Liver disease, frequently caused by hepatitis B virus (HBV) and/or hepatitis C virus (HCV) co-infection, is a significant cause of global health burden. About 130–170 million people are infected with HCV, representing ~3 per cent of the world's population. NS5B is responsible for the synthesis of negative-sense RNA and subsequently of positive-sense RNA that is incorporated into progeny virions. Importantly, the selective pressures that shape non-structural regions of the viral genome are distinct from those targeting structural genomic regions. For instance, highly conserved secondary RNA structures limit NS5B diversity. Immune-mediated selection pressures contribute to NS5B polymorphism, and HLA-restricted epitopes may overlap with sites of drug resistance. Immune- or drug-selected mutations in NS5B dramatically reduce viral replication in vivo, although compensatory mutations may develop. NS5B variability also impacts pathogenesis as a higher mutation rate is associated with elevated ALT levels, and NS5B enzymatic activity positively correlates with ALT levels. As additional non-structural gene inhibitors are developed, characterization of the factors that shape HCV diversity in vivo will be necessary to limit HCV replication and increase the effectiveness of new antiviral agents. The HIV Epidemiologic Research Study (HERS) was established in 1993 to prospectively define the biological, psychological, and social effects of HIV in US women. Serum samples were obtained from women who were HCV RNA positive but HIV negative, HIV/HCV co-infected with CD4 <350, and HIV/HCV co-infected with CD4 ≥350. HCV RNA was extracted using the QIAamp UltraSens Virus Kit and subjected to RT-PCR for the entire NS5B region (~1,798 bases). Next-generation sequencing was used to evaluate intra-patient and interpatient NS5B diversity. NGS data were visualized in Integrated Genome Viewer. Consensus sequences were aligned in ClustalX 2.1 and BEAST v1.8.4 under an uncorrelated log-normal relaxed molecular clock, the general time-reversible model with nucleotide site heterogeneity estimated using a gamma distribution, and a chain length of 100,000,000 with sampling every 10,000th generation.

A40 Genotypic diversity of HCV in Kosovo with an emphasis on phylogenetic investigation of subtype 4D

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Hepatitis C virus (HCV) infection is a global health problem, affecting up to 3 per cent of the world's population, with the highest prevalence found among intravenous drug users and patients on hemodialysis. In Kosovo, a small developing country at Balkan Peninsula, there is lack of data on the prevalence of HCV genotypes in specific risk groups. The aim of the study was to determine HCV genotypes circulating in Kosovo and to further examine the spread of HCV genotype 4 in the country by employing phylogenetic analysis. A total of 437 HCV RNA positive hemodialysis patients, intravenous drug users and other patients were selected for genotyping by sequencing core region of HCV genome and using NCBI Genotyping tool. For the purpose of investigating the molecular epidemiology and transmission routes of genotype 4 in Kosovo, the HCV NS5b region was also sequenced. Major clusters were identified in a quick neighbor joining tree and HCV control sequences selected employing HCV BLAST search tool. Finally, maximum likelihood phylogenetic trees were constructed by using PhyML 3.0, with automatic substitution model selection Smart Model Selection. Transmission clusters were identified according to approximate likelihood ratio test (aLRT) branch support values obtained. In 383 out of 437 HCV RNA positive patients the HCV core region was successfully sequenced. The following HCV subtypes were determined: 1a (227/383; 59.3 per cent), 4d (95/383; 24.8 per cent), 1b (28/383; 7.3 per cent), 3a (28/383; 7.3 per cent), 2c (4/383; 1.0 per cent), and 2k (1/383; 0.3 per cent). A total of eighty-eight partial NS5b sequences were obtained, mostly from hemodialysis patients. This indicates that subtype 4d is epidemiology distinct from that of subtypes 1a and 3a, since genotype 4d has not been observed among injecting drug users in Kosovo so far. The phylogenetic tree of subtype 4d obtained revealed several clusters, suggesting several introductions of this subtype into dialysis units. In conclusion, 4d is the second most prevalent HCV subtype in Kosovo. Phylogenetic analyses showed several introductions of this subtype to the country and further spread among dialysis units thus demanding an urgent change in infection control practices in order to prevent further transmission of HCV.

A41 Characterization of NS5 coding region resistance associated substitutions from DAA-naïve GT1 HCV-infected patients in a Portuguese cohort

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Hepatitis C virus (HCV) is considered to be the leading cause of hepatocellular carcinoma (HCC) and other co-morbidities. During recent years, several highly effective regimens of direct-acting antivirals (DAAs) with excellent rates of success became available. However, therapeutic failure may occur in up to 10 per cent of treated individuals, and one of the main causes for this failure is the presence of resistance-associated substitutions (RASs) present before treatment initiation. Our aim was to study the profile and prevalence of baseline RASs in the NS5 coding region of DAA-naïve GT1 HCV infected patients, and then understand the impact of the found RASs in the response to treatment by ascertaining an association between treatment failure and the presence of major NS5 RASs. Plasma RNA from eighty-one GT1 HCV infected patients was extracted using the NucliSens® easyMAG system, followed by an in-house nested RT-PCR of the NS5 coding region. PCR products were purified and subsequently sequenced with the 3130xl ABI PRISM Genetic Analyzer. Sequences were finally aligned and edited using ChromasPro v1.7.6, and analyzed online with hcv.geno2pheno.org. NS5A RASs were present in 28.4 per cent (23/81) of all GT1 infected patients, with GT1a showing the highest prevalence followed by GT1b (17 vs. 11 per cent, respectively). Major NS5A RASs were detected in 23.6 per cent (13/55) of GT1a infected patients (M28V, Q30H/R, L31M, and Y93C/H) and in 15.4 per cent (4/26) of GT1b infected patients (L31M and Y93H). The most commonly detected NS5A RAS was Y93C/H with a prevalence of 9.9 per cent (8/81) in all GT1 infected patients, followed by L31M and Q30H/R with a prevalence of 8.6 per cent (7/81) and 6.2 per cent (5/81), respectively. Furthermore, Y93C/H showed a higher prevalence in GT1b patients than in GT1a, namely 11.5 per cent (3/26) vs. 9.1 per cent (5/55), respectively. NS5B RASs