

The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province

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SUMMARY

Genotype 5 (G5) was initially discovered and is still mainly diagnosed in South Africa. No cases of G5 have ever been reported from the Middle East countries. The aim of the study was to determine the hepatitis C virus (HCV) genotype distribution in Syria and the prevalence of G5 in this country. Genotyping of HCV was performed in 636 consecutive HCV patients referred to eight medical centres in Syria over a 3-year period. Genotype 4 was the most frequent genotype (375 patients, 59%) followed by genotype 1 (181 patients, 28.5%) and G5 (64 patients, 10%). The majority of G5 patients (56 cases, 87%) live in the north of Syria, including 21 cases (33%) from Azaz, a small city close to Turkey. No obvious epidemiological reason for such high prevalence of G5 was found.

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of liver disease, cirrhosis and hepatocellular carcinoma with an estimated 200 million infected persons worldwide [1].

Its prevalence varies from <1% to >20%, as in Egypt [2]. In Syria, the prevalence of HCV infection is about 1% for a general population of 19 million people.

HCV is classified into six different genotypes [3]. While genotype 1 (G1) is the most prevalent in North America [4] and Northern Europe, genotype 4 (G4) is by far the most common in the Middle East and North Africa. G5 has been initially reported in South Africa where it represents 40% of all HCV cases [5, 6]. A few recent reports have indicated its (rare) presence in France, Belgium, Canada, Brazil, Saudi Arabia,

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† See Appendix for details of Syrian Working Group for the Study of Viral Hepatitis.

and Spain [7–13]. No cases of G5 have been reported from the neighbouring countries of Syria, e.g. Turkey, Lebanon, Jordan, Egypt and Israel. The importance of determining HCV genotypes is related to their respective response to therapy and different treatment duration.

After the discovery of a few cases of G5 in Syria, the present study was conducted in order to determine the exact HCV genotype distribution and the prevalence of G5 in Syria and to present the epidemiological data for the group of patients carrying this specific strain.

METHODS

Subjects

All HCV patients, referred to eight major medical centres in Syria from January 2004 to December 2006 were prospectively included in the study. Age, gender, location, family history, alcohol use and risk factors for HCV transmission were determined and recorded during the initial visit. If necessary, further information was collected by personal or phone interviews. Anti-HCV testing was proposed for the spouses and children of all patients and, when positive, HCV-RNA and genotyping were performed.

For the purpose of this study, Syria was divided geographically into four regions: north, centre, east and south (Fig. 1).

Virological tests

Virological tests were performed in three different laboratories in Syria using the same methods. HCV genotyping was performed by INNO-LIPA[®] version 2.0 (Innogenetics, Zwijndrecht, Belgium). HCV viral load was measured by real-time PCR using Cobas Amplicor[®] version 2.0 or Lightcycler[®] version 2.0 methods (both from Roche Diagnostics, Basel, Switzerland) with a lower limit of detection of 50 IU/ml.

Statistics

Statistical analysis was performed using SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL). Continuous variables are reported as mean and standard deviation (s.d.), while categorical variables are shown as count and proportion. Two-sample *t* tests, Mann–Whitney *U* tests and χ^2 tests were used to



Fig. 1. Map of Syria showing the four regions of the study area: north, centre, east and south.

compare between groups as appropriate. For all tests, two-sided *P* values were calculated and the results were considered statistically significant if $P < 0.05$.

Informed consent

The study was conducted in accordance with the Helsinki Declaration for the protection of human subjects. Informed consent was obtained from all patients and allowed a review of patients' medical records for research purposes.

RESULTS

Demographic data

During the study period, 636 HCV-RNA-positive patients were evaluated. The age of the patients varied between 2 years and 80 years with a mean (\pm s.d.) age of 42 ± 17 years. A total of 356 patients (56%) were female. There were 255 patients from the east region (40%), 207 from the north region (32.5%), 95 patients from the south region (14.5%) and 67 from the centre region (10.6%). Twelve patients were of undetermined region since they had moved a number of times between regions. In total, 109 patients (17%) had received a blood transfusion and 99 (15.6%) were under haemodialysis.

Genotype distribution

The genotype distribution for the whole country was: 181 patients (28.5%) with G1, five (0.8%) with genotype 2 (G2), 11 (1.8%) with genotype 3 (G3),

Table 1. Geographical distribution of HCV genotypes in Syria

Genotype	North (n=207)	East (n=255)	Centre (n=67)	South (n=95)	Undetermined (n=12)	Total (n=636)
1	50 (24.1%)	19 (7.5%)	44 (65%)	64 (67%)	4	181 (28.5%)
2	4 (2%)	0	1 (1.5%)	0	0	5 (0.8%)
3	2 (1%)	3 (1%)	0	5 (5.3%)	1	11 (1.8%)
4	95 (46%)	227 (89%)	21 (31%)	25 (26.3%)	7	375 (59%)
5	56 (27%)	6 (2.4)	1 (1.5%)	1 (1%)	0	64 (10%)

375 (59%) with G4, and 64 (10.1%) with G5 (Table 1).

G1 was most prevalent in the centre (65%) and south (67%) regions, in patients under haemodialysis (67%) and in patients who have received blood transfusions (58%), while G4 was most prevalent in the east (89%) and north (46%) regions.

For the patients under haemodialysis, G1 was the most prevalent (67%) followed by G4 (29%) and G5 (2%). Only 63/99 patients under dialysis had received a blood transfusion. Among the 109 patients who had received blood transfusions, G1 was the most prevalent (58%) followed by G4 (32.5%) and G5 (8%). In the 375 G4 patients, 4c/4d, 4a, 4h subtypes were found in 66, 17 and 16 patients, respectively.

In the whole group of 636 patients, a probable mode of transmission was determined in 295 cases (46%). This includes blood transfusions and/or haemodialysis in 145 cases, tattooing in 130 cases, and intravenous drug use in one case. A strong family history (≥ 2 first-degree family members) of HCV was present in an additional 19 cases. Many patients had received dental care but a definite relationship could not be established.

G5 patients

Sixty-four patients had G5; 56 cases (87.5%) were from the north, six patients (9.3%) from the east, and one patient each from the south and centre regions. G5 represents 27% of all genotypes in the north but only 2.4%, 1.5%, and 1% of the genotypes in the east, centre and south regions. In the patients from the north region, 21 patients (33% of all G5 cases) were from the small city of Azaz (population 30 000) in northern Syria close to the border with Turkey. Thirty-nine of the G5 patients were female (61%), 25 (39%) were male and were all circumcised. Age varied between 41 years and 80 years with a mean age of 52 ± 10 years. The mode of transmission was determined in only 30%. This included nine cases related

to the transfusion of blood products and/or haemodialysis, five cases of tattooing and five cases with a strong family history of HCV (Table 2). No case of intravenous drug use was reported in this group. The spouses of all married infected patients were also tested. Only one was positive but with a different genotype (G4). Of the 64 patients, 20 had a liver biopsy and it showed advanced fibrosis or cirrhosis in 15 patients. No hepatitis B virus or HIV co-infection case was diagnosed.

The difference in mean age between G5 patients and the other genotypes (non-G5) was statistically significant (52 years vs. 40 years, $P < 0.0001$) while the gender distribution was comparable (Table 2). The non-G5 group had a higher rate of identified mode of transmission (48% vs. 29.6%, $P = 0.006$), a higher proportion of tattooing (20.4% vs. 7.8%, $P = 0.008$) and a trend towards a higher proportion of dialysis/blood transfusion cases (22.8% vs. 14%, $P = 0.07$). A higher rate of positive family history of HCV (7.8% vs. 2%, $P = 0.004$) was noted in the G5 group.

DISCUSSION

HCV G4 is the most prevalent genotype in Egypt, the Middle East and North Africa. It has been extrapolated that all countries in the area have the same high prevalence of G4 [14]. The estimated prevalence of HCV in Syria is 1% [15]. The only previous study on HCV genotype distribution in Syria [16], published in 1998, showed a prevalence of 54% and 46% for G4 and G1, respectively, in a group of 37 patients. However, the patients included in that study were all under haemodialysis in the same hospital in the south of the country. The present study was conducted in eight centres covering all of Syria and included haemodialysis patients. We, therefore, consider our results of 59%, 28% and 10% for G4, G1 and G5, respectively, more accurate than the previous study. On the other hand, in our group of 99 patients (15.6%) under haemodialysis, the most prevalent

Table 2. Comparison between all HCV genotypes and genotype 5 (G5) patients

	All HCV genotypes (n=636)	Non-G5 (n=572)	G5 (n=64)	P value*
Age, years (\pm s.d.)	42 \pm 17	40 \pm 10	52 \pm 10	<0.0001
Gender				
Female	356 (56%)	317 (55%)	39 (61%)	0.35
Male	280 (44%)	255 (45%)	25 (39%)	
Mode of transmission	295 (46%)	276 (48%)	19 (29.6%)	0.006
Dialysis/transfusion	145 (22.8%)	136 (24%)	9 (14%)	0.07
Tattooing	130 (20.4%)	125 (22%)	5 (7.8%)	0.008
Family history	19 (3%)	14 (2%)	5 (7.8%)	0.004
Intravenous drug use	1 (0.15%)	1 (0.17%)	0 (0%)	0.74

s.d., Standard deviation.

* For comparisons between G5 patients and non-G5 patients.

genotype was G1 (67%) and this prevalence was comparable in haemodialysis patients from the four regions (south, north, centre, east) of the country: 72%, 65%, 67% and 67%, respectively.

Finally, we found that the most prevalent G4 subtype was 4c/4d, which was four times more prevalent than subtype 4a. This is an interesting finding since in Egypt, the country with the higher prevalence of HCV and G4, the most frequent subtype is 4a [17]. On the other hand, the prevalence of G4 is increasing in Europe mainly with 4d subtype [18, 19]. There is a recent trend to consider the G4 subtypes as an important predictive factor to the response to treatment [20].

In the present study, we found that 64 (10%) of HCV patients in Syria are G5 and of these 64 patients, 56 (87%) originated from the northern province around the city of Aleppo. Only six cases were from the eastern part of Syria where HCV is most prevalent and G4 is almost exclusively found. The usual causes of transmission (blood transfusion, intravenous drug use and tattooing) could only be identified in 30% of our HCV G5 cases while no obvious cause was found to explain the contamination of the others. Twenty-five patients (39%) were male and were all circumcised (circumcision is practised at birth and not by physicians). Two of these patients had received blood products. We do not believe that the contamination of the remaining 23 males can be attributed to circumcision in every single case as they are from different social and hygienic backgrounds.

There were five cases from the same family (a mother who died from liver cirrhosis, two sons and two daughters out of 10 siblings) suggesting that intrafamilial transmission may occur, probably in

infancy. On the other hand, we have not found any cases of transmission between spouses (the only infected husband–wife pair carried different genotypes) confirming that sexual transmission of HCV is rare. Finally, the use of reusable contaminated needles was considered but there was no convincing evidence for this hypothesis. In all reported cases of HCV G5 from different countries [7–9], blood transfusion and nosocomial transmission were almost the only causes of contamination, however, this is not the case in our series. Our patients, although from a limited geographical area, are from different educational, social, financial and hygienic backgrounds and do not receive their health care at the same medical facilities.

The duration of infection with HCV G5 could not be precisely determined since the source of contamination was identified with certainty in only 14 cases. The five cases of tattooing were all from the east and the tattooing was performed more than 20 years ago. Of the nine cases of blood transfusion, six were from the north (three from Azaz). Screening of blood products for HCV in Syria commenced in 1995 and all nine patients had received blood transfusions prior to 1986. Fifteen patients had advanced fibrosis or cirrhosis on liver biopsy. Four of them are among those who received blood transfusions more than 20 years ago. Based on the natural history of HCV infection, we speculate that the 11 remaining patients with advanced fibrosis or cirrhosis have also been infected for more than 20 years. In conclusion, in at least 25 patients (39%) with G5, the duration of infection is more than 20 years.

Twenty-one cases of HCV G5 (33%) originated from Azaz and a total of 39 HCV cases were reported

from this city in our study. Therefore, G5 represents 54% of cases while the 18 remaining (46%) are G4. No cases of G1 were reported from Azaz although it represents 28% of cases at the national level.

We also note that there was a statistically significant lower rate of identifiable risk factors for transmission in the G5 group suggesting the presence of an unknown cause of transmission for HCV G5 in the northern area of Syria.

Why and how G5 exists in the northern region of Syria, so far from South Africa, remains an unsolved problem especially when we consider that there is absolutely no immigration from Africa to Syria and that no HCV G5 cases have been reported from Turkey or any other of Syria's neighbours, e.g. Lebanon [21], Jordan, Iraq or Egypt.

CONCLUSION

This is the first report describing the presence of HCV G5 in Syria and the Middle East. No cases were reported from neighbouring countries. One small town has a very high prevalence rate. We were unable to discover an explanation for these unexpected findings. The source of contamination was determined in a minority of patients. More epidemiological studies are needed to determine the reason for the high prevalence rate in a small area.

APPENDIX

The authors of this study are members of the Syrian Working Group for the Study of Viral Hepatitis (SWGSH) whose objectives are to conduct research and studies on viral hepatitis and to publish Syrian national guidelines for the management of viral hepatitis.

Institutions participating in the study: St Louis Hospital, Aleppo, Syria; Ibn Nafis Hospital, Damascus, Syria; Deir Al Zor National Hospital, Deir Al Zor, Syria; Homs National Hospital, Homs, Syria; Mouassat Hospital, Damascus, Syria; Squelbiye National Hospital, Squelbiye, Syria; Lattakia National Hospital, Lattakia, Syria; Sweida National Hospital, Sweida, Syria.

DECLARATION OF INTEREST

None.

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