Prevalence of HCV/HIV co-infection among haemophilia patients in Baghdad

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معدل انتشار العدوى المشتركة بفيروس التهاب الكبد «سي» وفيروس الإيدز بين المصابين بالناعور وقار عبد القهار الكبيسي، خالد طارق النائب، منال حبيب

الخلاصة: لتقدير معدل الانتشار المصلي للعدوى بفيروس التهاب الكبد « سي » بين المصابين بالناعور، ولمعرفة النمط الجيني الأكثر انتشاراً، تم فحص 47 من المصابين بالناعور وبعدوى فيروس الإيدز لتحرِّي أضداد فيروس التهاب الكبد « سي ». وقد أسفر إجراء تفاعل سلسلة البوليميراز والمقايسة المناعية الإنزيمية للدنا (DNA) عن اكتشاف رنا (RNA) فيروس التهاب الكبد « سي » والتنميط الجيني الخاص به. وقد بلغ معدل الانتشار المصلي لأضداد فيروس التهاب الكبد « سي » 66٪. ومن بين المصابين بالعدوى المشتركة بفيروس التهاب الكبد « سي » وفيروس الإيدز، والبالغ عددهم 31 مريضاً، لم يكن لدى 21 منهم (أي نسبة 7.76٪) سوابق نقل دم. وقد اكتشفنا أربعة من الأنماط الجينية لفيروس التهاب الكبد، وهي: 1أ، 1ب، 4، و4 المختلط مع 3أ. وكان النمط 1ب هو الأكثر تواتراً. وقد يكون العامل الثامن (عامل التجلُّط) الملوَّث هو المسؤول عن اكتساب المرض.

ABSTRACT To estimate the seroprevalence of HCV infection among HIV-infected haemophiliacs and to demonstrate the most prevalent HCV genotype, 47 HIV-infected haemophilia patients were screened for anti-HCV antibodies. By performing polymerase chain reaction and DNA enzyme immunoassay, HCV-RNA was detected with subsequent genotyping. Seroprevalence of anti-HCV antibodies was 66.0%. Of 31 HCV/HIV co-infected patients, 21 (67.7%) had no history of blood transfusion. We detected 4 HCV genotypes: 1a, 1b, 4 and 4 mixed with 3a, HCV-1b being the most frequent. Contaminated factor VIII (clotting factor) could be responsible for disease acquisition.

Prévalence de la co-infection VHC-VIH chez des patients hémophiles à Bagdad

RÉSUMÉ Afin d'estimer la séroprévalence de l'infection par le VHC chez des hémophiles infectés par le VIH et de connaître le génotype du VHC le plus répandu, on a procédé à une recherche d'anticorps anti-VHC chez 47 patients hémophiles infectés par le VIH. La *polymerase chain reaction* (PCR) et une méthode de révélation immunoenzymatique d'ADN ont été utilisées pour détecter l'ARN du VHC et un génotypage a été ensuite effectué. La séroprévalence des anticorps anti-VHC était de 66,0 %. Sur les 31 patients co-infectés VHC-VIH, 21 (67,7 %) n'avaient pas d'antécédents de transfusion sanguine. On a détecté 4 génotypes du VHC : 1a, 1b, 4 et 4 mixte avec 3a, le VHC-1b étant le plus fréquent. Le facteur VIII contaminé (facteur de coagulation) pourrait être responsable de l'acquisition de la maladie.

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Introduction

The introduction of factor VIII concentrates in 1970 has improved the treatment of patients with coagulopathy. Early administration of these concentrates has resulted in a longer life span and a better quality of life. However, a rise in cases of acute viral hepatitis was observed following the use of these factor VIII concentrates [1,2]. The high incidence of hepatitis after such treatment was first identified by Kasper and Kipnis in 1972 [2]. Since the 1980s, the rate of transmission of hepatitis C virus (HCV) to patients with haemophilia was reported to be 100% until effective procedures to inactivate the virus were introduced [3–5].

Studies on HCV genotypes in people with haemophilia have identified types 1, 2, 3 and 4, in addition to genotype 5, which is found in only a few people. Such studies have lead to speculation about the source of some commercial factor VIII concentrates [5]. Hepatitis C virus and human immunodeficiency virus (HIV) co-infection in haemophilia patients is common and causes a complex interaction. Replication of HCV is accelerated in the presence of HIV (probably as a result of immunodeficiency): the level of HCV-RNA in co-infected haemophilia patients has been found to be 58 times greater than in those infected with HCV alone [6]. Telfer et al. found the relative risk of developing liver failure in HCV infection increased 21-fold after HIV infection [7].

