Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia

W.A. Al-Kubaisy,[1] K.T. Al-Naib[1] and M. Habib[2]

معدل انتشار أضداد فيروس التهاب الكبد «سي» في المصل بين الأطفال العراقيِّين المصابين بالثلاسيمية

وقار عبد القهار الكبيسي، خالد طارق النائب، منال عدنان حبيب

الخلاصـة: أُجريت دراسة لمعدل انتشار فيروس التهاب الكبـد «سي» وتـوزُّع الأنمـاط الجينية في مـصل 559 مـن الأطفال العراقيِّين المصابين بالثلاسيمية ممَّن تكرَّر نقل الدم إليهم. وقد تم اكتشاف أضداد التهاب الكبـد «سي» في 376 عينة مصلية (أي بنسبة 67.3٪) باستخدام الجيل الثالـث مـن المقايـسة المناعيـة الإنزيمية ومقايسة اللطخـة المناعية التوكيدية. وتبيَّن في 48 عينة من عينات المصل المنتقاة عشوائياً البالغ عـددها 78 عينـة (أي بنـسبة 61.5٪) أنها إيجابية المصل للرَّنا (RNA) الخاص بفيروس التهاب الكبد «سي». كما وُجد النمط المصلي 1a لفيروس التهـاب الكبد «سي» في ثلاث عـشرة عـينة (أي بنـسبة 27.1٪)، والـنمط المـصلي 1b في إحـدى عـشرة عـينة (أي بنسبة 22.9٪)، والنمط المصلي 4 في سبع عشرة عينة (بنسبة 35.4٪)، ومزيج من النمط المصلي 1b والنمط المصلي 4 في سبع عينات (أي بنسبة 11.6). وتوصي الدراسة بضرورة اتخاذ تدابير صارمة لمكافحة انتشار فيروس التهاب الكبـد «سي»، عن طريق إدخال التقنيات المتقدِّمة لتحرِّي المتبرعين بالدم.

ABSTRACT The seroprevalence of hepatitis C virus (HCV) specific antibodies and HCV genotypes distribution were studied among 559 Iraqi children with thalassaemia in receipt of repeated blood transfusions. HCV-specific antibodies were detected in 376 (67.3%) serum samples using third-generation enzyme immunoassay and confirmatory immunoblot assays. Of 78 randomly selected sera, 48 (61.5%) were HCV-RNA positive. HCV genotypes 1a, 1b, 4 and mixed 1b and 4 were demonstrated in 13 (27.1%), 11 (22.9%), 17 (35.4%) and 7 (14.6%) sera respectively. Strict measures for the controlling the spread of HCV are needed by introducing advanced techniques for blood donor screening.

Séroprévalence des anticorps spécifiques du virus de l'hépatite C chez des enfants iraquiens thalassémiques

RÉSUMÉ On a étudié la séroprévalence des anticorps spécifiques du virus de l'hépatite C (VHC) et la distribution des génotypes du VHC chez 559 enfants iraquiens thalassémiques recevant des transfusions sanguines répétées. Des anticorps spécifiques anti-VHC ont été détectés dans 376 échantillons de sérum (67,3 %) au moyen de tests immuno-enzymatiques de troisième génération et d'immunoblots de confirmation. Sur les 78 sérums choisis au hasard, 48 (61,5 %) étaient positifs pour l'ARN du VHC. Les génotypes 1a, 1b, 4 et mixtes 1b et 4 du VHC ont été mis en évidence dans 13 (27,1 %), 11 (22,9 %), 17 (35,4 %) et 7 (11,6 %) sérums respectivement. Des mesures strictes pour enrayer la propagation du VHC sont nécessaires en introduisant des techniques perfectionnées de dépistage des donneurs de sang.

