

## SUPER INFECTION WITH TWO GENOTYPES OF HEPATITIS C VIRUS IN SYRIAN HEMODIALYSIS PATIENTS

العدوى الفائقة بنمطين جينيين من فيروس التهاب الكبد C

عند مرضى التحال الدموي في سوريا

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### ملخص البحث

**هدف البحث:** يشكل التهاب الكبد الفيروسي C سبباً رئيسياً وعالمياً لأمراض الكبد المزمنة، غالبية المرضى المصابين بالتهاب الكبد الفيروسي C غير عرضيين، لفيروس التهاب الكبد C ستة أنماط جينية رئيسية تبعاً للجينوم الفيروسي ترقم من 1 إلى 6، يكون التهاب الكبد الفيروسي C أكثر شيوعاً عند مرضى المراحل النهائية للمرض الكلوي المعالجين بالتحال الدموي بالمقارنة مع عامة السكان. إن مرضى المراحل النهائية للمرض الكلوي المعالجين بالتحال الدموي والمصابين بنمط جيني واحد من فيروس التهاب الكبد C لديهم خطورة أعلى للإصابة بنمط جيني آخر (العدوى الفائقة) عن طريق مراكز التحال، توجد عدة أسباب متهممة بهذه العدوى منها قلة تدريب العاملين بمراكز التحال بمجال ضبط العدوى، وجود العديد من المعدات الملوثة بفيروس التهاب الكبد C بمراكز التحال. يهدف هذا البحث إلى دراسة العدوى الفائقة بنمطين جينيين من التهاب الكبد الفيروسي C عند مرضى المراحل النهائية للمرض الكلوي المعالجين عبر التحال الدموي في بسوريا، والأسباب التي أدت لحصول هذه الإصابة.

**طرق البحث:** تم خلال سنة فحص 25 مريضاً من مرضى المراحل النهائية للمرض الكلوي المعالجين بالتحال الدموي عن طريق الاستخراج الآلي باستعمال جهاز Roche MagNA pure. تم اختبار عينات الحمض الريبي النووي RNA سوية مع عينات شواهد سلبية عن طريق آلة الجينات الدوارة باستعمال تقنية Sacace Real Time HCV genotype. شملت العينة المستهدفة بالبحث المرضى المصابين بالقصور الكلوي بالمراحل النهائية المعالجين بالتحال الدموي بمراكز التحال بسوريا.

**النتائج:** لوحظ من أصل 25 مريضاً من مرضى المراحل النهائية للمرض الكلوي وجود إصابة عند 4 مرضى (بنسبة 16%) بنمطين جينيين من فيروس التهاب الكبد C (3 مرضى مصابين بالنمطين 1a-4 (بنسبة 12%) ومريض واحد مصاب بالنمطين 1b-4 (بنسبة 4%) في ثلاث مراكز للتحال الدموي بسوريا.

**الاستنتاجات:** لوحظت زيادة نسبة العدوى بفيروس التهاب الكبد C عند مرضى القصور الكلوي بالمراحل النهائية الموضوعين على التحال الدموي، مع احتمالية إصابتهم بأكثر من نمط جيني واحد بنفس الوقت (العدوى الفائقة) أثناء علاجهم بالتحال الدموي. توجد أسباب متعددة لانتقال فيروس التهاب الكبد C لدى هؤلاء المرضى أثناء تواجدهم بمراكز التحال الدموي للعلاج، والتي يمكن تفاديها من خلال المراقبة الروتينية للإنتانات بمراكز التحال الدموي وإتباع تعليمات مركز ضبط العدوى فيما يخص البرامج المسحية عن هذا الفيروس عند مرضى القصور الكلوي بالمراحل النهائية المعالجين بالتحال الدموي، وتدريب العاملين في مراكز التحال الدموي روتينياً ببرامج ضبط العدوى.

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## ABSTRACT

**Objective:** Hepatitis C virus is a global and major cause of chronic liver disease. Most of the infected HCV carriers are asymptomatic. HCV has six major genotypes according to its viral genome, numbered one to six. HCV is more common among patients with end-stage renal disease (ESRD) that require hemodialysis compared to the general population. ESRD patients with one genotype HCV infection have a high risk of developing infection with another HCV genotype (super infection) from dialysis centers. Many reasons appear to be involved including: many centers are administered by untrained staff of infection control and a lot of contaminated equipment with HCV in dialysis centers. The aim of this work was to investigate the super infection with two genotypes of hepatitis C virus (HCV) in patients with end-stage renal disease, who presented to dialysis centers in Syria, and the causes that lead to this super infection.

**Methods:** During one year (from January 2018 till January 2019), we examined 25 ESRD patients who were under hemodialysis by using Roche MagNA pure (automated extraction). RNA samples were tested together with negative controls on Roter-Gene machine using Sacace Real Time HCV genotype. The target population includes patients who underwent hemodialysis in dialysis centers in Syria.