Interferon alpha remains the most promising treatment for hepatitis C. For patients who do not have haemophilia, a liver biopsy is essential in deciding who will benefit from treatment, but this is a hazardous procedure for a patient with haemophilia. Hence, knowledge of other variables, such as HCV genotype and viral load, may be helpful as patients with type 2 and 3 and those with lower viral loads have the greatest chance of responding [8]. Therefore, we conducted this study in order to identify the prevalence of HCV among HIV patients in Baghdad and to determine the most prevalent genotypes among this group.

Methods

All 47 HIV-infected haemophilia patients attending Ibn Al-Khatib hospital in Baghdad From January 1998 to June 1998 were selected for this study. The age range was 12–46 years. Serum samples from each participant were dispensed into 2 screw-capped vials; 1 was stored at -20 °C and the other at -70 °C. The former was used for detection of HCV-specific antibodies and the latter was used for molecular analysis. Informed consent was obtained from all participants, or from the mother for those under 18 years. There were no refusals to participate.

For determination of anti-HCV antibodies we used a third generation enzyme immunoassay (HCV EIA, United Biomedical Inc., Hauppauge, New York). Positive results were confirmed by third generation immunoblot assay (Lia-Tek-III kit, Organon, Amsterdam). All assays were carried out at the Central Health Laboratory, Baghdad.

Twenty anti-HCV positive sera (stored at -70 °C) were transferred in dry ice to the laboratories of Sorin Biomedica, Saluggia, Italy. There, they were tested for HCV-RNA positivity and subsequent HCV genotyping using an advanced molecular method based on a combination of 2 well-established techniques, the polymerase chain reaction and DNA enzyme immunoassay. First, RNA was extracted from 140 µL of the serum sample according to the method used by Garson et al. [9] and was subjected to reverse transcriptase. The cDNA developed was amplified at the 5'UTR region

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by single step polymerase chain reaction according to the manufacturer's method. The amplified cDNA was then hybridized to specific oligonucleotide probes fixed to a solid phase through an avidin-biotin bridge, using an avidin-coated plate from Genetik (Sorin Biomedica, Saluggia, Italy). The hybrids were then detected by a standard enzyme linked immunosorbent assay (Sorin Biomedica) using monoclonal antibody specific for double stranded DNA. All steps were carried out according to the manufacturer's instructions. Positive and negative control samples were included throughout the assay. The absorbance of the coloured reaction was read at 450 nm and 630 nm.

Using the same DNA enzyme immunoassay method but 6 different oligonucleotide probes, HCV genotypes as well as their different subtypes were detected (Sorin Biomedica). The test was then carried out as described above.

The HCV genotypes/subtypes were classified according to Simmond's nomenclature [10]. Statistical analysis was performed by using chi squared and *t*-test with P < 0.05 significant.

history of blood transfusion was reported among 21 (44.6%) haemophilia patients infected with HIV; only 10 of these (32.3%) were co-infected with HCV (Table 1).

Of the 26 patients who had no history of blood transfusion, 21 (67.7%) were HIV/ HCV co-infected. Co-infection was significantly higher among these patients ($\chi^2 = 4.3$, P < 0.005) (Table 1).

Of the 31 sera confirmed positive for anti-HCV antibodies, 20 were randomly selected for molecular analysis. Only 14 of these (70.0%) demonstrated HCV-RNA positivity. In 13 HIV/HCV co-infected patients with positive results for HCV-RNA, we detected 4 different genotypes/subtypes: single (1a, 1b and 4) and mixed (3a plus 4) (Table 2). The highest proportion of patients (61.5%) was infected with HCV genotype 1b. Genotype 3a was detected in the mixed pattern of HCV infection only. A single RNA sample failed to reveal a positive signal based on DNA enzyme immunoassay analysis with any of the investigated primers for HCV specific genotypes.

Discussion

Results

Overall seroprevalence of anti-HCV antibodies among Iraqi haemophilia patients infected with HIV was 66.0% (31/47). A The haemophilia community, already hit by HIV infection, is now facing the problem of HCV infection [11]. Extensive seroepidemiological studies have shown that 60%-91% of patients with haemophilia

Table 1 Association of history of blood transfusion with HCV infection in haemophilia patients infected with HIV							
Anti-HCV	History of blood tr		od trans	fusion	Total		
status	No.	%	No.	%	No.	%	
Positive	10	32.3	21	67.7	31	66.0	
Negative	11	68.8	5	31.3	16	34.0	
Total	21	44.7	26	55.3	47	100.0	

 $\chi^2 = 4.3$ (with Yates correction); P < 0.005.

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Table 2 HCV genotype distribution amongHCV-RNA positive sera of haemophiliapatients infected with HIV					
HCV genotype	HCV-RNA No.	positive sera %			
1a	2	15.4			
1b	8	61.5			
4	2	15.4			
3a & 4	1	7.7			
Total	13ª	100.0			

^aOne HCV-RNA positive sample was untypeable.

have antibodies to HCV [12-14]. Other studies have recorded a rate of 85%-98% [15,16]. However, the prevalence of HCV infection in HIV-infected patients varies widely in different studies and depends, above all, on the distribution of the various risk factors for acquiring HIV in each population. In our study, the overall prevalence of anti-HCV antibodies among HIV infected patients was 66.0% a finding that is in agreement with results recorded by others [12-14] but much higher than the 3.21% detected among pregnant Iraqi women by Al-Kubaisy [17]. The high prevalence of HIV/ HCV co-infection may be related to the fact that both viruses share the parenteral route as the main portal of entry [18].

Although, the risk of acquiring HCV and HIV infection by blood transfusion is greatly reduced in the developed countries [19], it is still considered a major risk factor for acquiring such infections in the developing countries [20] owing to a lack of new detection techniques based on determination of viral genetic material before the appearance of antibodies (window period).

In addition to blood transfusion, unscreened factor VIII is considered a major source of HCV transmission. Overall, 55.3% of haemophilia patients infected with HIV and 67.6% of those co-infected with HIV/HCV had no history of blood transfusion. This finding supports the theory that factor VIII could be the source of infection of both HIV and HCV in our country. There has been speculation that the route of HCV transmission could be one of the determinants of outcome, as patients who receive a greater viral inoculum are those infected by transfusion of blood and blood products [21,22].

Also, 70% of 20 haemophilia patients who were co-infected displayed HCV-RNA positivity. The fluctuating pattern of HCV viraemia is one explanation; cure from HCV infection cannot, however, be excluded. There is conflicting opinion about the outcome of HIV/HCV co-infection. Some studies have, however, indicated that there is a greater progression of HIV in the presence of HCV infection [23,24] and that for every 10-fold increase in HCV-RNA level there was a 1.6-fold increase in the risk of progression to acquired immune deficiency syndrome (AIDS) [25].

There are few data available at the moment on HCV genotypes which predominate in HIV/HCV co-infected patients. We detected 4 different genotypes. Patients treated with multiple batches of clotting factor concentrate will have been exposed to a large amount of virus as well as to many viral genotypes [26], which may explain why we found mixed HCV genotypes in 1 of our patients. However, the presence of an untypeable HCV genotype was probably a result of the limited availability of HCVspecific primers for genotype analysis [27].

In recent years several studies have analysed the distribution of HCV genotypes in different populations and have found a clear predominance of genotype 1, especially 1a and 1b [28,29], which is compatible with the finding in this study. A global genotype 1 prevalence of 83.3% was found in HIV patients in a recent American study;

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type 3 was found in 9% [30,31]. Studies on co-infected patients in Europe had, however, reported a higher probability of finding genotype 3. It was suggested that this was related to the low rate of intravenous drug users in the United States of America compared to Europe.

There has been much speculation about whether some HCV genotypes can themselves be a factor in poor prognosis. In most cases, genotype 1b (the most prevalent in our study) has been identified as being associated with a worse outcome and with greater prevalence of cirrhosis. It has been speculated that genotype 1 might be an

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inducer of T-cell helper type 2 immunologic response instead of type 1, which is the most effective for correct control of the disease [25]. On the other hand, the association has not been found in all studies [26].

We suggest that contaminated factor VIII may be the source of transmission of HCV to haemophilia patients infected with HIV, and further studies should be carried out to investigate this. Further studies are also necessary to evaluate the impact of HCV infection on HIV survival rates and, if possible, to evaluate the benefits of treating HCV infection in HIV seropositive patients.

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