[1]Department of Community Medicine; [2]Department of Microbiology, Al-Nahrain University College of Medicine, Baghdad, Iraq (Correspondence to W.A. Al-Kubaisy: waqar_abd@yahoo.co.uk).
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Introduction

Before the introduction of screening of blood donors for hepatitis C virus (HCV), the risk of acquiring HCV infection as a result of transfusion was about 10% [1]. Repeated blood transfusion in thalassaemia patients is necessary for their survival; however, such transfusions increase their exposure only to not HCV but also to other blood-borne viruses (hepatitis B, hepatitis G, human immunodeficiency virus, transfusion transmitted virus) [2]. HCV infection is the leading cause of post-transfusion hepatitis worldwide [3]. The prognosis of thalassaemia major has improved greatly over the last 20 years owing to intensive blood transfusion regimens and chelation therapy [3].

Testing for HCV-ribonucleic acid (RNA) and genotyping is the most reliable indicator for HCV infection and it correlates with infectivity, disease progression and response to treatment. It also confirms the presence of infection in patients with inconclusive antibody reactivity. So the aims of this study were to identify the seroprevalence of hepatitis C virus specific antibodies among children with thalassaemia in receipt of blood transfusions in a teaching hospital in Baghdad and to investigate the HCV genotype distribution among these patients.

Methods

A cross-sectional study was done on 559 thalassaemic children ranging from 2–10 years. Those patients were chosen randomly from Ibn Al Balady teaching hospital, Baghdad, during 1998. A blood sample of 5–10 mL was obtained from each subject.

Sera were separated immediately (within 1 hour) to prevent viral RNA degradation and each serum sample was then dispensed into 2 screw-capped vials stored at –20 ºC and –70 ºC. The former was utilized for detection of anti-HCV antibodies while the latter was used for HCV-RNA detection and subsequent genotyping/subtyping.

For anti-HCV antibody determination, we used a third-generation enzyme immunoassay kit (EIA-3) (UBI HCV EIA, United Biomedical, USA) at the baseline evaluation. The positive anti-HCV reactivity was then confirmed by a third-generation immunoblot assay (LiaTek-III kit, Organon, Amsterdam), which carries 6 different antigens in separate bands from core1, core2, E2/NS1, NS3, NS4 and NS5 proteins. Samples were considered positive if they were reactive to at least 2 bands, negative when not reactive and indeterminate when reactive to a single band (following the manufacturer's instructions).

Of 559 sera, 78 samples were selected randomly and transferred in an ice card to the laboratories of Sorin Biomedica, Saluggia, Italy. These included 50 positive samples, 12 negative samples and 16 with indeterminate results by confirmatory immunoblot assay (LiaTek-III) test. They were tested for HCV-RNA positivity and subsequent HCV-genotyping using an advanced molecular method. This method is based on a combination of 2 well-established techniques: the polymerase chain reaction (PCR) and DNA enzyme immunoassay (DEIA). At the beginning, RNA was extracted from 140 µL of serum sample according to Garson et al. [6] and was subjected to reverse transcription using reverse transcriptase from avian myeloblastosis virus (Promega Corporation, USA). All the reagents required for RNA extraction were provided with the kit (Qiagen, USA). The developed cDNA was amplified at the 5UTR region by single-step PCR according to the laboratory's method (Sorin Biomedica). The amplified cDNA was then hybridized to a specific oligonucleotide

probe fixed to solid phase through an avidin-biotin bridge, using avidin-coated plates (Genentech, San Francisco, USA). The formed hybrids were then detected by a standard enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody specific for double-stranded DNA. All the steps were carried out following the manufacturer's instructions (Sorin Biomedica). Positive and negative control samples were included throughout the assay. Finally, the absorbance of the coloured reaction was read at 450 nm and 630 nm.

The sequence variability in the 5UTR and core regions of the amplified cDNA products was investigated by the same DEIA method, using 6 different oligonucleotide probes, corresponding to their HCV genotypes as well as their different subtypes (Sorin Biomedica). The test was then carried out as described previously. The HCV genotypes/subtypes were classified according to Simmond's nomenclature [7]. Statistical analysis was performed using chi-squared tests.