**Results:** Of the 25 patients enrolled in this study (4 patients) 16% have two genotypes {genotypes 1a-4 found in 3 patients (12%), and genotypes 1b-4 found in one patient (4%)}, from 3 different hemodialysis centers.

**Conclusions:** There were an increasing infection with HCV among ESRD patients who underwent hemodialysis and the possibility to have infection with more than one genotype of HCV (super infection) during dialysis session. There are many reasons associated with HCV transmission within a facility that could be avoided by routine monitoring for infection in the facilities, follow CDC recommendations for HCV screening of hemodialysis patients, train the dialysis staff routinely in a program for preventing infection.

## INTRODUCTION

Hepatitis C virus (HCV) is a global and major cause

of chronic liver disease. Current estimates suggest that between 130 and 170 million people worldwide are infected with HCV, and more than 350,000 people die from HCV-related illnesses each year. Most of the infected HCV carriers are asymptomatic.<sup>1</sup>

HCV is an enveloped RNA virus with a high degree of genomic variability. HCV has six major genotypes according to its viral genome,<sup>2</sup> numbered one to six. Most of HCV patients in Syria have infected with genotype 4.<sup>3</sup>

HCV is a blood-borne pathogen, and transmission modes of the disease are parenteral and to a lesser extent sexual. Another important ways of transmission include intravenous drug misuses, needle stick injury and sharing tooth brushes/razors.<sup>4</sup> Patients in hemodialysis units have an increased risk for contracting HCV, and high prevalence rates found in hemodialysis units around the world.<sup>5</sup>

HCV is more common among patients with end-stage renal disease (ESRD) who require hemodialysis compared to the general population.<sup>4-8</sup> The ESRD patients with one genotype HCV infection have a high risk of developing infection with another HCV genotype (super infection) from dialysis centers.<sup>9</sup> Many reasons appear to be involved including: many centers are administered by untrained staff of infection control and a lot of contaminated equipment with HCV in dialysis centers.<sup>4</sup> There are a few studies in this field and they have insufficient data to determine the exact percentage.<sup>10</sup>

The aim of this cross-sectional study is to investigate the super infection with two genotypes of HCV in ESRD patients who presented to dialysis centers in Syria, and the causes that lead to this super infection. There are no previous studies in Syria about this topic. This research could lead to more efforts to be done to limit the incidence of HCV transmission in this group of patients.

## METHODS

This study is a cross-sectional study conducted

in dialysis centers during a year (from January 2018 till January 2019) in Damascus, Syria. The target population includes patients who underwent dialysis in renal replacement therapy units in Syria. All of patients signed the free and informed consent form, responded to a sociodemographic and behavioral questionnaire, and submitted a 5-milliliter sample of venous blood.

**Detection, genotyping and the RNA-HCV viral load:** The HCV viral load of all 25 ESRD patients was not presented at the beginning of hemodialysis. After agreeing and signing a consent form by each participant, 5 ml of venous whole blood was obtained.

Each participant was interviewed and answered a comprehensive questionnaire. It includes questions on: gender, age, place of birth, marital status, working department, current position, having intravenous drug antecedents, weather needle stick injured, surgical intervened, underwent blood transfusion or hemodialysis. It also includes lifestyle habits such as sharing tooth brushes, razors or piercing. Circumcision status, household of family member with HCV, history of dealing of HCV infected patients, history of occupational injury from HCV infected patient.

Five milliliters sample of blood (5 ml) was collected from the patients before hemodialysis session using a vacuum tube containing EDTA (ethylenediaminetetraacetic acid), and was immediately

centrifuged. The viral RNA was extracted from 200 ul sample of plasma using Roche MagNA pure (automated extraction). The assay was performed according to the manufacturer’s instructions. RNA samples were tested together with negative controls on Roter-Gene machine using Sacace Real Time HCV genotype. Statistical analysis was carried out using Microsoft Access Database.

## RESULTS

Of the 25 patients enrolled in this study, 4 patients (16%) have two genotypes {genotypes 1a-4 found in 3 patients (12%), and genotypes 1b-4 found in one patient (4%)}, from 3 different dialysis centers around Syria.

## DISCUSSION

This study reveals an increasing infection with HCV among ESRD patients who underwent hemodialysis, and the possibility to have infection with more than one genotype of HCV (super infection) during dialysis session. There are many reasons associated with HCV transmission within a facility including: the dialysis machine may have a role in transmission of HCV and that is not well understood,<sup>11,12</sup> blood transfusion during dialysis, the duration of hemodialysis.

