inhibits HBV replication (*Shih et al., 1993; Shih et al., 1995; Schüttler et al., 2002; Chen et al., 2003*).

Since the prevalence of HBV and HCV infection among HD-patients is highly variable, this study aimed to estimate the prevalence of HBV, HCV and HBV/HCV co-infection in HD-patients in Ismailia governorate, and to find out the impact of HCV on the HBV infection in HD-patients if any.

Methods:

The present study was conducted on 150 HD-patients’ serum attending the main HD-units in Ismailia governorate: Suez Canal University Teaching Hospital, General Hospital and Suez Canal Authority Hospital. The samples collection continued from January 2015 to January 2016.

Serological assessment: Serum IgG antibodies for HCV were detected by indirect ELISA technique using AccuDiag™ HCV-Ab ELISA kit, Cat.No.1707-12, supplied by Diagnostic Automation/Cortez Diagnostics, Inc. Serum HBV surface antigen (HBsAg) were detected by sandwich ELISA technique using DIAsource HBsAg ELISA kit, Cat.No.#KAPG4SGE3, supplied by DIAsource ImmunoAssays SA, Inc. Both ELISA tests were done according to the manufacturer’s instructions.

Molecular assessment: HBV viral load (copies/mL) was measured using real-time polymerase chain reaction (RT-PCR) according to the manufacturer’s instructions, using the commercially available Artus HBV RG RT-PCR Kit, Cat.No.4506263, Supplied by Qiagen, Germany. The Artus HBV RG PCR Kit constitutes a ready-to-use system for the detection of HBV DNA using RT-PCR. The HBV RG/TM Master contain reagents and enzymes for the specific amplification of a 134 bp region of the HBV genome, and for the direct detection of the specific amplicon in

fluorescence channel Cycling Green of the Rotor-Gene 6000. The methodology were done as follows: Master Mix was prepared by mixing 15 µl of HBV RG/TM Master and 1 µl of HBV RG/TM IC (internal control) per reaction; 15 µl aliquots of master mix were pipetted in to each PCR tube; 10µl of the eluted DNA samples were added to the respective sample PCR tubes to

reach final volume of 25 µl; 10 µl of the quantitation standards (HBV RG/TM QS 1–5) were added to standard tubes and used as positive controls, and 10 µl of PCR grade water were added to the non-template control tubes (NTC) and used as negative control. The amplification conditions were setup as shown in Table (1).

Table 1: HBV amplification settings on Rotor Gene 6000.

	Hold	Cycles		
Temperature (°C)	95	95	55	72
Time (min:s)	10:00	00:15	00:30	00:15
Repeats		n=45		

Results

The study population comprised of 150 HD-patients, 79 were males (52.7 %) and 71 were females (47.3%). Their ages arranged between 10 and 70 years [mean ± SD: (46.7 ± 11.45) years]. Out of 150 patients, 39 were positive for only anti-HCV (26%), 14 patients were positive for HBsAg (9.3%) and dual infection was observed in another three patients (2%).

The total HD-patients were classified according to the presence of Anti-HCV in to two groups: group {1} which included HD-patients with HCV infection (n=42) and Group (2) which included HD-patients without HCV infection (n=108). There was non-statistically significant difference between group {1} and group {2} regarding HBsAg prevalence (p-value= 0.24).

As, the frequency of HBsAg in the serum in group {1} was found to be 7.1%, whereas in group {2} it was 13%. In addition, the mean of HBV viral load in group {1} was 4578999 copies/mL, whereas it was 2683504 copies/mL in group {2}. Thus, there was a non-significant difference between the HD-patients with and without HCV infection regarding the HBV viral load [P-value=0.36].

Discussion

The prevalence of HBV, HCV and the dual HBV/HCV coinfection is variable from hemodialysis center to center, region to region and country to country. In a study by *Saravanan et al. (2009)*, out of 251 patients; 67 (26.7%) were positive for anti-HCV, 112 (44.6%) were positive for HBV, 15 (5.9%) had

dual infection, and 57 (22.7%) were non-HBV/non-HCV. Other studies reported the prevalence of HBV, HCV, and HBV/HCV co-infection as 7, 46, and 37% (*Bhaumik and Debnath, 2012*); 11, 30 and 3% (*Jain and Nijhawan, 2008*); 2.6, 31.1, and 1.2% (*Alashek et al., 2012*); 1.5%, 33.5%, and 0.8% (*Malhotra et al., 2016*), respectively. This can be attributed to variations in the implementation of infection control measures in the HD-units (*El-kader et al., 2010*). Thus, regular evaluation may play an essential role due to this variation in results and also to reduce the burden of HBV and HCV infections among HD-patients.

