Distribution of hepatitis C virus genotypes in haemodialysis patients of Guilan, northern Islamic Republic of Iran

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توزُّع الأنماط الجينية من فيروس الالتهاب الكبدي سي بين مرضى الديال الدموي في جيلان، شمال جمهورية إيران الإسلامية

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الخلاصة: من المعلوم أن معدل حدوث العدوى بالتهاب الكبد سي بين مرضى الديال الدموي مرتفع. وتهدف هذه الدراسة إلى التعرُّف على الأنماط الجينية لفيروس الالتهاب الكبدي سي بين مرضى الديال الدموي المزمن والإيجابيين لفيروس الالتهاب الكبدي سي في مقاطعة جيلان في شمال جمهوريةِ إيران الإسلامية. وقد استخدم الباحثون مقايسة الإنزيم المرتبط بالممتز المناعي (إليزا) والتفاعل السلسلي للبوليميراز. واتَّضح أنه من بين 514 مريضاً أُدرجوا في الدراسة وُجدت نسبة 11.9٪ إيجابية مصلياً لفيروس الالتهاب الكبدي سي، و6.2٪ إيجابية لـ (رَنَا) فيروس الالتهاب الكبدي سي HCV-RNA. وقد عيّن الباحثون الأنماط الجينية لفيروس الالتهاب الكبدي سي باستخدام مشروعات نوعية لها. وكانت أكثر الأنماط الجينية شيوعاً هي –1أ: (59.4٪)، و–3أ: 40.6٪. ولم يشاهد أيُّ تَرَابُط يعتد به بين الأنماط الجينية للفيروس وبين العمر أو الجنس. وقد كان نموذج الأنماط الجينية لفيروس الالتهاب الكبدي سي بين المرضى الذين يعالجون بالديال الدموي في مقاطعة جيلان مختلفاً عن سائر بلدان الشرق الأوسط، وأكثر شبهاً ببلدان شمال أمريكا وأوروبا حيث يسود النمط الجيني 1.

ABSTRACT The incidence of hepatitis C virus (HCV) infection among patients undergoing haemodialysis is high. The aim of this study was to identify the HCV genotypes among chronic haemodialysis patients who were HCV positive in Guilan province, northern Islamic Republic of Iran. All patients on haemodialysis at Guilan haemodialysis centres were enrolled. Enzyme-linked immunosorbent assay and polymerase chain reaction testing were performed. Out of 514 enrolled patients, 11.9% were serologically HCV positive and 6.2% had positive HCV-RNA. HCV genotypes were determined with HCV genotype-specific primers. The most frequent genotypes were 1a (59.4%) and 3a (40.6%). There was no significant relationship between virus genotypes and sex or age. The HCV genotype pattern among haemodialysis patients in Guilan province was different from other countries of the Middle East and was more similar to North American and European countries where genotype 1 is predominant.

Répartition des génotypes du virus de l'hépatite C chez des patients hémodialysés à Guilan (Nord de la République islamique d'Iran)

RÉSUMÉ L'incidence de l'infection par le virus de l'hépatite C chez les patients hémodialysés est élevée. La présente étude visait à identifier les génotypes du virus de l'hépatite C chez des patients hémodialysés infectés dans la province de Guilan (Nord de la République islamique d'Iran). Tous les patients hémodialysés dans les centres spécialisés de Guilan ont été recrutés. Un test immuno-enzymatique et une amplification en chaîne par polymérase ont été réalisés. Sur 514 patients recrutés, 11,9 % étaient sérologiquement positifs au virus de l'hépatite C et 6,2 % étaient positifs à l'ARN-VHC. Les génotypes du virus de l'hépatite C ont été déterminés avec des amorces spécifiques de génotypes du virus. Les génotypes 1a (59,4 %) et 3a (40,6 %) étaient les plus fréquents. Aucune relation significative n'a été retrouvée entre les génotypes du virus et le sexe ou l'âge des patients. La répartition des génotypes du virus de l'hépatite C chez les patients hémodialysés dans la province de Guilan était différente de celle des autres pays du Moyen-Orient. Elle ressemblait davantage à la répartition observée en Amérique du Nord et dans les pays européens où le génotype 1 prédomine.

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Introduction

The incidence of hepatitis C virus (HCV) infection among patients undergoing haemodialysis is high. Patients with renal disease are at increased risk of acquiring HCV because of prolonged vascular access and the potential of exposure to infected patients and contaminated equipment. Hepatitis C is the most prevalent liver disease among haemodialysis patients. Meanwhile, liver disease itself is a significant cause of morbidity and mortality in patients with end-stage renal disease (ESRD) treated by dialysis or transplantation [1–3].

HCV is classified into 6 major genetic groups (designated genotypes 1 to 6), each comprising multiple subtypes (designated a, b, c, etc.). These genotypes have different geographical distributions. Genotype has been shown to be a predictor of the response to antiviral treatment. Furthermore, the genotyping of HCV is a useful tool in molecular studies carried out to establish the source of nosocomial infection outbreaks in haemodialysis centres [4,5].

HCV genotype prevalence varies in different parts of the world and is a considerable epidemiologic concern [6]. Although the relationship between virus genotype and disease severity is controversial, there is some evidence to suggest that patients with HCV subtype 1b have a poorer response to treatment with interferon alpha [6,7].

Previous studies from Islamic Republic of Iran have shown that genotypes 1a and 3a are the most common genotypes in our country [6,8–10]. This is different from the dominant genotypes of other countries in the Eastern Mediterranean region [6,11,12]. The aim of this study was to reveal the distribution of different HCV genotypes among haemodialysis patients in Guilan province which is located in the north of the Islamic Republic of Iran.

Methods

This cross-sectional survey was carried out at 11 haemodialysis units in Guilan from January to May 2008. A total of 514 patients with ESRD were under treatment of haemodialysis in Guilan within this period. They were asked to take part in this study and informed consent was obtained from all participants. The diagnosis of HCV was made on the basis of the presence of anti-HCV antibodies in sera detected by 3rd generation enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic BioProbes srl), and positive HCV-RNA tested by polymerase chain reaction (PCR) amplification of 5'-UTR with nested primers (Artus Qiagen GmbH). Positive ELISA samples were frozen at –70 °C for PCR evaluation. HCV genotypes were determined with HCV genotype specific primers using HCV genotype kit (Biodiversity srl).

Statistical evaluations were performed using *SPSS*, version 14. Prevalences and 95% confidence intervals (CI) were calculated. The chi-squared test was used to analyse data and *P*-value < 0.05 was considered statistically significant.

Results

The serological data collected showed that 61/514 (11.9%) patients had positive anti-HCV by ELISA. The prevalence of anti-HCV ranged from 0% to 25% in different areas of the province (data not shown). The test was confirmed by PCR in 32/514 (6.2%) patients.

Of the HCV-RNA positive patients 24 (75.0%) were male and 8 (25.0%) were female and the most prevalent detected genotypes were genotypes 1a (59.4%, 95% CI: 42.4%–76.4%) and 3a (40.6%, 95% CI: 23.6%–57.7%). Among 24 males with HCV-RNA+ (positive PCR) 15 (62.5%) were genotype 1a and 9 (37.5%) were genotype 3a. Among females 50.0% were genotype 3a and 50.0% were 1a. There was no significant relationship between sex and HCV genotype ($P > 0.05$).

One interesting result was the high rate of genotype 3a to 1a (64.7% versus 35.3% respectively) among haemodialysis patients of Rasht, the capital city of the province, compared with other haemodialysis centres of Guilan (13.3% versus 86.7% respectively) ($P = 0.005$) (Table 1).

Patients were divided into 3 age groups (Table 1) but no significant relationship between HCV genotype and age group was detected ($P > 0.05$). The mean (SD) age of patients with genotype 1a was 49.3 (SD 15.6) years and with genotype 3a was 54.1 (SD 12.4) years ($P = 0.37$).

Discussion

This is the first study to investigate the seroprevalence and genotypes of HCV in haemodialysis patients in Guilan, Islamic Republic of Iran. The prevalence of anti-HCV positive ELISA test in our study was 11.9%, which is lower than the mean prevalence of anti-HCV among a haemodialysis population in Tehran (21.0%, range 2.9%–55.9%) [9]. In another study in a neighbouring province (Mazandaran), Taziki et al. reported a similar prevalence of 12% in 2006 [13]. In Saudi Arabia, Pakistan and Turkey the prevalences of HCV among haemodialysis patients were 84.6%, 68.0% and 19.1% respectively [14,15].

The predominant genotypes among our studied patients were 1a (59.4%) and 3a (40.6%). This is in accordance with Zali et al.'s study, the first study on prevalence of HCV genotyping from Iranian population, conducted on Tehran haemodialysis patients [8]. Later, Samimi-Rad et al., in a survey on Iranian anti-HCV positive patients from Tehran and 5 other cities of the country, showed that genotype 1a was predominant (47%) and genotypes 3a,

Table 1 Hepatitis C virus (HCV) subgenotypes according to location in Guilan province and age group

Variable	HCV genotype				Total	*P*-value
	1a		3a			
	No.	%	No.	%	No.	
Location						0.005
Rasht (capital city)	6	35.3	11	64.7	17	
Other areas	13	86.7	2	13.3	15	
Sex						> 0.05
Male	15	62.5	9	37.5	24	
Female	4	50.0	4	50.0	8	
Age group (years)						> 0.05
20–40	6	75.0	2	25.0	8	
40–60	7	53.8	6	46.2	13	
> 60	6	54.5	5	45.5	11	
Total	19	59.4	13	40.6	32	

1b and 4 were detected in 36%, 8% and 7% of the patients respectively [10]. In another study at 45 haemodialysis centres of Tehran, Hosseini-Moghaddam et al. revealed that 3a (30.3%) and 1a (28.8%) were the most prevalent genotypes, and 1b, 3b and 4 accounted for 18.2%, 3.0%, and 16.7% respectively [9]. More recently, Somi et al. in East Azerbaijan reported 74.6%, 5.5% and 5.5% for genotypes 1a, 3a and 1b respectively [6]. Keyvani et al. also reported the same predominant genotypes in HCV-positive cases acquired from different sources [16]. Although genotype 4 is mainly reported from the Middle East and central Africa [17], only 2 studies in Islamic Republic of Iran reported genotype 4 among the top 4 common genotypes [9,10].

Table 2 Worldwide distribution of hepatitis C virus (HCV) genotypes and subtypes

Region	Reference	Most common genotype/subtype
Arab countries		
Egypt	[26]	4
Saudi Arabia	[27,28]	4
Syrian Arab Republic	[29]	4
Kuwait	[30]	4
Lebanon	[31]	4
Islamic Republic of Iran		
Tehran	[8]	1a
Tehran	[9]	3a
Tehran	[10]	1a
East Azerbaijan	[6]	1a
Guilan	Current study	1a
North America		
United States	[32,33]	1a
Europe		
North	[32,33]	1a
South and East	[34]	1b
Africa		
Tunisia	[35]	1b
Morocco	[36]	1b
Cameroon	[37]	4
South Africa	[38]	5

Similar to the previous studies on the genotype distribution in the Islamic Republic of Iran, none of our patients was infected by genotype 2. This finding is in contrast with the global HCV genotype distribution [9]. Generally, genotypes 1a and 3a are the most common genotypes in Islamic Republic of Iran and the current study confirmed this.

A noticeable point was the high prevalence of genotype 3a compared with 1a (almost twice as many) in Rasht haemodialysis centres. This is the reverse of other haemodialysis centres of the province, where genotype 1a was the most prevalent genotype. These differences in the predominant HCV genotype between the capital city and other cities in the province may reflect different sources of infection. This illustrates the value of genotyping in investigating HCV infection routes. More studies are needed to determine the sources of infection and where to focus infection control measures.

In comparison with other countries the pattern of our genotypes was different from other Middle East countries, where genotype 4 has been found to be the most prevalent subtype [4,18,19]. In fact the pattern was more similar to those reported from Brazil, France [20–22], England, Australia and Scotland [10]. In western neighbours of the Islamic Republic of Iran, such as Turkey, genotype 1b is prevalent. However, in eastern border countries such as Pakistan and India, genotype 3a (which is endemic in South-East Asia) is prevalent [8]. In North America and Northern Europe 1a is the most common subtype followed by 2b and 3a [18]. Worldwide the distribution of HCV genotypes and subtypes are shown in Table 2.

In our study, like the studies of Somi et al. [6] and Kabir et al. [22] there was no significant difference in genotype distribution in terms of age group and sex. This is different from reports in some countries such as Turkey and Korea, where genotype 1b was the prevalent genotype among older patients [23,24] and genotype 2 was the common HCV genotype among younger patients [25]. Only one study in the Islamic Republic of Iran has reported the relationship between age and HCV genotypes and showed that study 1b was the common genotype among older patients [16]. Generally, whenever the genotype of HCV is related to age, it is common to find genotype 1b in older patients.

Conclusion

The HCV genotype pattern among haemodialysis patients in Guilan province was different from other countries of the Middle East and was more similar to North American and European countries where genotype 1 is predominant.

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References

1. Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and patients with end-stage renal disease. *Hepatology*, 2002, 36:3–10.
2. Chong VH, Zinna HS. Hepatitis C virus infection and haemodialysis: experience of a district general hospital in Brunei Darussalam. *Singapore Medical Journal*, 2008, 49:916–920.
3. Tokars JI et al. National Surveillance of dialysis-associated diseases in untied states, 2000. *Seminars in Dialysis*, 2002, 15:162–171.
4. Freitas SZ et al. Prevalence, genotypes and risk factors associated with hepatitis C virus infection in hemodialysis patients in Campo Grande, MS, Brazil. *Memorias do Instituto Oswaldo Cruz*, 2008, 103:405–407.
5. Zein NN. Clinical Significance of hepatitis C virus genotypes. *Clinical Microbiology Reviews*, 2000, 13:223–235.
6. Somi MH et al. Hepatitis C virus genotypes in patients with end-stage renal disease in East Azerbaijan, Iran. *Saudi Journal of Kidney Disease and Transplantation*, 2008, 19(3):461–465.
7. Berg T et al. Prognostic relevance of hepatitis C virus genotype for responsiveness to interferon-alpha. *Zeitschrift fur Gastroenterologie*, 1995, 33:426–430.
8. Zali MR et al. Hepatitis C virus genotypes in the Islamic Republic of Iran: a preliminary study. *Eastern Mediterranean Health Journal*, 2000, 6:372–377.
9. Hosseini-Moghaddam SM et al. Distribution of hepatitis C virus genotypes among hemodialysis patients in Tehran—a multicenter study. *Journal of Medical Virology*, 2006, 78:569–573.
10. Samimi-Rad K et al. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS 5B region. *Journal of Medical Virology*, 2004, 74:246–252.
11. Selcuk H et al. Distribution of HCV genotypes in patients with end-stage renal disease according to type of dialysis treatment. *Digestive Diseases and Sciences*, 2006, 51:1420–1425.
12. Bozdayi G et al. Hemodiyaliz hastalarinda hepatit C virus (HCV) enfeksiyon varliginin arastirilmasi ve HCV genotip dagiliminin belirlenmesi [The presence of hepatitis C virus (HCV) infection in hemodialysis patients and determination of HCV genotype distribution]. *Mikrobiyoloji bulteni*, 2002, 36:291–300.
13. Taziki O, Espahbodi F. Prevalence of hepatitis C virus infection in hemodialysis patients. *Saudi Journal of Kidney Diseases and Transplantation*, 2008, 19:475–478.
14. de Paula Farah K et al. Hepatitis C, HCV genotypes and hepatic siderosis in patients with chronic renal failure on haemodialysis in Brazil. *Nephrology, Dialysis, Transplantation*, 2007, 22:2027–2031.
15. Makhlough A, Jamshidi M, Mahdavi R. Hepatitis C prevalence studied by polymerase chain reaction and serological methods in haemodialysis patients in Mazandaran, Iran. *Singapore Medical Journal*, 2008, 49:921–923.
16. Keyvani H et al. Distribution frequency of hepatitis C virus genotypes in 2231 patients In Iran. *Hepatology Research*, 2007, 37:101–103.
17. Ramia S, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. *International Journal of Infectious Diseases*, 2006, 10:272–277.
18. Elsawy EM et al. Stereotyping of hepatitis C virus in hemodialysis patients. Comparison with a standardized genotyping assay. *Diagnostic Microbiology and Infectious Disease*, 2005, 51:91–94.
19. Al-Faleh FZ et al. Hepatitis C virus genotypes in patients with chronic liver disease and hemodialysis patient from Saudi Arabia. *Journal of Viral Hepatitis*, 1995, 2:293–296.
20. Esporoto-Santo MP et al. Genotyping hepatitis C virus from hemodialysis patients in central Brazil by line probe assay and sequence analysis. *Brazilian Journal of Medical and Biological Research*, 2007, 40:545–550.
21. Tamalt C et al. Genomic and phylogenetic analysis of hepatitis C virus isolates a survey of 535 strains circulating in southern France. *Journal of Medical Virology*, 2003, 71:391–398.
22. Kabir A, Alavian SM, Keyvani H. Distribution of hepatitis virus genotypes in patients infected by different sources and its correlation with clinical and virological parameters. *Comparative Hepatology*, 2006, 5:40.
23. Altuglu I et al. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in western