In a study for Nguyen DB, et al showed that failure to properly clean and disinfect priming receptacles between patients, handling blood specimens near

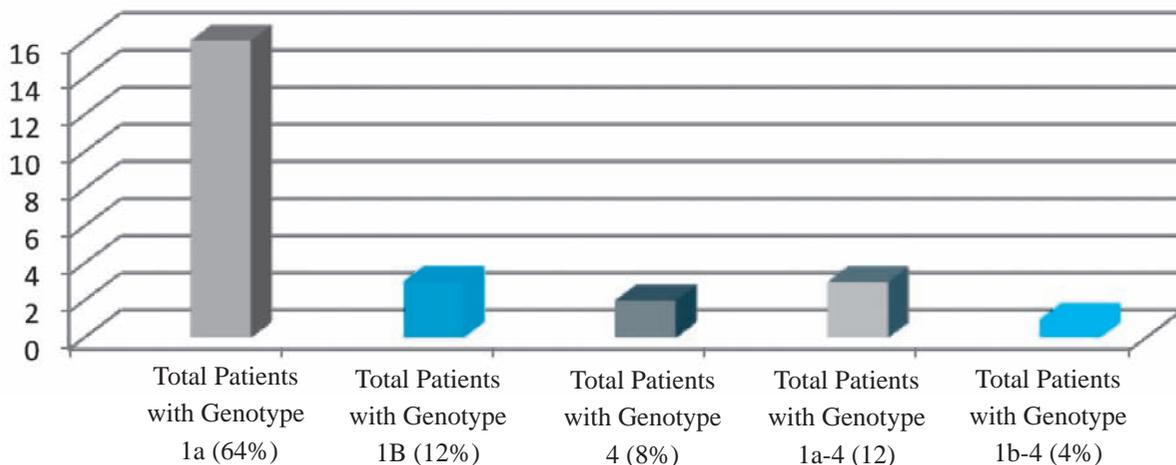


Figure 1. The incidence of HCV among hemodialysis patients.

medication preparation area and clean supplies, use of a mobile cart to deliver injection medications, and failure to clean and disinfect high-touch surfaces on dialysis machines between patients. Failure to clean and disinfection of equipment and environmental surfaces, adherence to hand hygiene and glove use, vascular access care, and medication preparation and administration. Other facility factors likely contribute to the failure of maintaining optimal infection control practices. Examples that have been determined to be significant include small or cluttered treatment areas that do not allow adequate space and separation between dialysis stations, quick turnovers between shifts and rushed treatment schedules that do not allow adequate time for cleaning and disinfection, high staff turnover, and staff lacking infection control training and supervision. All of these factors can contribute to non-adherence to proper infection control practices.<sup>13</sup> All ESRD patients on dialysis should be screened for anti-HCV antibodies at the time of admission to dialysis center to detect any infection with HCV. They have to test HCV-RNA-PCR (polymerase chain reaction) if the anti-HCV antibodies' result is positive, and if the HCV-RNA-PCR's result is positive, they should take treatment, but if the anti-HCV antibodies test is negative, they should be monitored every 6 months for anti-HCV antibodies.

A patient who has previous healed infection with HCV (i.e., achieved sustained viral response), his/her anti-HCV antibodies may still positive, and in this situation he/she should do screening via HCV-RNA-PCR to detect any new infection. Any detected HCV infection must be reported to local public health agencies. In addition, anti-HCV negative patients should have a monthly ALT monitoring, and an HCV detecting test should be done if any unexplained increase in ALT occurs.<sup>13</sup>

False negative anti HCV-antibodies results are common in dialysis patients, and that could be related to immune dysfunction in such patients. In order to prevent these results, we lowered the threshold to distinguish between anti HCV core-positive and anti HCV core-negative in this study in a way that does not compromising test specificity.<sup>14</sup> Considering the relapse hypothesis, the authors speculate that HCV viral load was not presented in the serum at the time of

testing for 25 patients, because of either treatment or disappearance of HCV-RNA to extra-vascular sites. In a study for Teresa CA and her colleagues showed that regarding the hypothesis of re-infection, the patients could be infected with multiple HCV strains, and one strain would prevail as the viremic virus. After the end of a successful treatment, one displaced strain would become predominant and influence the outcome of therapy although some studies have described super infection in intravenous drug users and hemodialysis patients, this event is uncommon and probably cannot explain the switch in genotype between the patients.<sup>15</sup> It can be differentiated between the re-infection and super infection by using the nucleic acid-based techniques.<sup>15</sup>

The disadvantage of this study is that a few numbers of patients were enrolled, so we cannot make a precise percentage of HCV transmission among ESRD patients.

Solutions to prevent transmission of HCV in dialysis facilities; adherence to recommended infection control and practicing are needed, training the dialysis staff routinely in a program for preventing infection, follow CDC recommendations for HCV screening of hemodialysis patients, evaluate the facility routinely for infection. The previous recommendation steps should be done at all dialysis facilities to prevent transmission of HCV among their patients.

## CONCLUSIONS

We investigate the super infection with two genotypes hepatitis C virus (HCV) infection in 25 patients with ESRD and found that 4 of 25 patients (16%) have two genotypes from 3 different dialysis centers around Syria. There were an increasing infection with HCV among ESRD patients who underwent hemodialysis and the possibility to have infection with more than one genotype of HCV (super infection) during dialysis session. There are many reasons associated with HCV transmission within a facility that could be avoided by routine monitoring for infection in the facilities, Follow CDC recommendations for HCV screening of hemodialysis patients, train the dialysis staff routinely in a program for preventing infection.

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