

Genotype of HCV-virus among high-risk groups in Albania

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Abstract

Aim: To determine the prevalence and HCV genotype among high risk groups in Albania.

Methods: In this study we analyzed 172 blood samples collected from various high risk groups for the presence and genotype of HCV virus with HCV-RNA (Cobas Amplicor HCV Test v2.0, Roche), HCV genotype (Line Probe Assay-LiPA, Bayer). The data analysis was done using SPSS, version 16.0.

Results: A total of 172 blood samples were screened including thalasemic patients, dialysis patients, and injecting drug users (IDUs). Initial screening was done for anti-HCV using ELISA-test. Confirmation of active HCV infection was carried out with the help of Cobas Amplicor test V 2.0. Out of 172 samples, 121 (70.34%) were both HCV positive for anti-HCV and HCV RNA. Prevalence of active HCV-RNA infection in individual risk groups was: 20 (41.66%) for 48 thalasemics patients, 74 (88%) for 84 cases with dialysis and 27 (67.5%) for 40 cases in injection drug users, respectively. Four major genotypes (1, 2, 3, 4) and their subtypes (1a, 1b, 1a/ b, 3a, 4a, 4e) were identified. The most frequent HCV genotypes were 1 and 3. There was no significant relationship between virus genotypes and sex, age and risk-group.

Conclusions: The prevalence of HCV-RNA was higher in these population groups. Genotype 1, subtype 1b was the most prevalent in dialysis patients and genotype 3 was the most prevalent between the IDUs group, but more detailed studies with a larger number of patients are necessary to understand the HCV dynamics in this population subgroup of Albania.

Keywords: haemodialysis, HCV genotype, HCV-RNA, Hepatitis C virus, prevalence.

Introduction

Hepatitis C is an infectious disease affecting the liver, caused by the hepatitis C virus (HCV). Choo and co-workers in 1989 discovered HCV, a member of the Flaviviridae family, as a new viral agent causing non-A, non-B hepatitis (1). WHO estimated the global prevalence of Hepatitis C at 3% (2).

The genome of HCV is highly variable. Hepatitis C virus has been classified into 6 major genotypes (1-6) and into several subtypes (3) and additional genotypes have been recently proposed (4,5). The genotypes have a geographically distinct distribution (6,7) which carries important implications such as treatment decisions, possible transmission route and vaccine development. HCV is transmitted through contaminated blood transfusion, surgery, surgical instruments, dental surgery, sexual contacts, drug abuses, sharing of the household items such as razors, toothbrushes and shaving from the barber (8). Genotype 3 was shown to be more common in Europe among young injecting drug users, compared to genotype 1b, which is associated with transfusion-related HCV (9). Major risk factors associated with the transmission of HCV were never investigated at molecular level in Albania. In this study, we have analysed patients belonging to various risk groups for the prevalence and genotyping of HCV infection. The risk groups included thalasemics, dialysis group and IDUs (Injection Drug Users).

Materials and Methods

Patients

Plasma of 172 high-risk for HCV infection consecutive patients (115 men and 57 women, 5 - 68 years of age) from different departments of our hospital (Dialyses Centre and Hematology Unit of University Hospital Centre "Mother Teresa" Tirana-Albania) during January 2010 -December 2012 was included in this study. Patients' plasma was examined for Hepatitis C to Molecular Biology Laboratory, Institute of Public Health, Tirana, Albania, and were assigned to three groups:

A) 84 Dialysis Patients (DP), 55 men and 29 women, 18-68 years of age. These patients acquired chronic Hepatitis C, contaminated by intravenous catheters or other equipment and solutions.

B) 40 IDUs (Injection Drug Users), all male, 18-35 years of age. Sharing of contaminated needles was the main risk factor in this group.

C) 48 multi transfused patients with Beta Thalassemia Major (BTMP), 20 men and 28 women, 5-28 years old, transfused since their childhood, as part of their therapy. Each individual duly signed a proforma containing information about his/her previous exposure to a risk factor, age, sex etc.