Results

Out of 559 children with thalassaemia, 445 were classified as seropositive for HCV-specific antibody using EIA-3. When subjected to the confirmatory test (LiaTek-III),

376 (67.3%) of these samples were seropositive for HCV-specific antibody and 39 (8.8%) were seronegative; the remaining 30 (6.7%) gave indeterminate results.

A total of 78 serum samples were further investigated for HCV-specific RNA, followed by genotyping/subtyping. Out of those, 48 (61.5%) sera displayed HCV-RNA positive results. Interestingly, 2 (16.7%) of the 12 negative anti-HCV sera, and 8 (50.0%) of the 16 sera with indeterminate results, demonstrated positive HCV-RNA. However, 12 (24.0%) of 50 anti-HCV positive sera displayed HCV-RNA negative results (Table 1).

Those 48 HCV-RNA positive sera were subjected to HCV-genotyping/subtyping. Genotypes 1a, 1b, 4 and mixed 4 and 1b were detected in 13 (27.1%), 11 (22.9%), 17 (35.4%) and 7 (14.6%) samples respectively, with a predominance of genotype 4 (50.0%) as a single or mixed pattern of infection. Moreover, the 2 anti-HCV seronegative samples that were positive for HCV-RNA were of HCV genotype 4.

Discussion

The incidence of transfusion-associated hepatitis has been substantially reduced after the implementation of screening of blood donors for HCV-antibodies [4]. Nev-

Table 1 **Hepatitis C virus (HCV) antibody seroprevalence in 78 sera of children with thalassaemia**

Immunoblot assay results	No. tested	HCV results			
		No. positive	%	No. negative	%
Positive	50	38	76.0	12	24.0
Negative	12	2	16.7	10	83.3
Indeterminate	16	8	50.0	8	50.0
Total	78	48	61.5	30	39.5

$\chi^2 = 15.5$; df = 2; P = 0.0004.
RNA = ribonucleic acid.

Table 2 **Hepatitis C virus (HCV) genotype distribution among HCV-positive sera of children with thalassaemia**

HCV-genotype	HCV-positive	
	No.	%
1a	13	27.1
1b	11	22.9
4	17	35.4
1b & 4	7	14.6
Total	48	100.0

ertheless, chronic transfusion recipients such as thalassaemia patients still suffer a high frequency of liver disease due to transfusion-related iron overload and infection with blood-borne agents [5]. HCV is responsible for the majority of cases of post-transfusion non-A non-B hepatitis in patients with thalassaemia major [6]. The prevalence of blood-borne viral infections in multi-transfused patients is related to the prevalence of the viruses in the community [2].

In Iraq, HCV-antibody seroprevalence among pregnant women has been recorded as 3.2%, a figure that reflects the seroprevalence among the normal population [8]. In the current study, the seroprevalence of HCV-antibody among multi-transfused children with thalassaemia was 84.5%. Surveys on thalassaemic patients worldwide have found variable rates of anti-HCV-antibody seroprevalence. Moreover, Iraqi children with thalassaemia have demonstrated a higher rate than that reported in other countries, such as 40.7% in Jordan [2], 40% in Saudi Arabia [3], 14% in Turkey [9], 17% in Taiwan [10], 10% in Sri Lanka [11], 68% in India (as cited by Jan-shin et al. [12].

Technical reasons are unlikely to account for this difference, since a third-generation EIA and third-generation confirmatory im-

munoblot assay were used for evaluating HCV-antibody seropositivity by all the above workers. It would be interesting to find out whether age and geographical differences could account for such variations. Notably, differences in sample size and the introduction of blood donor screening with restricted policy in other countries may resulted in a lower prevalence than that reported in the current study. Two important reasons can be suggested as an explanation for such a high rate reported in this study. First, screening of blood for HCV in blood banks in Iraq started in 1996 and secondly, shortage of blood and other supplies in the health services as a result of the international sanctions against Iraq in the 1990s resulted in lower standards of cleaning and sterilization of medical instruments and a shortage of disposable syringes and needles. However, Parti et al. in 1999 [4] reported a similarly high rate of HCV-positivity (85.2%) that may be related to the larger sample size in that study. In addition, different HCV genotypes may induce different levels of HCV-antibodies [13].

