Distribution of hepatitis C virus genotypes among injecting drug users in Albania

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Abstract

Aim: The aim of this study was to determine the prevalence of active infection (presence of HCV-RNA) among injecting drug users (IDUs) in Albania, in order to establish the current prevalence of HCV genotypes in this group.
Methods: During the period 2012-2013, we analyzed 96 IDUs (95 male and 1 female) in Albania. Mean age was 26.6 years (range from 19 to 42 years). All samples were tested for anti-HCV positivity by enzyme-linked immunoassay (ELISA). The samples that resulted positive by ELISA were analyzed for presence of HCV-RNA (active infection of HCV) and HCV genotypes.
Results: The prevalence of anti-HCV was 55.2%. The positivity for HCV-RNA was 79.2%. Genotypes 1 (85.7%) and 3 (9.5%) were detected in the IDUs group.
Conclusion: The predominance of genotype 3 makes the situation in Albania similar to that among IDUs in Europe. Nonetheless, more detailed studies with a larger number of patients are necessary to understand the HCV dynamics in this population sub-group in transitional Albania.

Keywords: genotypes, HCV-RNA, hepatitis C virus, injecting drug users.

Introduction

Hepatitis C virus (HCV) infection is a worldwide problem in health. This infection is a major cause of chronic liver disease, hepatocellular carcinoma, and the single most common indication for liver transplantation. Approximately 2-3 percent of the world's population is infected with HCV (1,2). According to the WHO, the prevalence of HCV infection is 2% (ranging from 0.6% to 2.3%) in North America, Northern and Western Europe, and Australia. In Albania, the prevalence of HCV infection is about 1% in the general population (3). HCV is a blood-borne pathogen and can be transmitted through blood products and infected syringes, and infection rates are typically high among the injecting drug users (IDUs). Nowadays, IDU is the major risk factor for HCV infection (4). The incidence of HCV infection among IDUs varies from 31% to 89% in different parts of the word (5,6). Genotype 3a was shown to be more common in Europe among young IDUs (7).

HCV is one of the few microorganisms for which genotyping, besides providing epidemiological information, also provides information with regard to treatment. Treatment is more frequently successful in cases of infection with genotype 2 or 3 than in cases of infection with other genotypes (8). From a public health perspective, it is important to know how HCV spreads in the community and how IDUs contribute to the transmission of this infection.

In Albania, the hepatitis C prevalence among IDUs obtained by the Albania Behavioural and Biological Surveillance Study Report, third round conducted in 2011, was found to be significantly higher, 28.8% (9) and the infection is emerging due to needle sharing among this group. But, there are limited data about the type of HCV virus (genotype) circulating within IDUs group (10).

The aim of this study was to determine the prevalence of active infection (presence of HCV-RNA) among injecting drug users (IDUs) in Albania, in order to establish the current prevalence of HCV genotypes in this group.

Methods

Patients

Blood samples of 96 Injecting Drug Users (IDUs) collected from five districts of Albania (Korça, Tirana, Durrës, Fier, Elbasan) during the period 2012-2013, were analysed in Molecular Biology Laboratory at Public Health Institute, Tirana, Albania.

Anti-HCV detection and RNA-extraction

All samples were tested for anti-HCV by Enzyme-linked Immunoassay (ELISA BioRad). Anti-HCV positive samples were subjected to RNA extraction using Cobas Amplicor HCV Test v2.0 (Roche) for further qualitative HCV detection and genotyping. Five milliliters of peripheral blood was taken from each patient into EDTA-containing tubes. Plasma was separated from whole blood and immediately stored at -80°C. Viral RNA was extracted from 200 µL of plasma using a Cobas Amplicor HCV Test v2.0 (Roche) according to the manufacturer's instructions. For this study, we avoided RNA degradation by using only those aliquots that had not been thawed more than once prior to molecular testing.

HCV qualitative detection and genotyping

Cobas Amplicor Hepatitis C Virus Test, version 2.0 was used for qualitative in vitro diagnostic HCV detection in the RNA extracted samples using Cobas Amplicor Analyzer (Roche). This test is based on five major processes: specimen preparation; reverse transcription of the target RNA to generate complementary DNA (cDNA); PCR amplification of target cDNA using HCV specific complementary primers; hybridization of the amplified product to oligonucleotide probes specific to the target; and detection of the probe-bound amplified products by colorimetric determination according to the manufactures instruction. Qualitatively positive HCV aliquots of denatured amplicon by Cobas Amplicor were genotyped by reverse hybridisation using Linear Array Hepatitis C Virus Genotyping Test (Roche) which is coated with a series of oligonucleotide

probes specific for various HCV genotypes. The developed bands obtained were analysed for genotype using a reference strip (11).