Turkey. *International Journal of Infectious Diseases*, 2008, 12:239–244.

24. Pena MJ et al. Prevalencia de genotipos del virus de la hepatitis C: epidemiologia y caracteristicas histologicas. [Prevalence of hepatitis C virus genotypes: epidemiology and histology characteristics.] *Enfermedades Infecciosas y Microbiologia Clinica*, 1998, 16:456–460.
25. Lee H et al. Distribution of hepatitis C virus genotypes in Jeju Island. *Korean Journal of Hepatology*, 2008, 14:28–35.
26. Ray SC et al. Genetic epidemiology of hepatitis C virus throughout Egypt. *Journal of Infectious Diseases*, 2000, 182:698–707.
27. Shobokshi OA, Serebour FE, Skakni LL. Hepatitis C genotypes/subtypes among chronic hepatitis patients in Saudi Arabia. *Saudi Medical Journal*, 2003, 24(Suppl. 2):S87–91.
28. Osoba A. Hepatitis C virus genotypes in the Kingdom of Saudi Arabia. *Saudi Medical Journal*, 2002, 23:7–12.
29. Abdulkarim AS et al. Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis V virus subtypes. *American Journal of Tropical Medicine and Hygiene*, 1998, 50:571–576.
30. Pasca A et all. Genotypes of hepatitis C virus in Kuwait. *Medical Principles ad Practice*, 2001, 10:55–57.
31. Ramia S et al. Hepatitis C virus genotypes and hepatitis G virus infection in Lebanese thalassemics. *Annals of Tropical Medicine and Parasitology*, 2002, 96:197–202.
32. Mahaney K et al. Genotypic analysis of hepatitis C virus in American patients. *Hepatology*, 1994, 20:1405–1411.
33. McOmish F et al. Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serologic reactivity and rate of alanine aminotransferase abnormalities. *Transfusion*, 1993, 33:7–13.
34. Viazov S et al. Hepatitis C virus genotypes in different regions of the former Soviet Union (Russia, Belarus, Moldova, and Uzbekistan). *Journal of Medical Virology*, 1997, 53:36–40.
35. Djebbi A et al. Genotypes of hepatitis C virus circulating in Tunisia. *Epidemiology and Infection*, 2003, 130:501–505.
36. Cacoub P et al. Étude épidémiologique et virologique des infections par le virus de l'hépatite C au Maroc [Epidemiologic and virologic study of hepatitis C virus infection in Morocco]. *Gastroenterologie Clinique et Biologique*, 2000, 24:169–173.
37. Njouom R et al. High rate of hepatitis C virus infection and predominance of genotype 4 among elderly inhabitants of a remote village of the rain forest of South Cameroon. *Journal of Medical Virology*, 2003, 71:219–225.
38. Smuts HE, Kannemeyer J. Genotyping of hepatitis C virus in South Africa. *Journal of Clinical Microbiology*, 1995, 33:1679–1681.

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