5 mL of blood sample was collected in EDTA-tubes in each case and immediately transported to Molecular Biology Laboratory, Institute of Public Health, for plasma isolation. Plasma samples were aliquot and stored at -70°C until use.

HCV qualitative detection and HCV genotyping

COBAS AMPLICOR Hepatitis C Virus Test, version 2.0 (v2.0) was used for qualitative in vitro diagnostic HCV detection in the RNA extracted samples using COBAS AMPLICOR Analyzer (Roche). Qualitatively positive HCV aliquots of denatured amplicon by Cobas Amplicor were genotyped using a line probe assay, LiPA (Line Probe Assay-LiPA, Bayer), a reverse hybridization test that can associate the 5' NCR genome in products of a post PCR amplification of DNA amplicons. This assay allows differentiation of 6-major HCV genotypes (1-6) and 15 subtypes (1a, 1b, 2a/ 2c, 2b, 3a, 3b, 3c, 4a, 4b, 4c4d, 4e, 4f, 4h, 5a, 6a) according to (10,11). Sub-type 1a/1b, 1a was referred as genotype 1, 2a/ 2c as genotype 2 while 4a, 4e as genotype 4 in this study.

The data was analyzed with SPSS version 16.0 for windows or Microsoft Excel. Frequencies of hepatitis C in different risk groups were calculated in percentages.

Results

A total of 172 blood samples were screened including thalassemic patients, dialysis patients, and injection drug users. All samples were positive for anti-HCV. Confirmation of active HCV infection was carried out with the help of RT-PCR. Out of 172 samples, 121 (70.34%) were HCV positive both for anti-HCV and HCV RNA. Prevalence of active HCV infection in individual risk groups was 41.66%, 88%, and 67.5% in thalasemics, dialysis, and injection drug users respectively (Table 1).

Table 1. Prevalence of HCV in individual risk groups

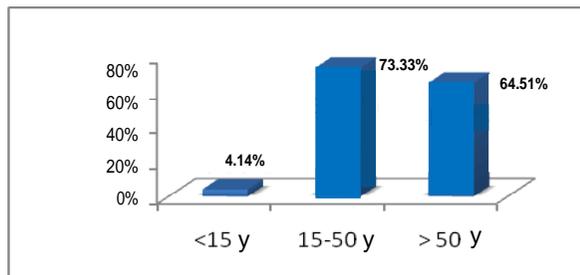
S. No	Major Risk groups (N)	HCV-RNA positive
1	Thalassemia (48)	20 (41.66%)
2	Dialysis (84)	74 (88%)
3	IDUs (40)	27 (67.5%)
Total	172	121 (70.34%)

Exposure to various HCV risk factors is preferentially gender specific in our country mainly due to our social set up. Exposure of females to some of the risk factors is limited. Among the observed population, 115 (66.87%) were male and 57 (33.13%) were female out of which 84 (73.04%) males and 37 (64.91%) females were positive for

anti-HCV as well as HCV RNA (Table 2). In terms of age distribution, the subjects were grouped into three categories. Lowest prevalence 4.14% was recorded in the case of patients with age 15 years or below, while in the case of older age group (50 years and above), the highest HCV prevalence 73.33% was recorded (Figure 1).

Table 2. Sex wise prevalence of active HCV infection

Sex	Total samples	HCV-RNA positive	HCV-RNA negative	Prevalence (%)
Male	115	84	31	73.04%
Female	57	37	20	64.91%
Total	172	121	51	70.34%

Figure 1. Percentage according to age distribution

Out of the total 172 samples from different risk groups included in the study, 11.62% were positive in the cases of thalassemia, 43.02% in dialysis, and 15.69% in injection drug users. Highest prevalence was observed in dialysis patients (Table 3). Major risk factors for HCV transmission in our study turned out to be dialysis followed by Injection Drug Use and Thalassemia.