Data analysis

The data were analysed using SPSS version 16.0 for Windows.

Results

A total of 96 IDUs samples belonging to five districts of Albania, were studied. The majority of participants were male, composing 98.95% (95/96) of IDUs. Only one (1.04%) participant in the IDUs group was female. The mean age was 26.6 years with a range from 19 to 42 years. The prevalence of anti-HCV with serological method (ELISA) was 55.2% (Table 1).

Table 1. Anti-HCV and HCV-RNA positivity among the IDUs

District	Total nr of IDUs	Anti-HCV Positive	HCV-RNA positive
Durrës	5	1 (20%)	1 (20%)
Tiranë	26	5 (19.2%)	4 (15.4%)
Korçë	44	40 (90.9%)	32 (72.7%)
Fier	7	2 (28.6%)	1 (14.3%)
Elbasan	14	5 (35.7%)	4 (28.6%)

The samples that resulted positive by ELISA were analyzed for presence of HCV-RNA (active infection of HCV) and HCV genotypes. The positivity for HCV-RNA was 79.2%. Active HCV was more prevalent in the district of Korça with 32 samples (72.7%), followed by the district of Elbasan with 4 samples (28.6%).

Genotypes 1 and 3 were detected in IDUs group in Albania. Genotype 3 was predominant (85.7%), followed by genotype 1 (9.5%) (Table 2).

Table 2. Positivity of HCV infection and genotypes by sex

Sex	Number of IDUs	Anti-HCV positive	HCV-RNA positive	Genotypes	
				1	3
Male	95	52	42	4	36
Female	1	1	0	0	0
Total	96	53 (55.2%)	42 (79.2%)	4 (9.5%)	36 (85.7%)

There were no patients with mixed infection. Two participants with active HCV displayed an undetected genotype.

Discussion

Epidemiological studies in different regions of the world show a wide variation in HCV prevalence patterns (2). Injecting drug users have now become the predominant source of HCV infection in the world (12). According to a survey conducted among IDUs in Albania in the year 2011, the prevalence of anti-HCV in this group was 28.8% (9). It is very important to manage the HCV infection in this population subgroup in order to have under control the HCV infection in the overall popu-lation of Albania.

In the current study, the prevalence of anti-HCV among the IDUs was 55.2%, but the prevalence of active infection was 79.2%. Compared with similar studies from different parts of the world including Europe (13,14) and North America (15), the prevalence of HCV-RNA detected in Albanian anti-HCV positive IDUs patients is higher.

IDU is uncommon among Albanian females where social constraints do not allow free mix-ups with the males and where social interaction among

opposite sexes is limited. In our study we obtained evidence of one female participant who was positive for anti-HCV, but was negative for HCV-RNA.
The distribution of HCV genotypes in the IDUs group in Albania showed that HCV genotype 3 was predominant (85.7%), followed by genotype 1 (9.5%). The predominance of genotype 3 makes the situation in Albania similar to the situation among IDUs in Europe (13,16). Our findings are similar to the results of a previous report from Albania that revealed the predominance of HCV genotype 3 (57.8%) in Albanian IDUs. Worldwide prevalence of HCV-3 and mechanisms of its transmission are not accurately defined; therefore, large epidemiologic studies are needed for better understanding of this issue. A future phylogenetical analyse of HCV genotype 3 samples from Albanian IDUs, in order to find origin, spread and similarity between the pattern of HCV genotype in IDUs in Albania and Europe is definitely needed. One of the limitations of this study includes the lack of information about the socio-economic status of participants including income and education, risk factors, and the mobility – among others.

Conclusion

This study showed a high prevalence of HCV-RNA in chronic HCV infected IUDs in Albania. The genotype 3 was the most prevalent among the IDUs group, but more detailed studies with a larger number of patients are necessary to understand the HCV dynamics in this population subgroup in Albania. Considering that HCV anti-viral therapy varies significantly with the genotype, this finding may affect dis-ease treatment and control.

Conflicts of interest: None declared.

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