Regarding the assessment of the impact of HCV on HBV among the studied HD-population, the present study showed that there was non-significant difference between group (1) and group (2) regarding the HBV-DNA viral load (p-value=0.45) and HBsAg frequency (p-value= 0.24). In fact, the reason(s) for significantly higher values of HBV viral load found in HBV patients with and without HCV infection in the present study is not clear. As, HBV-DNA levels have been reported to remain low and stable over time, which can probably explain the low mortality rates due to liver disease in HD-patients in developed countries (*Fabrizi et al., 2002*). This was conflicted with the present study findings. But, the relatively low

response rates to HBV vaccination in this group of patients might contribute to the ongoing HBV transmission and reactivation in the dialysis setting (*Mina et al., 2010*). In addition, this high HBV viremia may be due to the immune system alteration in the studied HD-patients.

After using molecular technique (RT-PCR) to detect the HBV infection, a new reassessment of the HBV/ HCV coinfection was done, as the incidence percentage of HBV/ HCV dual infection was increased to be 16.7% (n=7) instead of 2% (n=3), this strongly support the importance of using molecular techniques besides the serological ones to detect HBV in the dialysis units.

In Conclusion:

HCV infection was the most prevalent one than the HBV infection and the dual infection was rare in the studied HD-patients in Ismailia governorate. In addition, there was a non-significant difference of both HBV incidence and viral load (copies/mL) between the HD-patients with and without HCV infection [P-value=0.36]. Thereby, HCV did not have a significant impact on HBV infection in HD-patients.

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تقييم الإصابة بفيروس الإلتهاب الكبدي (بي) في مرضي الغسيل الكلوي المصابين وغير المصابين بفيروس الإلتهاب الكبدي الوبائي (سي)

يعتبر مرضى غسيل الكلى هم أكثر الأشخاص عرضه لعدوى الإلتهاب الكبدي الفيروسي بسبب إرتفاع عدد جلسات نقل الدم وكذلك بسبب التعامل و التعرض لآلات الغسيل الكلوي الملوثة حيث وجد أن عدوي كلاً من فيروس الإلتهاب الكبدي المزمن (سي) و (بي) من أكثر عدوى المستشفيات الشائعة التي تسبب إرتفاع معدلات الوفيات في مرضى الغسيل الكلوي عنه في عموم الأصحاء، ووجد أنه تختلف نسبة الإصابة بهذين الفيروسين في مراكز الغسيل الكلوي من منطقة إلى أخرى ، وبالتالي كان الهدف من هذه الدراسة هو تحديد معدل الإلتشار المصلي لفيروس الإلتهاب الكبدي (بي) بين مرضي الغسيل الكلوي بالإسماعيلية سواء المصابين أو غير المصابين بفيروس الإلتهاب الكبدي المزمن (سي)، وذلك عن طريق عمل تحليل سيروولوجي للمستضد السطحي لفيروس الإلتهاب الكبدي (بي) (HbsAg) بإستخدام تقنية الإليزا، بالإضافة الي استخدام تقنية تفاعل البوليميراز المتسلسل (RT-PCR) ، وذلك للتقدير الكمي لفيروس الإلتهاب الكبدي (بي). وتضمنت هذه الدراسة مائه و خمسون من مرضى الغسيل الكلوي الذين تم اختيارهم عشوائياً من أهم وحدات الغسيل الكلوي الحكومية بالإسماعيلية وهما : مستشفى جامعة قناة السويس، مستشفى الإسماعيلية العام، مستشفى هيئة قناة السويس. وتم تقسيمهم إلى مجموعتين علي حسب الإيجابية لوجود الاجسام المضادة لفيروس الإلتهاب الكبدي المزمن (سي)، وبينت الدراسة انه فيما يتعلق بالإنتشار المصلي للإصابة بفيروس الإلتهاب الكبدي (بي) والوبائي (سي) والإصابة المزدوجة بالفيروسين معاً بين مجموعة الدراسة الأتي : كانت عدوى فيروس الإلتهاب الكبد الوبائي (سي) هي الأكثر انتشاراً حيث بلغت نسبة الإصابة 26%، وكانت نسبة الإصابة بفيروس الإلتهاب الكبدي (بي) 9.3%، بينما كانت الإصابة المزدوجة بكلاً الفيروسين نادرة حيث بلغت 2% فقط من المرضى الذين شملتهم الدراسة في محافظة الإسماعيلية، بالإضافة الي عدم وجود فرق ذو دلالة إحصائية فيما يخص الإنتشار المصلي أو التقدير الكمي لحينوم فيروس الإلتهاب الكبدي (بي) بين مرضي الغسيل الكلوي المصابين و غير المصابين بفيروس الإلتهاب الكبدي الوبائي (سي) مما يدل علي انه ليس لفيروس الإلتهاب الكبدي الوبائي (سي) تأثير علي معدل إنتشار الإصابة بفيروس الإلتهاب الكبدي (بي) في المرضي المتضمنين في الدراسة.