Hepatitis viruses in populations at risk

PhD thesis

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2015
Introduction

Hepatitis B and Hepatitis C viruses (HBV, HCV) spread through infected blood and body fluids, both viruses can establish persistent infection and trigger chronic hepatitis. Millions of people are infected causing a great burden for the healthcare systems worldwide. Virus carriers are often symptomless, thus screenings are important in diagnosis. Active and passive immunizations are available against HBV, while there is no vaccine against HCV. This may due to the great variability of the HCV. It has 7 genotypes and several subtypes, genotypes have a certain geographical distribution. Antiviral therapy exists against both viruses, which can reduce virus transmission and disease progression as well. HCV genotypes have different susceptibility to treatment.

The prevalence of HCV and HBV in Hungary and in most part of Europe is relatively low. Although publications suggest that in certain at-risk populations the burden of the infections is high.

Aims

Our goal was to determine

- the prevalence of HCV and HBV infections in the Hungarian prisons to examine whether Hungarian inmates form a population at risk in relation to HCV and HBV,
- whether the presence of anti-HCV antibody and HBV surface antigen has a correlation with age or sex in the Hungarian detention centres,
- whether there is an extra risk for infections of prison staff compared to the general population,
- which risk behaviours play role in HBV infections in prisons,
- what is the HCV genotype distribution among Hungarian people who inject drugs (PWID),
- what HCV subtypes circulate among Hungarian PWID,
whether HCV genotypes and subtypes of PWID have a certain geographical
distribution within Hungary,

whether there is a relation between HCV genotypes and the duration of drug use, and
between genotypes and the age of the PWID,

the evolutionary relations among genotype 3 viruses of Hungarian PWID and other
genotype 3 sequences published worldwide.

Materials and Methods

Prison study: A national, multicentre, cross-sectional study was conducted between June
2007 and June 2009 in 20 Hungarian prisons, which geographically covered the entire
country. Participation was voluntary. Out of a total of 14,331 inmates in the prison system at
that time, 4,894 (34.2%) participated in this study. In order to have a comparison group,
prison staff was also asked to take part in the survey voluntarily.

DBS (Dried Blood Spots) study: From 2006 to 2011, capillary blood samples were
collected through the use of self-retracting single-use lancets from 2,133 PWID appearing at
22 needle exchange sites and drug outpatient services in all seven Planning and Statistical
Regions of Hungary. As a comparison group (referred to as the general population), 89
treatment-naïve HCV carriers seen by hepatologists were randomly selected between 2006
and 2011 from all Statistical Regions of the country.

Serological tests: Each DBS, cut out for serology, was placed into 200 μl of PBS
containing 0.05% TWEEN 20 and 0.08% Na-azide, vortexed and eluted overnight at 4 °C.
For anti-HCV antibody detection, HCV Ab (Dia.Pro) ELISA was used. The results were
confirmed by Innotest HCVAb IV ELISA or HCV Inno-LIA (Innogenetics). In the prison
study we used the same kits for HCV antibody detections. For the detection of HBsAg,
Hepanostika HBsAg Ultra and Confirmatory tests (Biomerieux) were used.

Questionnaires: After informed consent was obtained, anonymous questionnaires on risk
behaviours relating to the transmission of hepatitis infections were filled in voluntarily by
1,553 participating inmates between June 2008 and June 2009 in seven prisons. PWID could
only participate in the DBS study if they filled in an anonymous questionnaire on risk behaviours relating to the transmission of HCV.

**Molecular biological techniques:** In the prison study nucleic acid isolation was carried out using QIAmp Minelute™ virus Spin Kit (QIAGEN), in the DBS study we used TRI REAGENT BD (Sigma). RNA was subjected to reverse transcription through the use of a GeneAmp RNA PCR kit with random hexamers (AppliedBiosystems). The presence of HCV RNA was determined by nested PCR (Sigma REDTaq ReadyMix reagents), which detects all known genotypes with primers specific for the 5′UTR. PCR products were purified by Viogene PCR-M kit, and were sequenced on a MegaBACE 1000 (Amersham Biosciences) machine after utilising DYEnamic ET Dye Terminator Cycle Sequencing Kit (General Electric Company). The kit based on the selective incorporation of differently labelled chain-terminating dideoxynucleotides by Taq polymerase *in vitro*, developed by Frederick Sanger and his colleagues.

**Prison study:** 5′UTR sequences were aligned with reference sequences of known genotypes with the Clustal W online tool. To reveal evolutionary relations and the genotype, the Molecular Evolutionary Genetics Analysis (Mega) software 5 (using the neighbour-joining process) was applied with 1000 bootstrap replicates. Evolutionary distances were computed by the Maximum Composite Likelihood Method.