In thalassaemia major there is a greater likelihood of progression to chronicity and the occurrence of cirrhosis is also more frequent. Detection of HCV-RNA in serum and liver tissue through the use of RT-PCR is currently the most sensitive and specific method for detecting active infection. It overcomes two other problems: the serological test cannot differentiate between acute and chronic infection and cannot detect evidence of infection during the window period (usually 6–10 weeks and occasionally up to 9 months) between acquiring infection and the detectable marker [14]. In addition, the existence of indeterminate results by the confirmatory serological test makes it more difficult for the researcher to give a clear definitive interpretation of the serological assay.

A molecular assay based on HCV-RNA detection might be the answer to these obstacles. In the current study, 78 serum samples were subject to molecular analysis. Interestingly, the positive predictive value of serum samples was 76% (38 of 50 samples) with respect to HCV-RNA, whereas 12 (24%) of LiaTek-III positive sera failed to display HCV-RNA with RT-PCR. These negative PCR findings might be explained as follows. First, the initial level of viraemia may be low and below detectable levels for PCR assay at the time of sampling [15]. Secondly, there may be a pattern of fluctuating viraemia or intermittent viraemia [16]. Thirdly, patients might be have been cured of HCV infection at the time of sampling. The fourth possibility is that the results of LiaTek-III might be a false positive.

Therefore the final interpretation of the immunoblot findings is that a positive PCR result obtained from a reliable test indicates a chronic infection and potential infectivity, while negative results do not eliminate this possibility [17]. Our results also showed that 50% of sera (8 out of 16) that were considered as indeterminate and about 16.7% of sera (2 out of 12) that were considered as negative by LiaTek-III were all found to be positive by RT-PCR. A possible interpretation of this finding is that sample taking might be during the window period [14]. Moreover, false negative or indeterminate results may be obtained, especially if individuals were infected by HCV other than genotype 1 which was used as a source of antigen in the assay [13].

Identifying geographical distribution of various genotypes of HCV is useful for understanding the epidemiological status, detecting the mode and source of infection, designing the control programmes, evaluating the response to treatment and developing diagnostic and vaccine production methods [18]. Based on the RT-PCR/DEIA assay, the most common HCV-genotypes circulating among Iraqi pregnant women (which reflects the normal population) are 4, 1, 1b, 1a and 3a, in decreasing order [8]. In the current study, the most predominant HCV-genotype among children with thalassaemia was type 4 followed by 1a, 1b and mixed 1b and 4, in order of frequency. This finding is in accordance with other reports concerning HCV genotype distribution in the Middle East [18] and in Greece [19] where the predominance HCV genotype among thalassaemic patients was type 4.

However, others have reported different observations, with a predominance of HCV-genotypes 3a in more than 50% of HCV-infected thalassaemia patient in Switzerland [20] or mixed genotypes 2/1b in Taiwan [21]. Mixed genotypes in thalassaemia can be explained if the patient is infected concomitantly or successively by 2 different genotypes of HCV usually through multiple blood transfusions [11]. However, there might also be a condition in which a variant of HCV occurs during infection, which reacts to different specific primers from a distinct sequence of domains, inducing the results of seemingly double infections [22].

In conclusion, this study has established the seroprevalence of HCV-specific antibody and the distribution of HCV-genotypes in thalassaemia children as a high-risk group. This data will serve as a baseline by which to monitor changes in the epidemiology of HCV and drifts in genotype distribution. Finally, until a vaccine against HCV becomes available, preventive measures such as blood donor screening using advanced techniques for detecting HCV infection before transfusion and strict infection control measures are crucial for the control of spread of HCV among these high-risk patients.

References

1. Minola E et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. *Blood*, 2002, 99(12):4588–91.

2. Al-Sheyyab M, Batieha A, El-Khateeb M. The prevalence of hepatitis B, hepatitis C and HIV markers in multi-transfused patients. *Journal of tropical pediatrics*, 2001, 47:239–42.

3. Al-Hawsawi ZM. Prevalence of HCV antibody. *Annals of Saudi medicine*, 2000, 20:488–9.

4. Sharara AI, Hunt CM, Hamilton JD. Hepatitis C. *Annals of internal medicine*, 1999, 125:658–68.

5. Garson JA et al. Demonstration of viraemia patterns in haemophilics treated with hepatitis-C-virus-contaminated factor VIII concentrates. *Lancet*, 1990, 336:1022–5.

6. Simmonds P et al. A proposed system for the nomenclature of hepatitis C virus genotypes. *Hepatology*, 1994, 19(5):1321–4.

7. Prati D et al. A multicenter prospective study on the risk of acquiring liver disease in anti-hepatitis C virus negative patients affected from homozygous β-thalassemia. *Blood*, 1998, 92:3460–6.

8. Al-Kubaisy WA. *Epidemiologic and genotypic distribution of hepatitis C in Iraqi pregnant women*. PhD thesis,1998.

9. Kebudi R et al. Seroprevalence of hepatitis B, hepatitis C, and human immunodeficiency virus infections in children with cancer at diagnosis and following therapy in Turkey. *Medical and pediatric oncology*, 2000, 34:102–5.

10. Chung JL et al. Hepatitis C and G virus infections in polytransfused children. *European journal of pediatrics*, 1997, 156(5):546–9.

11. Fernando S et al. Antibodies to HCV in patient who have has multiple transfusions in Sri Lanka. *Acta paediatrica*, 2001, 86:1135–7.

12. Wu JS et al. Genotype distribution of hepatitis C virus infection in Taiwan. *Journal of medical virology,* 1994, 44(1):74–9.

13. Pawlotsky JM et al. Serological determination of hepatitis C virus genotype: comparison with a standardized genotyping assay. *Journal of clinical microbiology*, 1997, 35(7):1734–9.

14. Sibal A, Mishra D, Arora A. Hepatitis C in childhood. *Journal of the Indian Medical Association*, 2002, 100(2):93–8.

15. Mison LM et al. Prevalence of hepatitis C virus and genotype distribution in an Australian volunteer blood donor population. *Transfusion*, 1997, 37:73–8.

16. Watson JP et al. Hepatitis C virus density heterogeneity and viral titre in acute and chronic infection: a comparison of immunodeficient and immunocompetent patients. *Journal of hepatology*, 1996, 25(5):599–607.

17. Skidmore S. Hepatitis C virus—epidemiology and serological diagnosis. *Journal of medical microbiology*, 1993, 39:321–2.

18. Simmonds P. Clinical prevalence of hepatitis C virus genotypes. *Gut*, 1997, 40:291–3.

19. Spiliopoulou M et al. Response to interferon alfa 2b therapy in multitransfused children with beta thalassaemia major and chronic hepatitis C. *European journal of clinical microbiology and infectious diseases*, 1999, 18:709–15.

20. Diamantis I et al. Genotype distribution of hepatitis C infection in Greece: correlation with different risk factors and response to interferon therapy. *European journal of*

gastroenterology and hepatology, 1998, 10(1):75–9.

21. Ni YH et al. Hepatitis C viral infection in thalassaemic children: clinical and mo-

lecular studies. *Pediatric research*, 1996, 39(2):323–31.

Hepatitis C

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily by direct contact with human blood. The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized.

No vaccine is currently available to prevent hepatitis C and treatment for chronic hepatitis C is too costly for most persons in developing countries to afford. Thus, from a global perspective, the greatest impact on hepatitis C disease burden will likely be achieved by focusing efforts on reducing the risk of HCV transmission from nosocomial exposures (e.g. blood transfusions, unsafe injection practices) and high-risk behaviours (e.g. injection drug use).

Source: WHO Fact sheet No. 164
/Available at: http://www.who.int/mediacentre/factsheets/fs164/en