Taking into account all HCV-RNA positive cases (121), genotypes 1 (31.4%) and 3 (13.95%) were

Table 3. Prevalence of HCV among the high-risk groups

Sex	Total Sample	HCV-RNA positive	Thalassemia	Dialysis	IDUs
Male	115	84	8	49	27
Female	57	37	12	25	
Total	172	121	20	74	27
		70.34%	11.62%	43.02%	15.60%

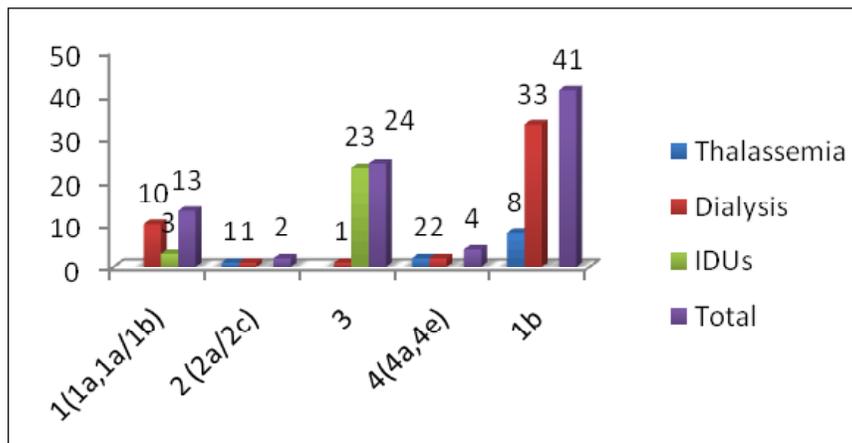
most common, while only 2.32% and 1.16% carried genotype 4 and 2, respectively. However, within each

risk group a different genotype distribution was observed (Figure 2). Genotype 1 especially subtype

1b (39.28%) was predominate among dialysis groups. Genotype 3 (57.8%) was predominant in the IDU group. The predominance of genotype 3

makes the situation in our country similar to that among IDUs in Europe (10).

Figure 2. HCV genotypes distribution according to the different risk groups



Discussion

Most Thalassemia patients are among the high-risk groups for HCV infection. In Albania, blood transfusion is still a major source of HCV transmission. Possible reasons for this include lack of resources, weak infrastructure, ill-equipped resources, inadequate policy implementation, frequent power breakdown and ineffective screening of blood donors for anti-HCV antibody.

In this study the prevalence of HCV RNA in individual group was 41.66% while among the high-risk groups it was 11.62% (Table 1, 3). Hemodialysis is considered to be one of the major risk factors for HCV transmission. Some earlier studies in Albania (Biological Surveillance of infectious Agent Among individual Multiple Transfusions) reported that the prevalence of HCV in hemodialysis patients was 29% (12). In this study the prevalence of HCV RNA were 88% in the individual risk group and 43.02% among the high-risk groups (Table 3). The highest prevalence of HCV in dialysis group indicates the limitations of the screening procedures used in these units for the detection of anti-HCV. According to a study conducted in injection drug use in Albania (The Behavioral and Biological surveillance survey 2011 third round) the prevalence

of HCV in this group was 30-31%. In this study, prevalence of HCV RNA among the IDUs was 67.5% in the individual group of IDUs while it was 15.69% in total among the high-risk groups. Data from the present study reveals that genotype 1 (31.4%) and 3 (13.95%) are the pre-dominant genotypes among chronically infected high-risk patients. Genotype 1 predominates among patients that acquired HCV infection by blood transfusion and other parenteral exposures before 1990. Subtype 1b was significantly higher in dialysis group. This is in accordance with literature (13). Genotype 3 (57.8%) was predominant in the IDU group in present study. In accordance with many studies of other European countries (14), the predominant HCV genotype between IDU is type 3.

Conclusion

The prevalence of HC-RNA was higher in these groups. Genotype 1 subtype 1b was the most prevalent in Dialysis patients and genotype 3 was the most prevalent between the IDUs group but more detailed studies with a larger number of patients are necessary to understand the HCV dynamics in this group population of Albania.

Conflicts of interest: None declared.

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