**DBS study:** For phylogenetic analysis 25 randomly selected genotype 3 HCV partial ns5b sequences, obtained from PWID, were amplified. PCR products were purified and directly sequenced as mentioned above. We selected further 49 sequences from 11 countries with NCBI nucleotide BLAST regardless the source was a PWID or not. With the help of MultAlin online tool the sequences were aligned and phylogenetic trees were constructed. The nucleotide substitution model that described the data best was chosen by ModelTest using PAUP* as implemented in FindModel. Phylogenetic trees were constructed by the Maximum Likelihood method using PhyML 3.0 program. Bootstrap analysis with 1000 replicates was performed to confirm tree topology. Evolutionary distance calculations were carried out using MEGA software version 5.0. A 3b sequence (accession number: D49374) was used as an outgroup.
HCV subtyping was carried out via Line Probe Assay (LIPA; Siemens Versant HCV Genotype 2.0 Assay).

**Statistics:** All analyses were conducted using STATA 11 software (StataCorp. 2010. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP) and Microsoft Excel. A p value of <0.05 was considered indicative of a significant difference.

**Thesis:**

1. We concluded that the Hungarian prison inmates are a population at risk regarding the HBV and the HCV, since both viruses were significantly more prevalent among inmates (anti-HCV: 4.9%, HBsAg: 1.5%) than among the general population and the prison staff (anti-HCV: 0.47%, HBsAg: 0.38%).

2. The prison staff did not differ significantly from the general population regarding HCV and HBV infections even after the data were adjusted to age and gender, which suggests low occupation-related virus transmission in Hungarian prisons.

3. The HCV was significantly more prevalent among female inmates (7.4%) than among males (4.7%), the reason of this finding needs further investigation.

4. HCV was found to be more prevalent in older age of PWID. Young age was correlated with higher proportion of injection drug users among prisoners. This may suggest that HCV burden will increase in the future in Hungarian prisons.

5. None of the questioned risk behaviours was statistically associated with the HBV carrier state among inmates, perhaps because HBsAg burden was low in the Hungarian detention centres. Anti-HBc antibody, which is usually more frequent than HBsAg, should be examined in the future for statistical analysis.

6. Based on our results and a collaborative network with clinicians, more than 60% of the HCV carriers started antiviral treatment. The opportunity to reach HCV infected people for treatment – including “hiding” injection drug user population – underlines the importance of screening programs for blood-borne viruses in prisons.
7. We determined HCV genotypes by examining DBS samples in Hungary. Genotype 3 HCV was significantly more prevalent among PWID (22.7%) than in the general population (2.2%). Genotype 1 was significantly more frequent in the general population (96.6% vs. 74.2%), while genotype 4 did not differ between the two populations. The high proportion of genotype 3 HCV among PWID can help drug abusers to accept and complete antiviral therapy (interferon-α + ribavirin) because of the shorter duration of treatment and the higher cure rate compared to genotype 1 HCV.

8. Subtype 1a was the dominant subtype in PWID (72.1%), but it was rare in the general population (4.2%). The abundance of genotype 3 and subtype 1a among PWID was in accordance with data on PWID in industrialised nations worldwide. Since genotype 3 and subtype 1a are rare in the general Hungarian population, we suggest that Hungary is involved in the worldwide epidemic of these genotypes among PWID, and that genotype 3 and subtype 1a may possibly have entered the Hungarian population of PWID from abroad.

9. Younger PWID are most likely to present with a more recently acquired infection. A shorter duration of drug injection use was found to correlate with a higher prevalence of genotype 1 and a lower prevalence of genotype 3. These data suggest that the prevalence of HCV genotypes may drift towards genotype 1 in the future among Hungarian PWID. This is not a general trend in neighbouring countries.

10. Genotype 3 proved to be significantly more frequent among PWID who entered the study in the provincial towns than in Budapest. We also found differences among districts of the capital city in that regard. This suggests that different PWID communities exist in Hungary even within Budapest that have different genotype prevalences.

11. Phylogenetic analysis of the ns5b regions revealed that the HCV genotype 3 sequences of Hungarian PWID did not form a separate clade, but certain sequences were grouped together, forming at least three subclusters. This confirms previous findings that no separate clades were found among HCV sequences amplified from PWID of different geographical origin. Subclusters confirmed with strong bootstrap values suggest that members of the same cluster may have a common origin.
**Thesis based on the following publications:**


**Thesis based on the following presentations:**

1. **Tresó B.** Hepatitis C virus és vizsgálata hazai veszélyeztetett populációkban. 2014. szept. 29. Semmelweis Egyetem Orvosi Mikrobiológiai Intézet.


**Book chapter in connection with the thesis:**


**Other publications in connection with the thesis:**


Other presentations in connection with the thesis:

