Molecular Epidemiology of Hepatitis C virus in Prishtina region of Kosovo

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This dissertation was developed at the Department of Microbiology, National Institute of Public Health of Kosovo, Prishtina, Kosovo; the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia; and the University Hospital for Infectious Diseases 'Fran Mihaljević', Zagreb, Croatia.

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TABLE OF CONTENTS

List of symbols and abbreviations

aLRT	Approximate likelihood ratio test
Anti-HCV	Antibodies against hepatitis C virus
APR	Adjusted prevalence rate
BIOBSS	Biological and behavioral surveillance study
CI	Confidence interval
DAA	Direct acting antiviral
EMCDDA	European Monitoring Center for Drugs and Drug Addiction
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IDU(s)	Injecting drug user(s)
IFN	Interferon
IL	Interleukin
NBTCK	National Blood Transfusion Center of Kosovo
PR	Unadjusted prevalence rate
WHO	World Health Organization

1. INTRODUCTION AND BACKGROUND FOR THE PROPOSED RESEARCH

1.1 Global impact of HCV infection

Infection by the hepatitis C virus (HCV) is a global health problem (1). Since its discovery in 1989, HCV is one of the leading causes of acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (2, 3). Up to September 2016, the global prevalence of HCV infection was estimated to be between 2–3%, ranging from 1.3% in Americas up to 2.9% in Africa (4). According to prevalence estimates based on a comprehensive modelling study, the global prevalence of viremic HCV was estimated to be 1.0% in 2015, corresponding to 71.1 million (62.5–79.4) people with viremic infection (5). It was estimated that HCV caused 86,000 deaths in the World Health Organization (WHO) European Region and it was accounted for 35% of cirrhosis and 32% of liver cancer deaths (6).

HCV infection is unevenly distributed in the world. In the European Union, the prevalence of HCV infection among general population varies from 0.1% in the Western Europe (Belgium, Ireland, and the Netherlands) to 3.2% in Romania, and going up to 5.9% in some HCV hyper-endemic regions in Italy (7). In a number of Mediterranean countries, the HCV prevalence is around 3% and well over 10% in some regions of Asia and Africa, including Egypt with the highest prevalence of HCV in the world, estimated nationally at 14.7% (4, 8).

Infection with HCV most commonly happens through exposure to blood of HCV infected person. This could happen through transfusion of blood and its products, injecting drug use, hemodialysis, unsafe injection practices and surgical interventions, mother-to-fetus transmission, and among those with high-risk sexual behaviors. Transmission through sexual intercourse, though, is less likely and most commonly occurs among men who have sex with men and among people living with HIV (9).

Injection drug use and unsafe health-care procedures were the leading causes of new HCV infections, accounting for most of the 1.75 million new infections in 2015 (10).

Globally, 10 million injecting drug users (IDUs) are estimated to be infected with HCV, corresponding to a midpoint prevalence of 67% (11). It is estimated that IDUs have 47 times higher antibodies against hepatitis C virus (anti-HCV) prevalence compared to the general population (12). The HCV prevalence among IDUs in the European Union countries ranges from 13.8% in Malta to 84.3% in Portugal (6). The prevalence rate of HCV infection among IDUs in the USA aged 40–65 years was found to be 43.1%, whereas up to 90% of IDUs have been exposed to HCV in the past and 70–85% of those are estimated to be chronically infected and able to transmit HCV to others (13–15). Anti-HCV screening was positive for 54.6% of clients enrolled in the National Methadone Maintaince program in China (16). In Zanzibar, 33.5% of IDUs were found to be infected with HCV, with longer duration of drug injection and older age being independently associated with HCV infection (17).

Worldwide HCV prevalence varies also among patients on hemodialysis, ranging from 1.9% in Slovenia, 3.4% in the Netherlands, 4.7% in Lebanon, 5.6% in Senegal, 6.1% in Bejing, China, 7.7% in France, 8.4% in Brazil, 23.7% in Khartum, Sudan, 27.3% in Romania, 31.1% in Libya, and up to 60% in Morocco (18–28).

Patients on dialysis were recognized as a high risk group for hepatitis C virus (HCV) infection almost immediately after the identification of HCV in 1989. Both transfusional and nosocomial transmission of HCV were relatively frequent in dialysis units (29, 30). Correlation between HCV infection, blood transfusion and time duration on dialysis was noticed more than twenty years ago (31). In addition, higher prevalence in hemodialysis than peritoneal dialysis or home hemodialysis, and the highly variable prevalence from unit to unit, all suggested that nosocomial transmission has contributed to the high HCV prevalence (32).

HCV transmission in the hemodialysis environment still remains a substantial problem in low-resource countries compared to developed countries in which isolated small-scale HCV outbreaks in hemodialysis units are reported only occasionally (33–35).

1.2 Main features of HCV

HCV is an enveloped, single-stranded, positive-sense RNA virus (36). The virus is a member of the *Hepacivirus* genus in the virus family of *Flaviviridae*. The HCV genome with its 9.6 kb size, encodes a single polyprotein which is cleaved by cellular and viral proteases into at least ten different proteins: the structural proteins core, E1, E2, the ion channel p7 and the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B (37). They act with host proteins to mediate virus entry and to coordinate RNA replication and virus production (38). The hepatitis C genome has high genetic divergence, and the superfacing E1 and E2 proteins have the most changes (39). Not being a DNA virus, hepatitis C virus cannot enter the host genome, therefore does not proliferate with DNA; its half-life is around 2.5 hours (40). Genetically diverse variants of HCV seem to have circulated for hundreds of years in Central and West sub-Saharan Africa and South and South East Asia, where the origin of HCV could be traced (41).

There are 7 genotypes and 67 subtypes of hepatitis C virus (42, 43). In 2014, genotype 7 of HCV was identfied in four subjects originating from the Democratic Republic of Congo (44). It is estimated that HCV genotype 1 is most prevalent worldwide (approx. 46% of all HCV cases), followed by genotype 3 (with approx. 30%) and genotypes 2, 4, and 6 (with approx. 22.8%). HCV genotype 5 comprises less than 1% of global prevalence (45). Some subtypes of HCV (1a, 1b, 2a, 2b, and 3a) are widely distributed all over the world. Others have a more restricted distribution, such as genotype 4 in the Middle East and central Africa, subtype 5a in southern Africa, and genotype 6 in Southeast Asia (46). Infections with HCV subtype 1a and 3a have been shown to be significantly associated with injecting drugs (47-49). In the Netherlands, among patients with HCV subtype 1b, transfusion of blood/blood products was found to be the main route of transmission (50).

1.3. HCV infection and disease progression

In most cases, acute HCV infection remains without symptoms or with only unspecific symptoms such as fatigue, low-grade fever, myalgia or nausea. Jaundice appears only in 20% to 30% of the cases. On average, 26% of patients with acute hepatitis C infection experience spontaneous clearance of the virus, an event that occurs primarily during the first 3 months after clinical onset of disease and depends on several host and viral factors (51, 52). Individuals may remain asymptomatic for many years in case they fail to clear their HCV infection in this manner, entering into chronic infection and facing considerable risk of liver disease (53). Regardless of HCV genotype and of viral load, hepatic inflammation and fibrosis progression follow chronic infection (54). Death related to the complications of cirrhosis may occur, at an incidence of approximately 4% per year, whereas hepatocellular carcinoma (HCC) occurs in this population at an estimated incidence of 1–5% per year (55). Patients diagnosed with HCC have a 33% probability of death during the first year (56, 57).

1.4 HCV treatment

Until the introduction of direct acting antivirals (DAAs), the standard of care for HCV infection was based on pegylated interferon-α and ribavirin combination therapy (58). Currently, combination of DAAs, with/without ribavirin and/or interferon and treatment duration depends, in addition to clinical stage of HCV infection, also on HCV genotype (59).

Since 2011, the first DAAs, boceprevir and telaprevir, have been approved for use in the United States (60). DAAs are molecules that target specific nonstructural proteins of the virus, which results in disruption of viral replication and infection. Currently, there are four classes of DAAs, which are defined by their mechanism of action and therapeutic target. The four classes are: nonstructural proteins 3/4A (NS3/4A) protease inhibitors (PIs), NS5B nucleoside polymerase inhibitors (NPIs), NS5B non-nucleoside polymerase inhibitors (NNPIs), and NS5A inhibitors (61).

Availability of DAAs has transformed HCV treatment, enabling regimens that can be administered orally, with shorter duration (as short as eight weeks), resulting in cure rates higher than 90%, and being associated with fewer serious adverse events than the previous interferon containing regimens (62).

A number of studies have reported a genetic polymorphism near the interleukin (IL) 28B gene, encoding interferon-λ-3 (IFN-λ-3) associated with an approximately two-fold change in response to treatment with pegylated interferon (IFN) and ribavirin among patients with chronic hepatitis C, genotype 1 (63–65). Also, IL28B polymorphism (rs12979860 CC genotype) was associated with a sustained virological response in patients infected with HCV genotype 2 or 3 who did not achieve a rapid virological response (66). Therefore, information on the presence of IL28B polymorphism gives both patients and clinicians a possibility to make more informed decisions regarding the risk-benefit of treatment, raising the likelihood of success for any given individual (67). However, this test is not helpful in predicting response to DAA-based therapy, and thus is no longer one of the components of the pre-treatment evaluation in countries where these drugs are available for patients infected with HCV (62).

1.5 Kosovo and HCV infection

Kosovo is a small country (10908 km^2) in South Eastern Europe with approximately 1.8 million people. Administratively, it is divided into 7 regions, among them the Region of Prishtina being the largest with 27.5% of the total population (68).

Current information on HCV in Kosovo is scarce. There are no comprehensive data available or published on HCV prevalence among the general population, specific groups of population, and groups at a higher risk for exposure to HCV or genotype distribution among these groups. A few studies, with limited number of study participants or with limited geographical coverage for Kosovo, show the prevalence of HCV among the general population to be 0.5% – 0.7% (69, 70).

Blood donation in Kosovo is based on voluntary (approx. 80%) and family basis (approx. 20%), with approximately 23,000 donations per year. All blood donors are tested mandatorily on the presence of antibodies against HCV, human immunodeficiency virus (HIV), syphilis and hepatitis B surface antigen (HBsAg). Laboratory testing is centralized; blood donations given all around Kosovo are being tested only at the laboratory of the National Blood Transfusion Center of Kosovo (NBTCK) in Prishtina. Data from 70,438 blood donors at the NBTCK in the period 2000–2003 show the presence of HCV antibodies of 0.29% (71). An unpublished report from the NBTCK shows that there were 23,120 blood donations in 2011, of which 15,099 (65.3%) in Prishtina. There were 73,295 blood donations in Kosovo in the period 2011-2013, of which only 29 resulted anti-HCV positive, giving anti-HCV prevalence among blood donors of 0.04% (unpublished data from the National Blood Transfusion Center of Kosovo).

Labyrinth, a non-governmental organization working with drug users in Kosovo, reported an increase of drug use and estimated there were 3,000 IDUs in Kosovo in 2007, with half of them located in Prishtina. A biological behavioral surveillance study among IDUs in Prishtina and Prizren, conducted in 2014, showed anti-HCV prevalence of 27% among 300 IDUs in Prishtina and 12% among 199 IDUs in Prizren. The programatic mapping and size estimation of key populations in Kosovo with regard to HIV program, conducted in 2016, estimated 4,973 IDUs in Kosovo, of which 1,217 (range 966–1468) or 24.5% were from the municipality of Prishtina (72).

Hemodialysis in Kosovo is provided at 7 regional centers, with approximately 700 patients, among them 170-180 being located in the Region of Prishtina, Kosovo. A study by Telaku et al., based on data from 2008, shows anti-HCV prevalence of 42.9% among all Kosovo patients on hemodialysis (73). Also, Quaglio et al. reported anti-HCV prevalence at Peja dialysis center in 2005 to be 87% (69).

The treatment with pegylated interferon and ribavirin within the public health sector of Kosovo started in 2011, and it is still provided in one location only, at the Infectious

Disease Clinic, University Clinical Center of Kosovo in Prishtina. DAAs are not available through public funding (74). Due to lack of funds, a very limited number of people with chronic HCV infection are currently in the treatment program (approx. 10–15 new cases of HCV chronic patients per year).

2. HYPOTHESIS

1. HCV genotypes 1 and 3 are the most prevalent HCV genotypes in the Prishtina Region of Kosovo;
2. Phylogenetic analysis of HCV strains will reveal significant transmission clusters within different groups of population living in Prishtina, Kosovo.

3. AIMS AND PURPOSE OF THE RESEARCH

GENERAL AIM:

To determine genetic diversity of hepatitis C virus among different population groups in the Prishtina Region of Kosovo.

SPECIFIC AIMS:

To determine the distribution of HCV genotypes in different groups of population in Prishtina, Kosovo;

To assess IL28B genetic variations in HCV positive patients in Kosovo and their relationship to virus clearance outcome.

4. MATERIALS AND METHODOLOGY

With the purpose to reach goal of the study and considering the fact that we were dealing with different groups of population in Prishtina and overall in Kosovo, following approval by the Ethics Board of the Medical Faculty, University of Prishtina "Hasan Prishtina" in Kosovo, we have used materials and methodologies described below.

4.1 Hemodialysis patients

Blood samples of all 668 dialysis patients at Prishtina dialysis center and all other six regional dialysis centers of Kosovo, obtained between January–March 2013, were included in the study. We expanded the range of inclusion of dialysis patients beyond Prishtina dialysis center with all patients of regional dialysis centers of Kosovo to gain the overall picture of HCV infection at dialysis centers of Kosovo and to compare the situation in Prishtina dialysis center and other centers in Kosovo. Blood samples were initially tested on the presence of antibodies against hepatitis C virus (anti-HCV). The anti-HCV testing was conducted at the National Institute of Public Health of Kosovo in Prishtina with ELISA anti-HCV test (Axiom, Germany) on ELISA washer 2600 and ELISA reader 2100 (Awareness Technologies, USA) following the manufacturers' instructions. Positive anti-HCV samples were further tested for HCV RNA, HCV genotype, and HCV NS5B region sequenced for phylogenetic analyses. Additionally, in order to better understand the situation with HCV infection at dialysis units in Prishtina and overall in Kosovo, we implemented a questionnaire through face-to-face interviews with 618 out of 708 dialysis patients undergoing hemodialysis in Kosovo in December 2015. The questionnaire consisted of questions about the main demographic data of the patients undergoing dialysis in Kosovo, their knowledge about HCV, their anti-HCV status, the information on possible factors which could have played a role in HCV infection, and the information on inclusion within the treatment program. One part of the questionnaire was directed towards managers of the dialysis units in Kosovo, with

the aim to collect information about the issues related to management of the dialysis units.

4.2. Injecting drug users

We used 99 anti-HCV positive serum samples and data collected from interviews with 205 IDUs within the Biological and Behavioral Surveillance Study (BIOBSS) in the Prishtina Region of Kosovo. The BIOBSS was implemented in 2011 under the auspices of the Ministry of Health and funded by the Global Fund for AIDS, Tuberculosis and Malaria project in Kosovo using respondent-driven sampling methodology but there was no HCV genotyping or phylogenetic analysis conducted nor behavioral data analyzed with the focus on HCV infection. BIOBSS inclusion criteria were: (a) age 18–50; (b) fluency in Albanian language; (c) drugs injected at least once in the past month; and (d) living and/or working in Prishtina (or having injected drugs regularly for at least three months in the past year in Prishtina). Blood samples from IDUs were collected by staff of the non-governmental organization 'Labyrinth', at their premises in Prishtina, following the study protocol. This organization is known among IDUs since it provides on regular basis the methadone substitution therapy and needle exchange program for IDUs in the Prishtina Region. All eligible individuals were informed about the nature and requirements of the study and asked for informed consent. Following verbal consent, participants were interviewed and briefed about the biological testing (pre-test counseling). After blood drawing, participants received a primary incentive (10 €) and three coupons for peer recruitment. During the second visit, the participants were given post-test counseling, during which test results were communicated. A secondary incentive was given pending the successful recruitment of other participants (max. 3 x 2 €). Samples were tested at the Department of Microbiology, National Institute of Public Health of Kosovo using HCV Ab Sensitive ELISA test (Dialab, Austria) for the presence of antibodies against HCV, following the manufacturer’s instructions as part of the initial BIOBSS study. Positive anti-HCV samples were further tested for HCV RNA, HCV genotype, and HCV NS5B region sequenced for phylogenetic analyses. The behavioral component of the study included a questionnaire on drug use practices, sexual behavior, imprisonment and treatment information.

4.3. Chronic HCV patients and blood donors

We also included 50 blood samples from chronic HCV patients. As a part of treatment monitoring, samples were collected during the months January–June 2013, at the Infectious Disease Clinic of the University Clinical Center of Kosovo in Prishtina, the only institution that provides treatment to HCV infected patients in Kosovo. Those samples were further tested for HCV RNA, HCV genotype, and HCV NS5B region sequenced for phylogenetic analyses. Additionally, we implemented a questionnaire with 35 out of those 50 HCV chronic patients who agreed to be interviewed. The questionnaire included questions similar to the ones used with dialysis patients described above.

There were 73,295 blood donations in Kosovo in the period 2011–2013, of which 29 resulted anti-HCV positive through regular screening of blood for HCV infection at the Blood Transfusion Center of Kosovo. Only ten of those anti-HCV positive blood donors agreed to participate in the study. Due to the low number of anti-HCV positive blood donors agreeing to participate in the study, their data were analyzed in the same section with chronic HCV patients.

4.4 Molecular and phylogenetic testing

In total, 514 anti-HCV positive serum samples from all four study groups were further tested at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia, for HCV RNA detection, HCV genotyping, and sequencing of NS5B region of HCV for phylogenetic analysis. In addition, identification of genetic polymorphism of IL28B was conducted among all study groups, except for dialysis patients.

The presence of HCV RNA was tested using COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 (Roche Molecular Diagnostics, France).
Viral RNA was extracted from 200-400 µl of plasma, using the MagnaPure Compact Nucleic Acid Isolation kit (Roche Diagnostics, Germany) on MagNA Pure Compact

nucleic acid extraction instrument (Roche Diagnostics, Germany) yielding 50 µl of final eluate. The PCR was performed using SuperScript™ III One-Step RT-PCR System with Platinum® Taq High Fidelity (Invitrogen, USA) and primers Sc2/Ac2, as described previously (75). If no amplicon was obtained, nested PCR with S7/A5 primers (75) and FastStart High Fidelity PCR System (Roche Diagnostics, Germany) was employed. The acquired 441 bp or 355 bp amplicons of the core region of HCV genome were purified with the addition of enzymes Exonuclease I and FastAP (Thermo Fisher Scientific, USA) and sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with the nested S7 primer. A BigDyeXTerminator Purification Kit (Applied Biosystems) was used for the removal of unincorporated dye terminators from the sequencing reactions and after successfully obtaining the sequences on the 3500 Genetic Analyzer (Applied Biosystems, USA), HCV genotype was determined with the NCBI Genotyping tool (76) with the addition of the genotype reference set 2012 of the core region retrieved from HCV Sequence Alignments (77), since the default reference set, which did not include all subtypes, wrongly subtyped 4d sequences as subtypes 4a, 1c or 1c/4a.

For the purpose of investigating molecular epidemiology and transmission routes of HCV among patients in Kosovo, the NS5B region of the viral genome was sequenced as described for the core region. Samples that were determined as subtypes 1a and 1b, 3a and 4d were further analyzed with primers A1b/F1b and B1b/E1b for subtypes 1a and 1b, primers A3a/F3a and B3a/E3a for subtype 3a, and primers 4HCV-OS/4HCV-OA and 4HCV-IS/E1B for subtype 4d (78). Separate alignments of the obtained sequences of subtype 1a, 1b, 3a, and 4d sequences were made using ClustalW available in the BioEdit package (79). Quick neighbor joining tree was created using Mega 5.05 with 100 bootstrap replicates (80). Major clusters were identified and HCV control sequences were selected among GenBank available sequences, found most similar to the clustered sequences and also other more divergent sequences from Kosovo by employing HCV BLAST search tool (81–83). Find Best DNA/Protein Models tool in Mega 5.05 (80) was employed for the selection of the best fitted evolutionary model. Finally, maximum likelihood phylogenetic trees were constructed using PhyML 3.0 (84) and obtained phylogeny viewed with FigTree v1.4.3. Transmission clusters were identified according to approximate likelihood ratio test (aLRT) branch support values obtained.

The HCV positive samples were tested for the presence of IL28 polymorphism with LightMix kit IL28B (TIB MOLBIOL, Germany) and LightCycler® FastStart DNA Master HybProbe version 15 (Roche Diagnostics, Germany) on LightCycler® 480 Instrument (Roche), following the manufacturers' instructions.

4.5. Statistical analysis

The statistical analyses were performed using SPSS software, version 17.0. Data obtained from questionnaires were analyzed with the main focus on factors that could have had an impact on HCV infection.

Data are expressed as mean±SD, median and inter-quartile range, or frequencies, as appropriate. Differences between subgroups were assessed by $\chi 2$ (chi-squared) test, Fisher's exact test, Student's t-test and Analysis of Variance test. The Backward Poisson regression was used to assess the independent effect of a variable, and the prevalence ratios and their respective robust 95% confidence intervals were calculated. The Wald test was used as a statistical test.

5. RESULTS

5.1 HCV INFECTION AMONG DIALYSIS PATIENTS IN PRISHTINA AND OVERALL IN KOSOVO

5.1.1 Anti-HCV prevalence, HCV genotypes and phylogenetic analysis among dialysis patients

The study results showed that the mean prevalence of the antibodies against HCV (anti-HCV positive) in patients on dialysis in Kosovo was 53%. Prishtina dialysis center had the lowest anti-HCV prevalence (22.3%) among all dialysis centers of Kosovo. The highest percentage of anti-HCV positive patients on dialysis was recorded in Gjakova (91.1%), followed by Prizren, Peja, and Mitrovica, with very similar percentages (68.7%, 68.0%, and 67.6%, respectively). Almost half of the dialysis patients in Gjilan (49.4%) where anti-HCV positive as well as one third of patients at dialysis centers in Ferizaj (33.3%) (Table 1).

Table 1. Prevalence of antibodies against HCV among patients at dialysis centers of Kosovo in 2013

Dialysis center	Patients (n)	Anti-HCV positive (n)	Anti-HCV positivity (%)
Prishtina	188	42	22.3
Mitrovica	68	46	67.6
Peja	75	51	68.0
Prizren	163	112	68.7
Ferizaj	39	13	33.3
Gjilan	79	39	49.4
Gjakova	56	51	91.1
Total	**668**	**354**	**53.0**

The core region of HCV genome was detected among 275/354 (77.7%) anti-HCV positive dialysis patients in Kosovo. The highest percentage was observed among samples of Gjakova patients with 86.3%, followed by Gjilan patients with 84.6%. Very similar percentages of HCV core positive were detected among dialysis patients at

Prishtina, Prizren, Peja, and Mitrovica dialysis centers (76.2%, 77.7%, 80.4%, and 71.7%, respectively). The lowest percentage was found among anti-HCV positive patients at dialysis center in Ferizaj (38.5%) (Table 2).

Table 2. Anti-HCV positive patients at dialysis centers of Kosovo with presence of HCV core region and NS5B sequencing

Dialysis center	Anti-HCVpositive n	HCV core positive n (%)	NS5B sequenced n (%)
Prishtina	42	32 (76.2%)	29 (90.6%)
Mitrovica	46	33 (71.7%)	31 (93.9%)
Peja	51	41 (80.4%)	40 (97.6%)
Prizren	112	87 (77.7%)	80 (92.0%)
Ferizaj	13	5 (38.5%)	5 (100%)
Gjilan	39	33 (84.6%)	30 (90.9%)
Gjakova	51	44 (86.3%)	42 (95.5%)
Total	**354**	**275 (77.7%)**	**257 (93.5%)**

The HCV genotype was determined in 275 out of the total of 354 anti-HCV positive dialysis patients in Kosovo (Table 3).

The results showed that subtype 1a was the most frequent among patients on dialysis in Kosovo, with 62.2%; with the greatest frequency of occurrence at Ferizaj dialysis center (100%), followed by Gjakova (79.5%), Gjilan (78.8%), Mitrovica (75.8%), Prizren (63.2%), Prishtina (46.9%), and the lowest percentages of samples with subtype 1a were detected at Peja dialysis center (24.4%).

HCV subtype 4d was the second most frequent genotype among HCV patients at dialysis centers of Kosovo, with 33.1%. The frequency of occurrence of this subtype at the dialysis centers was: 73.2% in Peja, 43.8% in Prishtina, 34.5% in Prizren, 21.2% in Mitrovica. The lowest percentages of HCV subtype 4d were registered in Gjakova and Gjilan with 18.2% and 6.1%, respectively. HCV subtype 4d was not registered at Ferizaj dialysis center.

There were 10 samples with HCV subtype 1b, four in Gjilan (12.1%), followed by three (9.4%) in Prishtina, and one at each of the centers of Mitrovica, Peja, Prizren, and

Gjakova. Additionally, two patients had HCV genotype 2, one from Gjilan and one from Prizren (Table 3).

Table 3. HCV genotypes among patients at dialysis centers in Kosovo

Region	HCV genotype				
	Number of genotyped n	1a n (%)	1b n (%)	2 n (%)	4d n (%)
Prishtina	32	15 (46.9)	3 (9.4)	0	14 (43.8)
Mitrovica	33	25 (75.8)	1 (3.0)	0	7 (21.2)
Peja	41	10 (24.4)	1 (2.4)	0	30 (73.2)
Prizren	87	55 (63.2)	1 (1.1)	1 (1.1)	30 (34.5)
Ferizaj	5	5 (100)	0	0	0
Gjilan	33	26 (78.8)	4 (12.1)	1 (3.0)	2 (6.1)
Gjakova	44	35 (79.5)	1 (2.3)	0	8 (18.2)
Total	**275**	**171 (62.2)**	**11 (4.0)**	**2 (0.7)**	**91 (33.1)**

Out of 275 anti-HCV positive patients with sequenced HCV core region, we were able to obtain HCV NS5B region from 257 (93.5%) (Table 2). We obtained the following number of NS5B sequences: 162 sequences of subtype 1a (63.0%), 84 of subtype 4d (32.8%), and 11 of subtype 1b (4.3%) (Table 4).

Table 4. NS5B sequenced samples by dialysis center and HCV genotype

Region	Number of NS5B sequenced samples by HCV genotype			
	Number of NS5B sequences n	1a n (%)	1b n (%)	4d n (%)
Prishtina	29	14 (48.3)	3 (10.3)	12 (41.4)
Mitrovica	31	25 (80.6)	1 (3.2)	5 (16.1)
Peja	40	9 (22.5)	1 (2.5)	30 (75.0)
Prizren	80	51 (63,8)	1 (1.3)	28 (35.0)
Ferizaj	5	5 (100.0)	0	0
Gjilan	30	24 (80.0)	4 (13.3)	2 (6.7)
Gjakova	42	34 (81.0)	1 (2.4)	7 (16.7)
Total	**257**	**162 (63.0)**	**11 (4.3)**	**84 (32.7)**

The cladogram phylogenetic tree of the sequences gained from the NS5B region of the HCV subtypes 1a, 1b and 4d isolates from dialysis units in Kosovo showed a number of clusters which were mainly related to specific dialysis centers where dialysis patients had utilized the services.

Phylogenetic tree developed by the dialysis sample sequences of HCV subtype 1a showed three large clusters with aLRT>0.9. In the largest cluster (with aLRT>0.9) were 74 dialysis samples (74/162; 45.7%), of which 5 were from Prishtina dialysis center, 29 from Prizren, 25 from Gjakova, 8 from Peja, 4 sample sequences from Mitrovica dialysis center, and 3 from Gjilan. The second cluster (with aLRT>0.9) included 46 dialysis samples (46/162; 28.4%), of which 3 were from Prishtina dialysis center, 21 from Mitrovica, 20 from Prizren, 1 from Gjakova, and 1 from Gjilan dialysis center. The third cluster of dialysis samples with aLRT>0.9 inside cladogram of HCV subtype 1a consisted of 22 samples (22/162; 13.6%), of which 5 were from Prishtina dialysis center, 15 from Gjilan, and 2 from Ferizaj (Fig. 1). In general, all dialysis samples (100%) with HCV subtype 1a showed phylogenetic clustering with aLRT>0.9.

With regard to cladogram phylogenetic tree of the HCV isolates with subtype 1b at dialysis centers of Kosovo (n=11), there was one cluster of sample sequences with aLRT>0.9 consisted of 7 samples (7/11; 63.6%), of which 3 were from Prishtina dialysis center, 2 from Gjilan, and 1 from Prizren and Gjakova dialysis centers (Fig. 2). Almost 2/3 (63.6%) of sample sequences from dialysis patients with HCV subtype 1b showed phylogenetic clustering.

Phylogenetic tree developed by the NS5B sequences of the HCV subtype 4d isolated from dialysis patients showed three clusters with aLRT>0.9, 2 clusters with aLRT >0.8, and three clusters with aLRT<0.8. The largest cluster with aLRT>0.9 consisted of 13 sample sequences (13/84; 15.5%), of which 11 were from Peja, 1 from Prishtina, and 1 from Prizren dialysis center. The second cluster with aLRT>0.9 consisted of 11 sample sequences from Prizren dialysis center (11/84; 13.1%). The third cluster with aLRT>0.9, as part of the sample sequences from HCV subtype 4d, also consisted of 6 samples from Prizren dialysis center (6/84; 9.5%). Those sequences were part of a

larger cluster with aLRT>0.8, which additionally included 20 sample sequences from dialysis patients (20/84; 23.8%). Among them 9 were from Prishtina dialysis center, 2 from Gjilan, 4 from Prizren, and 5 from Mitrovica dialysis center. The second cluster with aLRT>0.8, as part of this cladogram, consisted of 15 dialysis samples (15/84; 17.8%), of which 6 were from Prizren, 5 from Peja, and 4 from Gjakova dialysis center (Fig. 3). In fact, 77.4% (65/84) of sample sequences from dialysis patients with HCV subtype 4d showed phylogenetic clustering with aLRT>0.8. If we add to this calculation also the samples with aLRT<0.8, then we have 96.4% (81/84) of sample sequences of HCV subtype 4d showing phylogenetic clustering.

Figure 1. The maximum likelihood phylogenetic tree of HCV subtype 1a. Sequences from different groups (including different dialysis centers) are colored differently. Clusters with aLRT>0.9 are highlighted in green and clusters with aLRT>0.8 are highlighted in yellow.

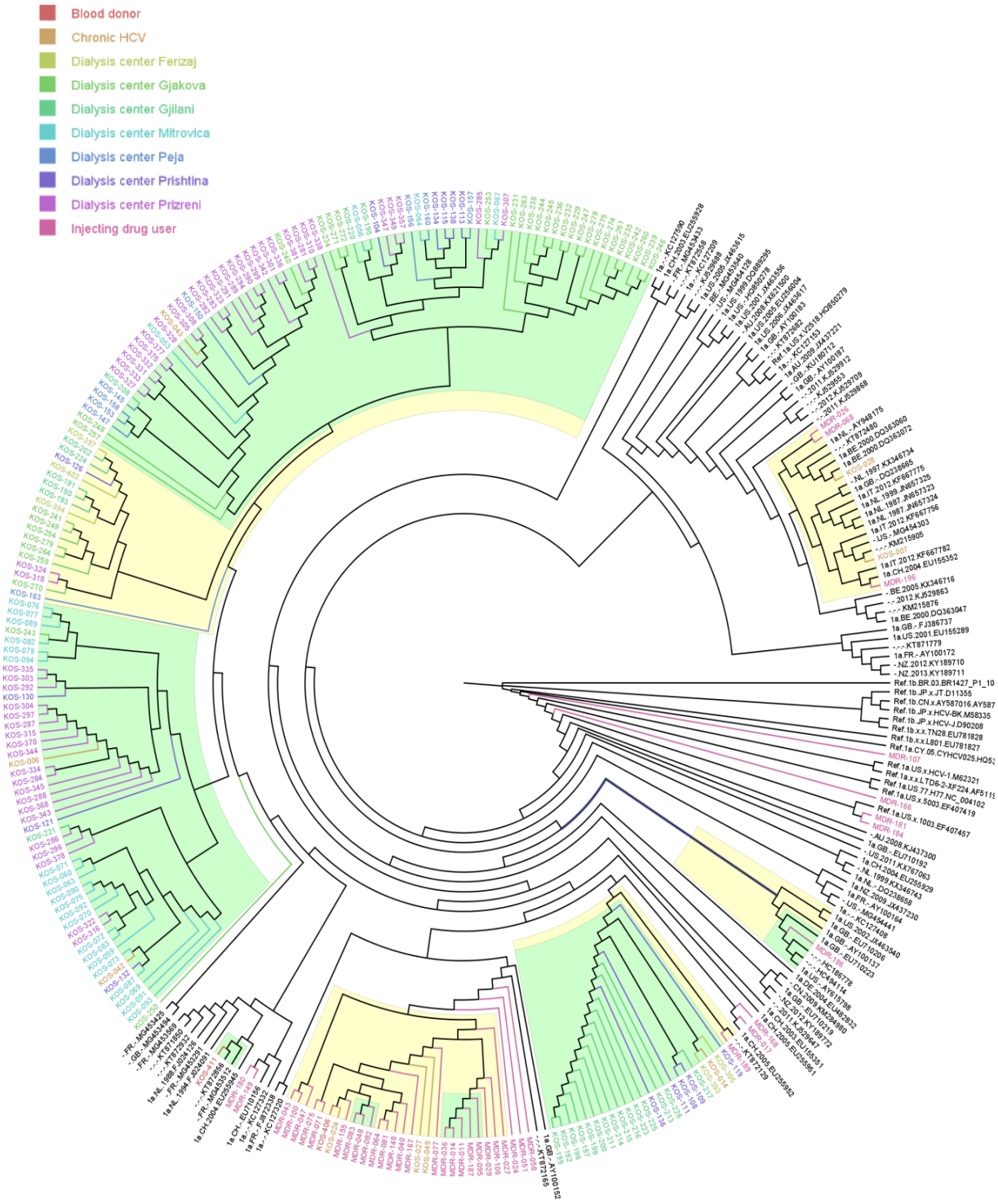


Figure 2. The maximum likelihood phylogenetic tree of HCV subtype 1b. Sequences from different groups (including different dialysis centers) are colored differently. Clusters with aLRT>0.9 are highlighted in green and clusters with aLRT>0.8 are highlighted in yellow.

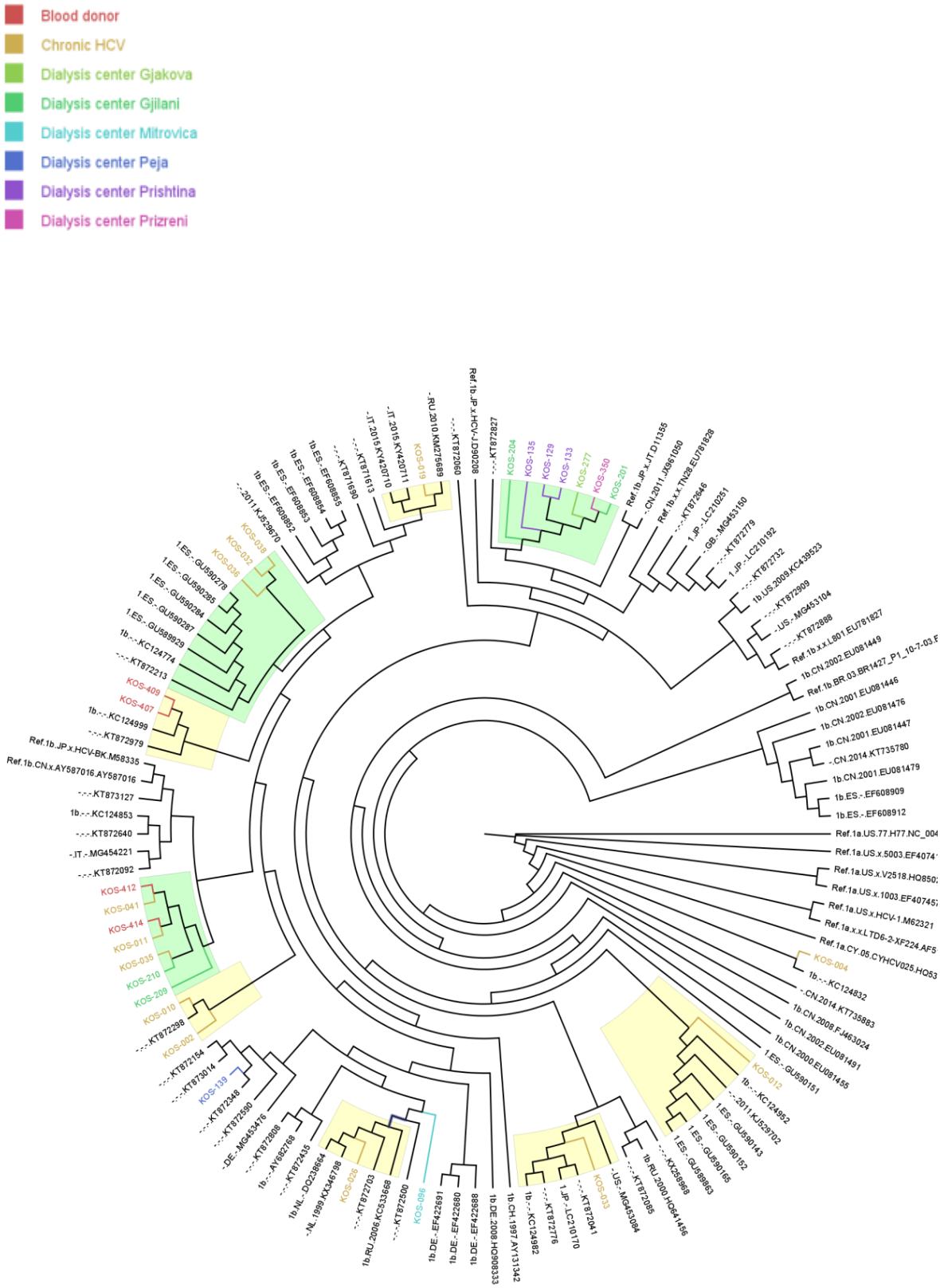
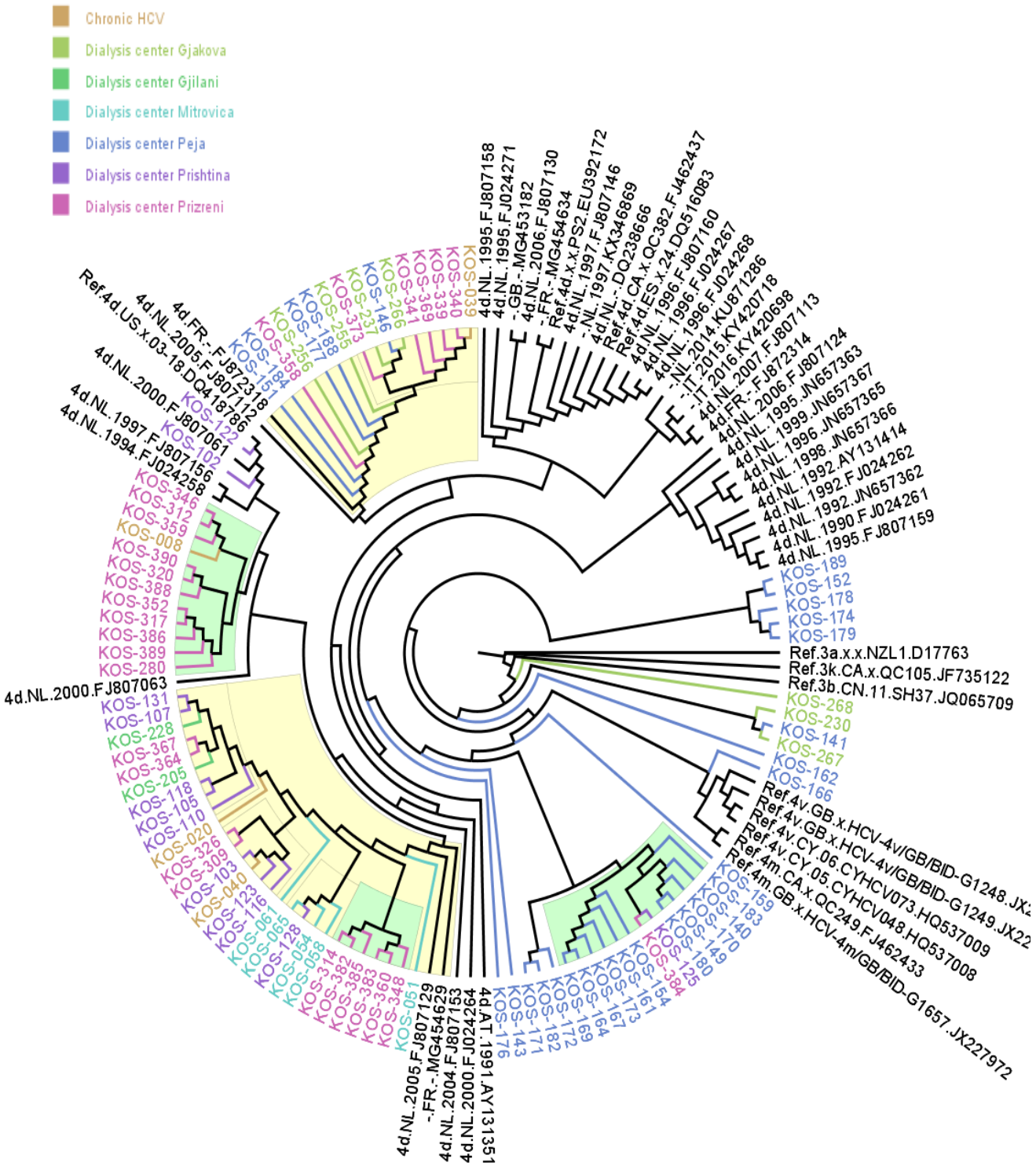


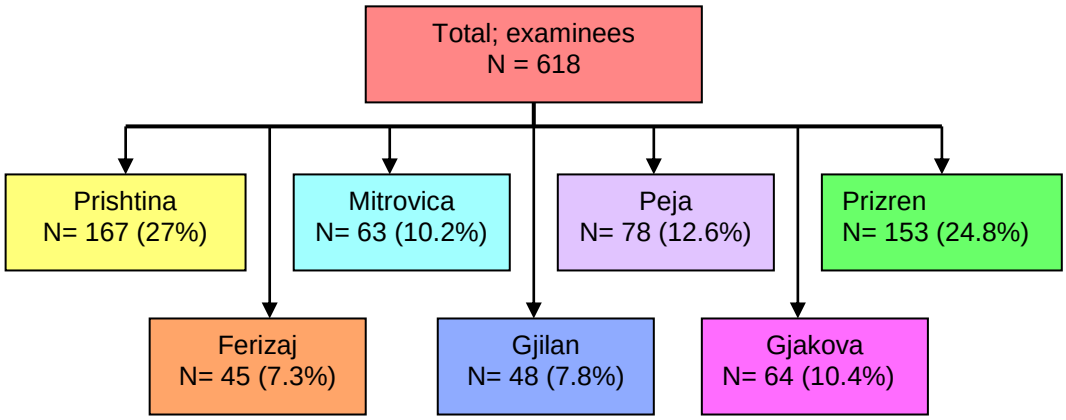
Figure 3. The maximum likelihood phylogenetic tree of HCV subtype 4d. Sequences from different groups (including different dialysis centers) are colored differently. Clusters with aLRT>0.9 are highlighted in green and clusters with aLRT>0.8 are highlighted in yellow.



5.1.2 Results from interviews with dialysis patients

Out of a total of 708 patients on dialysis at the dialysis centers in Kosovo in December 2015, 618 (87.2%) agreed to be interviewed and to participate in this study. Among them, 477 (77.1%) had been subjects of the initial study conducted in 2013.
Distribution of the examinees by regional dialysis centers showed that most patients were from Prishtina and Prizren dialysis centers (27% and 24.8%, respectively) (Fig. 4).

Figure 4. Distribution of dialysis patients by dialysis center in Kosovo



5.1.2.1 Socio-demographic data of dialysis patients in Kosovo

Among the study population, there were 299 (48.4%) females and 319 (51.6%) males, with a mean age of 57.7 ± 12.6 years (minimum age 20 years, maximum age 84 years). Most patients were aged 50–59 years, with 178 (28.8%) patients, and 60–69 years, with 180 (29.1%) patients. There were 52 patients aged up to 39 years (8.4%). As for marital status, married patients dominated – 508 (83.2%). As for educational level, there were 96 (15.5%) patients without formal education, 245 (39.6%) with primary education, 200 (32.3%) with secondary education, and the lowest number of patients with higher education – 60 (9.7%).

Among the study population, there were only 9 (1.5%) health workers, more than 50% of the examinees were unemployed – 337 (54.5%), and 212 (34.%) had a status of retired persons (Table 5).

Table 5. Demographic characteristics of dialysis patients in Kosovo

Variable		N (%)
Gender	female	299 (48.4)
	male	319 (51.6)
Age group	≤ 39	52 (8.4)
	40 – 49	96 (15.5)
	50 – 59	178 (28.8)
	60 – 69	180 (29.1)
	70 >	112 (18.1)
Marital status	single	49 (7.9)
	engaged	1 (0.2)
	married	508 (82.2)
	widow/er	59 (9.5)
	divorced	1 (0.2)
Education	no formal education	96 (15.5)
	primary school	245 (39.6)
	secondary school	200 (32.4)
	university	60 (9.7)
	other	17 (2.8)
Occupation	housewife	257 (41.6)
	farmer	76 (12.3)
	physical worker	55 (8.9)
	teacher	29 (4.7)
	engineer	6 (0.9)
	economist	17 (2.8)
	lawyer	6 (0.9)
	health worker	9 (1.5)
	other	163 (26.4)
Employment	unemployed	337 (54.5)
	employed in public sector	38 (6.1)
	employed in private sector	28 (4.5)
	employed in public sector, private sector	1 (0.2)
	retired	212 (34.3)
	missing	2 (0.3)

The gender structure at dialysis centers showed the highest numbers of females at Ferizaj (60%) dialysis center followed by the centers in Mitrovica, Gjakova, Prishtina, and Gjilan (55.6%, 53.1%, 52.1%, and 52.1%, respectively). In both regional centers,

Peja and Prizren, male patients were in majority (56.4% and 62.8%, respectively) (Table 6).

Table 6. Distribution of patients at dialysis centers by gender

Dialysis center		Gender	
		female n (%)	male n (%)
Prishtina	n = 167	87 (52.1)	80 (47.9)
Mitrovica	n = 63	35 (55.6)	28 (44.4)
Peja	n = 78	34 (43.6)	44 (56.4)
Prizren	n = 153	57 (37.3)	96 (62.7)
Ferizaj	n = 45	27 (60.0)	18 (40.0)
Gjilan	n = 48	25 (52.1)	23 (47.9)
Gjakova	n = 64	34 (53.1)	30 (46.9)
Total	N = 618	299 (48.4)	319 (51.6)

The average age was the lowest in the group of patients from Mitrovica **(**56.2 ± 12.6 years) and Prizren **(**56.2 ± 13.1 years) dialysis center, whereas patients from Ferizaj were the oldest, on average **(**62.4 ± 8.5 years). Age of Prishtina dialysis patients was similar to average age of dialysis patients in Kosovo (57.4 vs 57.7 years) (Table 7a).

Table 7a. Distribution of dialysis patients by age

Dialysis center	Age group (years)		Age (mean ± SD; min–max)
	Up to 59 n (%)	60 or older n (%)	
Prishtina	88 (52.7)	79 (47.3)	57.4 ± 14.3 (20–83)
Mitrovica	34 (54.0)	29 (46.0)	56.2 ± 12.6 (23–80)
Peja	39 (50.0)	39 (50.0)	58.9 ± 11.6 (27–84)
Prizren	92 (60.1)	61 (39.6)	56.2 ± 13.1 (23–81)
Ferizaj	15 (33.3)	30 (66.7)	62.4 ± 8.5 (41–79)
Gjilan	25 (52.1)	23 (47.9)	58.5 ± 11.9 (27–78)
Gjakova	33 (51.6)	31 (48.4)	58.6 ± 9.9 (29–80)
Total	326 (52.8)	292 (47.2)	57.7 ± 12.6 (20–84)

For study purposes, we divided patients from dialysis centers into 5 age groups: (1) up to 39 years; (2) 40 to 49 years; (3) 50 to 59 years; (4) 60 to 69 years; and (5) 70 years and older.

There were no patients in the youngest age group at Ferizaj dialysis center, while the greatest percentage of patients in this category (13.2%) was observed at Prishtina dialysis center. The lowest percentage of patients in age group 40 to 49 years was observed at Ferizaj dialysis center (6.7%), and the highest (22.2%) at Prizren dialysis center; the lowest percentage of patients in age group 50 to 59 years was observed at dialysis centers in Ferizaj and Mitrovica (26.8% and 27%, respectively), and the highest at Gjilan dialysis center (35.4%). The lowest percentage of patients in age group 60 to 69 years was found at Prizren dialysis center (21.6%), and the highest at Ferizaj center (46.7%). The lowest percentage of patients in the oldest age group was found among dialysis patients in Gjakova (10.9%), and the highest in Prishtina (22.2%) (Table 7b).

Table 7b. Distribution of dialysis patients by specific age groups

Dialysis center	**Age group (years)**				
	≤ 39 n (%)	**40 – 49 n (%)**	**50 – 59 n (%)**	**60 – 69 n (%)**	**>70 n (%)**
Prishtina	22 (13.2)	20 (12.0)	46 (27.5)	42 (25.1)	37 (22.2)
Mitrovica	6 (9.5)	11 (17.5)	17 (27.0)	20 (31.7)	9 (14.3)
Peja	4 (5.1)	11 (14.1)	24 (30.8)	26 (33.3)	13 (16.7)
Prizren	14 (9.2)	34 (22.2)	44 (28.8)	33 (21.6)	28 (18.3)
Ferizaj	0	3 (6.7)	12 (26.7)	21 (46.7)	9 (20.0)
Gjilan	4 (8.3)	4 (8.3)	17 (35.4)	14 (29.2)	9 (18.8)
Gjakova	2 (3.1)	13 (20.3)	18 (28.1)	24 (37.5)	7 (10.9)
Total	52 (8.4)	96 (15.5)	178 (28.8)	180 (29.1)	112 (18.1)

All 45 patients at Ferizaj dialysis center were married, as well as most patients at other regional dialysis centers (Table 8).

Table 8. Distribution of patients at dialysis centers by marital status

Dialysis center	**Marital status**				
	single n (%)	**engaged n (%)**	**married n (%)**	**widow/er n (%)**	**divorced n (%)**
Prishtina	17 (10.2)	0	139 (83.2)	11 (6.6)	0
Mitrovica	3 (4.8)	1 (1.6)	55 (87.3)	4 (6.3)	0
Peja	7 (9.0)	0	59 (75.6)	12 (15.4)	0

Prizren	15 (9.8)	0	118 (77.1)	19 (12.4)	1 (0.7)
Ferizaj	0	0	45 (100)	0	0
Gjilan	4 (8.3)	0	36 (75.0)	8 (16.7)	0
Gjakova	3 (4.7)	0	56 (87.5)	5 (7.8)	0
Total	49 (7.9)	1 (0.2)	508 (82.2)	59 (9.5)	1 (0.2)

The highest percentage of patients without formal education was observed at Ferizaj center (26.7%), with primary education in Prizren (52.3%), with completed secondary education in Prishtina (37.7%), and with higher education at Peja dialysis center (17.9%) (Table 9).

Table 9. Distribution of patients at dialysis centers by educational level

Dialysis center	**Educational level**				
	no formal education n (%)	**primary school n (%)**	**secondary school n (%)**	**university n (%)**	**other n (%)**
Prishtina	32 (19.2)	50 (29.9)	63 (37.7)	19 (11.4)	3 (1.8)
Mitrovica	12 (19.0)	23 (36.5)	17 (27.0)	8 (12.7)	3 (4.8)
Peja	11 (14.1)	30 (38.5)	21 (26.9)	14 (17.9)	2 (2.6)
Prizren	12 (7.8)	80 (52.3)	53 (34.6)	6 (3.9)	2 (1.3)
Ferizaj	12 (26.7)	16 (35.6)	15 (33.3)	2 (4.4)	0
Gjilan	4 (8.3)	20 (41.7)	14 (29.2)	3 (6.3)	7 (14.6)
Gjakova	13 (20.3)	26 (40.6)	17 (26.6)	8 (12.5)	0
Total	96 (15.5)	245 (39.6)	200 (32.4)	60 (9.7)	17 (2.8)

The regional distribution by patients' occupation showed a great number of housewives at all centers. Out of 9 health workers who participated in the study, 5 were from Prishtina, 2 from Mitrovica, and 1 health worker from Peja and Gjakova each (Table 10).

Table 10. Distribution of patients at dialysis centers by occupation

Dialysis centre	**Occupation**								
	Housewife n (%)	**Farmer n (%)**	**Physical worker n (%)**	**Teacher n (%)**	**Engineer n (%)**	**Economist n (%)**	**Lawyer n (%)**	**Health worker n (%)**	**Other n (%)**
Prishtina	75 (44.9)	9 (5.4)	8 (4.8)	10 (6.0)	2 (1.2)	6 (3.6)	3 (1.8)	5 (3.0)	49 (29.3)
Mitrovica	32 (50.8)	8 (12.7)	0	0	1(1.6)	2 (3.2.)	0	2 (3.2)	18 (28.6)

Peja	28 (35.9)	13 (16.7)	7 (9.0)	9 (11.5)	2 (2.6)	2 (2.6)	1 (1.3)	1 (1.3)	15 (19.2)
Prizren	54 (35.3)	27 (17.6)	22 (14.4)	5 (3.3)	0	0	2 (1.3)	0	43 (28.1)
Ferizaj	25 (55.6)	8 (17.8)	10 (22.2)	2 (4.4)	0	0	0	0	0
Gjilan	20 (41.7)	7 (14.6)	2 (4.2.)	1 (2.1.)	0	3 (6.2)	0	0	15 (31.2)
Gjakova	23 (35.9)	4 (6.2)	6 (9.4)	2 (3.1.)	1 (1.6)	4 (6.3)	0	1 (1.6)	23 (35.9)
Total	257	76	55	29	6	17	6	9	163

As for employment status, the lowest percentage of the unemployed was observed in Peja (39.5%), the highest in Mitrovica (69.8%). There were no patients employed in public and private sectors at Ferizaj dialysis center; the greatest percentage of the employed in public sector were from Peja (9.2%), and in the private sector from Gjilan (8.3%). The lowest percentage of the retired patients was found in Mitrovica (19.0%) and the greatest in Ferizaj (60.0%) (Table 11).

Table 11. Distribution of patients at dialysis centers by employment

Dialysis center	**Employment status**			
	Unemployed n (%)	**Employed in public sector n (%)**	**Employed in private sector n (%)**	**Retired n (%)**
Prishtina	90 (54.2)	12 (7.2)	8 (4.8)	56 (33.7)
Mitrovica	44 (69.8)	5 (7.9)	2 (3.2)	12 (19.0)
Peja	30 (39.5)	7 (9.2)	5 (6.6)	34 (44.7)
Prizren	94 (61.4)	7 (4.6)	7 (4.6)	45 (29.4)
Ferizaj	18 (40.0)	0	0	27 (60.0)
Gjilan	24 (50.0)	4 (8.3)	4 (8.3)	16 (33.3)
Gjakova	37 (57.8)	3 (4.7)	2 (3.1)	22 (34.4)
Total	337 (54.8)	38 (6.2)	28 (4.5)	212 (34.5)

Among the patients having antibodies against HCV, 52.5% were females, 78.7% had a partner (wife/husband), the majority (43.4%) had primary education, 2.0% were health workers, 61.5% were unemployed at the time of the study implementation, 57.0% were younger than 59 years.

Female patients on dialysis had non-significantly higher prevalence of antibodies against HCV compared to males (42.8% vs 36.4%).

The prevalence of antibodies against HCV of 42.6% was observed in the age group of patients up to 59 years, and it was non-significantly higher than in the group of patients older than 60 years, among whom 36.0% had antibodies against HCV. As for distribution by age groups: the prevalence of anti-HCV antibodies was 51.9% in the group of patients up to 39 years of age, 41.7% in the age group 40 to 49 years, 40.4% in the age group 50 to 59 years, 38.9% in the age group 60 to 69 years, and 31.2% in the group of patients aged 70 years and older. Statistically significant difference in the prevalence of anti HCV antibodies among the analyzed age groups (p=0.15) was not found.

In the groups of patients with and without a partner, the prevalence of antibodies against HCV differed non-significantly (47.7% and 37.7%, respectively).

Patients with different educational levels had non-significantly different prevalence of antibodies against HCV (p=0.3). The highest percentage of HCV positive patients was recorded among those with completed primary education (43.4%).

Patient's occupation was significantly correlated with the prevalence of antibodies against HCV (p=0.047). The presence of antibodies against HCV was found in 5 (55.6%) out of 9 health workers. The lowest prevalence of antibodies was found in the group of patients with higher education (teachers, lawyers, engineers, economists) at 34.5%.

The prevalence of antibodies against HCV significantly correlated with the employment status of patients on dialysis. The percentage of HCV positive patients was 44.5%, 34.3%, and 33.5%, in the groups of unemployed, employed and retired, respectively (Table 12).

Table 12. Demographic data of anti-HCV positive dialysis patients in Kosovo

Variable		**anti-HCV positive n (%)**	**% of positivity**	*p*-value
Gender	female	128 (52.5%)	42.8	0.1
	male	116 (47.5%)	36.4	
Age group	≤ 59	139 (57.0%)	42.6	0.09
	60 >	105 (43.0%)	36.0	
Age group	≤ 39	27 (11.1%)	51.9	0.15
	40 – 49	40 (16.4%)	41.7	
	50 – 59	72 (29.5%)	40.4	
	60 – 69	70 (28.7%)	38.9	
	70 >	35 (14.3%)	31.2	
Marital status	without partner	52 (21.3%)	47.7	0.4
	with partner	192 (78.7%)	37.7	
Education	no formal education	41 (16.8%)	42.7	0.3
	primary school	106 (43.4%)	43.3	
	secondary school	70 (28.7%)	35.0	
	university	22 (9.0%)	36.7	
	other	5 (2.0%)	29.4	
Occupation	housewife	113 (46.3%)	44.0	0.047*
	farmer	29 (11.9%)	38.2	
	physical worker	27 (11.1%)	49.1	
	teacher, engineer, economist, lawyer	20 (8.2%)	34.5	
	health worker	5 (2.0%)	55.6	
	other	50 (20.5%)	30.7	
Employment	unemployed	150 (61.5%)	44.5	0.024*
	employed	23 (9.4%)	34.3	
	retired	71 (29.1%)	33.5	

p (Chi-square) *$p<0.05$

5.1.2.2 Knowledge about HCV among dialysis patients in Kosovo

Results of this study showed that the anti-HCV positive patients on dialysis significantly more frequently heard about HCV infection than the anti-HCV negative patients

(p=0.001). Almost 65% of HCV positive patients heard about HCV infection vs. half of HCV negative patients.

The HCV infected and the noninfected patients on dialysis significantly differed in knowledge about HCV spread (p=0.02). Affirmative response to the question 'Do you know how the HCV is spread?' was given by 65.7% of HCV positive patients compared to 51.5% of HCV negative patients, and negative response by 18.6% of HCV positive versus 27% of HCV negative patients.

Surprisingly, 41.4% of laboratory confirmed HCV cases, responded 'No' to the question if they had been diagnosed with HCV, and in the same group 11.9% answered 'Don’t know'. In addition, 7.5% of HCV negative patients said they were HCV positive.

Affirmative response to the question 'Have you ever had skin jaundice?' was given by 36.6% of HCV positive and 9.8% of HCV negative patients, and negative response by 59.3% of HCV positive and 89.6% of HCV negative patients, while 4.1% of HCV positive and 0.5% of HCV negative patients did not know if such a situation happened to them. The difference in the distribution of responses between HCV positive and HCV negative patients with regard to this variable was shown to be statistically significant (p<0.0001).

Family history revealed family members infected with HCV among 10.2% of HCV positive and 8.0% of HCV negative patients. The HCV positive and negative patients on dialysis gave non-significantly different information for family history with HCV (p=0.17) (Table 13). Information on HCV infection of a mother or a father was given by 8 HCV positive and 5 HCV negative patients, of a wife or a husband by 2 HCV positive and 3 HCV negative patients, of a daughter or a son by 4 HCV positive and 10 HCV negative patients, of a brother or a sister by 5 HCV positive and 6 HCV negative patients.

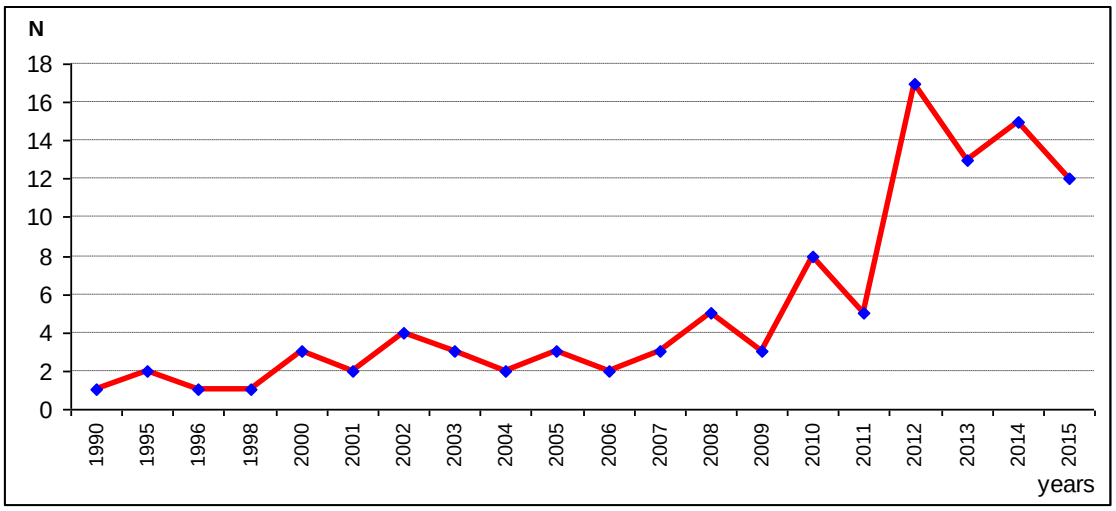
Table 13. Knowledge of HCV positive and HCV negative patients about HCV

Variable	HCV positive n (%)	HCV negative n (%)	*p*-value
Heard about hepatitis C virus			
Yes n = 348	158 (64.8)	190 (50.8)	0. 001 yes vs no 0.0003
No n = 256	79 (32.4)	177 (47.3)	
Don`t know n = 14	7 (2.9)	7 (1.9)	
Knew how hepatitis C virus is spread			
Yes n = 216	113 (65.7)	103 (51.5)	0.02 yes vs no 0.018 yes vs don`t know 0.046
No n = 86	32 (18.6)	54 (27.0)	
Don`t know n = 70	27 (15.7)	43 (21.5)	
Missing n = 246	72	174	
Diagnosed with hepatitis C infection			
Yes n = 142	114 (46.7)	28 (7.5)	< 0.0001 yes vs no < 0.0001 yes vs don`t know 0.000003
No n = 415	101 (41.4)	314 (84.0)	
Don`t know n = 61	29 (11.9)	32 (8.5)	
Skin jaundice			
Yes n = 125	89 (36.6)	36 (9.8)	< 0.0001 yes vs no < 0.0001 yes vs don`t know 0.0001
No n = 473	144 (59.3)	329 (89.6)	
Don`t know n =12	10 (4.1)	2 (0.5)	
Missing n = 8	1	7	
Family members infected with hepatitis C virus			
Yes n = 55	25 (10.2)	30 (8.0)	0.17
No n = 544	215 (88.1)	329 (88.0)	
Don`t know n =19	4 (1.6)	15 (4.0)	

p (Chi-square test)

Information on the time when HCV infection was diagnosed was given by 112 patients on dialysis. Diagnosis of HCV infection had been established in those patients, on average, 8.7 ± 11.3 years earlier (Fig. 5).

Figure 5. Distribution of anti-HCV positive dialysis patients by year of infection



5.1.2.3 Risk factors for HCV infection among dialysis patients in Kosovo

5.1.2.3.1 Blood transfusion as a risk factor for HCV infection

Patients on dialysis infected with HCV had received blood transfusion significantly more frequently compared to the noninfected ones ($p<0.0001$). The percentage of patients on dialysis who had received blood was 87.3% in the group of HCV positive and 70.3% in the group of HCV negative patients.

The University Clinical Center of Kosovo in Prishtina was the most frequent health institution where the dialysis patients from Kosovo had received blood transfusion (Table 14).

Table 14. Results of questionnaire about blood transfusion

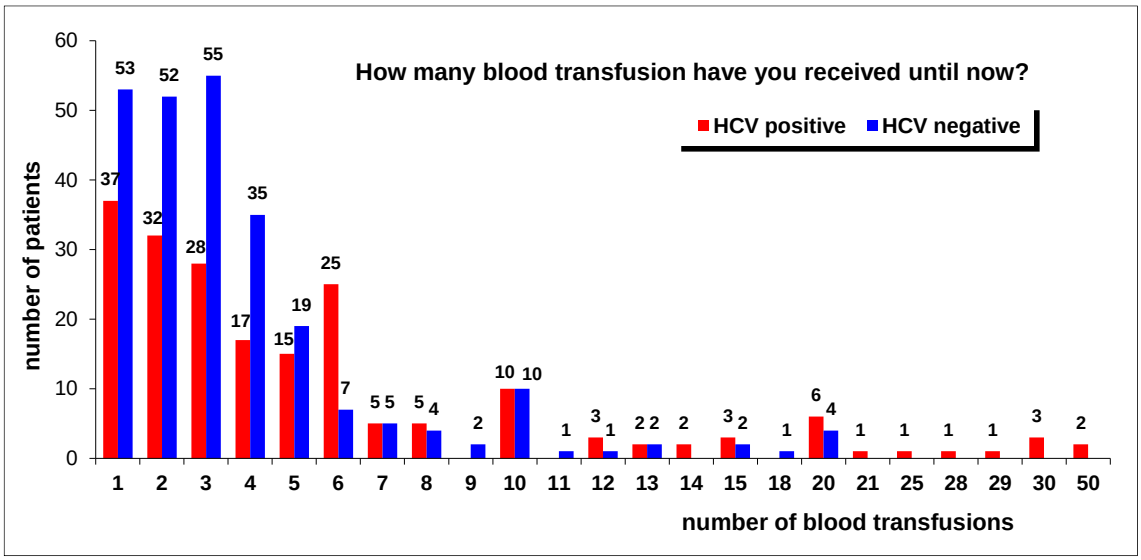
Variable	**HCV positive n (%)**	**HCV negative n (%)**	*p*-value
Blood transfusion			
Yes n = 476	213 (87.3)	263 (70.3)	0.000001
No n = 142	31 (12.7)	111 (29.7)	
Medical institution(s) where blood transfusion was performed			

University Clinical Center of Kosovo n = 198	80 (37.6)	118 (44.9)	
Regional hospital n = 164	67 (31.4)	97 (36.9)	
Private hospital n = 1	0	1 (0.4)	
Other n = 27	16 (6.6)	11 (4.2)	
University Clinical Center of Kosovo, Regional hospital n = 78	43 (20.2)	35 (13.3)	
Regional hospital, Other n = 8	7 (3.3)	1 (0.4)	

p (Chi-square test)

The number of blood transfusions received was significantly greater in the group of HCV positive patients vs. HCV negative patients (p=0.0013). Maximum number of blood transfusions received was 50 in the group of HCV positive, and 20 in the group of HCV negative patients. The average number of blood transfusions received in the HCV infected group was 4 (rank 2–6), and 3 in the noninfected group (rank 2–4) (Fig. 6).

Figure 6. Number of blood transfusions



Significant difference in the time of the first blood transfusion (p<0.0001) was observed between HCV infected and noninfected patients. HCV positive patients had their first blood transfusion earlier, on average 9.6 ± 7.6 years, compared to HCV negative patients, with 5.1 ± 6.5 years on average (rank 2–6) (Table 15).

Table 15. Year of first blood transfusion

Anti-HCV test result	N	Year of first blood transfusion		
		mean ± SD	min - max	median (IQR)
HCV positive	203	9.6 ± 7.6	1 - 50	7 (4 – 14)
HCV negative	248	5.1 ± 6.5	1 - 45	3 (2 – 6)
		$p < 0.0001$		

p (Mann-Whitney U test)

5.1.2.3.2 Dental services as a risk factor for HCV infection

The anti-HCV positive patients on dialysis used non-significantly more frequently dental services than the anti-HCV negative patients (p=0.36). Most patients in both groups had dental interventions (85.7% and 82.9%, respectively). The type of dental services and health institutions where they were provided are listed in Table 16. The HCV positive patients more frequently than the HCV negative patients underwent teeth extraction (49.9% vs 32.6%) and prosthetic interventions (12% vs 7.4%). Family Medicine Centers were the medical institutions in which most HCV positive patients utilized dental services (41.6%), whereas most HCV negative patients indicated private dentistry centers (47.1%) (Table 16).

Table 16. Use of dental services between HCV positive and HCV negative patients on dialysis

Variable	**HCV positive n (%)**	**HCV negative n (%)**	*p*-value
Dental services			
Yes n = 519	209 (85.7)	310 (82.9)	0.36
No n = 99	35 (14.3)	64 (17.1)	
Type of dental service			
Filling n = 28	8 (3.8)	20 (6.5)	
Extraction n = 204	103 (49.3)	101 (32.6)	
Prosthetics n = 48	25 (12.0)	23 (7.4)	

Other n = 11	7 (3.3)	4 (1.3)	
Filling, extraction n = 102	27 (12.9)	75 (24.2)	
Filling, prosthetics n = 5	1 (0.5)	4 (1.3)	
Filling, other n = 1	1 (0.5)	0	
Extraction, prosthetics n = 63	21 (10.0)	42 (13.5)	
Extraction, other n = 2	2 (1.0)	0	
Filling, extraction, prosthetics n = 55	14 (6.7)	41 (13.2)	
Medical institution where dental services were performed			
Family Medicine Center n = 182	87 (41.6)	95 (30.6)	
Private dentistry center n = 217	71 (34.0)	146 (47.1)	
University Clinical Center for Dentistry of Kosovo n = 13	4 (1.9)	10 (3.2)	
Other n = 41	21 (10.0)	20 (6.5)	
Family Medicine Center, Private dentistry center n = 61	24 (11.5)	37 (11.9)	
Family Medicine Center, University Clinical Center for Dentistry of Kosovo n = 2	2 (1.0)	0	
Family Medicine Center, University Clinical Center for Dentistry of Kosovo, Other n =2	0	2 (0.6)	

p (Chi-square test)

5.1.2.3.3 Surgical interventions as a risk factor for HCV infection

All patients on dialysis had a type of fistula placed. Beside this procedure, 36.1% of HCV positive and 36.6% of HCV negative patients on dialysis had other types of surgical interventions. Statistical analysis showed that patients on dialysis infected with HCV and the noninfected ones did not significantly differ in relation to surgical interventions (p=0.98).

Abdominal surgeries were the most frequent surgical interventions performed in both groups of patients (11.5% and 10.7%, respectively). Kidney transplantations were performed on 7 patients, 5 of which belonged to the group of HCV positive, and 2 to the group of HCV negative patients. In more than 50% of both HCV positive and HCV negative patients, surgical interventions were performed at the University Clinical Center of Kosovo in Prishtina (57.8% and 59.4%, respectively) (Table 17).

Table 17. Surgical interventions among HCV positive and HCV negative patients on dialysis

Variable	HCV positive n (%)	HCV negative n (%)	*p*-value
Surgical intervention			
Yes n = 225	88 (36.1)	137 (36.6)	0.98
No n = 393	156 (63.9)	237 (63.4)	
Type of surgical intervention			
Abdominal	28 (11.5)	40 (10.7)	
Orthopedics	6 (2.5)	14 (3.7)	
Ophthalmological	4 (1.6)	12 (3.2)	
Throat	3 (1.2)	1 (0.3)	
Urological	14 (5.7)	16 (4.3)	
Cardiovascular	3 (1.2)	14 (3.7)	
Gynecological	7 (2.9)	8 (2.1)	
Kidney transplant	4 (1.6)	2 (0.5)	
Thoracic		4 (1.1)	
Abdominal, orthopedics	1 (0.4)	1 (0.3)	
Abdominal, ophthalmological	2 (0.8)	2 (0.5)	
Abdominal, throat	1 (0.4)		
Abdominal, urological	2 (0.8)	2 (0.5)	
Abdominal, cardiovascular	2 (0.8)	3 (0.8)	
Abdominal, gynecological	1 (0.4)	1 (0.3)	
Abdominal, thoracic		1 (0.3)	
Orthopedics, ophthalmological	2 (0.8)	1 (0.3)	
Orthopedics, urological	2 (0.8)	1 (0.3)	
Orthopedics, cardiovascular		1 (0.3)	
Orthopedics, gynecological	1 (0.4)	1 (0.3)	
Orthopedics, kidney transplant	1 (0.4)		
Ophthalmological, throat		2 (0.5)	
Ophthalmological, urological		1 (0.3)	
Ophthalmological, cardiovascular		2 (0.5)	
Urological, cardiovascular		1 (0.3)	
Throat, gynecological	1 (0.4)	1 (0.3)	
Urological, gynecological	3 (1.2)	1 (0.3)	
Gynecological, kidney transplant	1 (0.4)		
Missing		3 (0.8)	
Medical institution where surgical intervention was performed			
University Clinical Center of Kosovo	141 (57.8)	222 (59.4)	

n = 363			
Regional hospital n = 62	24 (9.8)	38 (10.2)	
Private hospital n = 17	6 (2.5)	11 (2.9)	
Other n = 54	27 (11.1)	27 (7.2)	
University Clinical Center of Kosovo, Regional hospital n = 40	17 (7)	23 (6.1)	
University Clinical Center of Kosovo, Private hospital n = 14	8 (3.3)	6 (1.6)	
University Clinical Center of Kosovo, Other n = 47	15 (6.1)	32 (8.6)	
Regional hospital, Private hospital n = 3		3 (0.8)	
Regional hospital, other n = 9	3 (1.2)	6 (1.6)	
Private hospital, other n = 1	0	1 (0.3)	
Missing	1 (3.1)	4 (1.1)	

p (Chi-square test)

5.1.2.3.4 Tattoo, ear piercing, using others' shaving kit, imprisonment, and hemophilia as risk factors for HCV infection in dialysis patients

The HCV positive patients on dialysis non-significantly more frequently had had some of the following risk procedures: tattoo, ear/body piercing, injected drugs, used shaving kit belonging to someone else, and had been non-significantly more frequently imprisoned, or had hemophilia (p=0.37). The observed percentages of HCV positive and HCV negative patients with a registered high-risk procedure were 41.4% and 34.2%, respectively. Tattoo was registered in 2.9% of HCV positive and 4.3% of HCV negative patients; ear or other body piercing in 37.3% of HCV positive and 28.1% of HCV negative patients; used shaving kit belonging to someone else in 1.2% of HCV negative and 1.9% of HCV negative patients on dialysis. Experience as prisoners was registered in 4.9% of HCV positive and 8% of HCV negative patients, and the history of hemophilia was reported by 2.5% of HCV positive and 6.7% of HCV negative patients (Table 18).

Table 18. Tattoo, ear piercing, using others' shaving kit, imprisonment and hemophilia as risk factors for HCV infection in dialysis patients

Variable		HCV positive n (%)	HCV negative n (%)	*p*-value
Had tattoo, ear piercing, used shaving kit, imprisonment, hemophilia				
Yes n = 229		101 (41.4)	128 (34.2)	0.37
No n = 389		143 (58.6)	246 (65.8)	
High-risk behaviour				
Tattoo	Yes n = 23	7 (2.9)	16 (4.3)	0.06
	No n = 595	237 (97.1)	358 (95.7)	
Ear or other body piercing	Yes n = 196	91 (37.3)	105 (28.1)	0.016*
	No n = 422	153 (62.7)	269 (71.9)	
Used shaving kit belonging to someone else	Yes n = 10	3 (1.2)	7 (1.9)	0.78
	No n = 608	241 (98.8)	367 (98.1)	
Ever imprisoned				
Yes n = 42		12 (4.9)	30 (8.0)	0.13
No n = 574		232 (95.1)	344 (92.0)	
Hemophilia				
Yes n = 31		6 (2.5)	25 (6.7)	0.058
No n = 565		230 (94.3)	335 (89.6)	
Don`t know = 22		8 (3.3)	14 (3.7)	

p (Chi-square test) *p<0.05

5.1.2.3.5 Risk factor – Dialysis

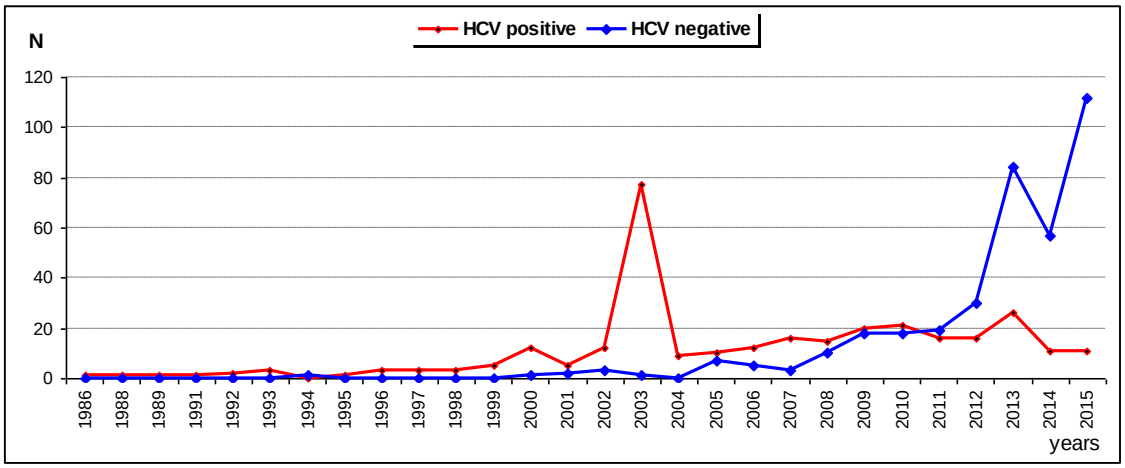
Results of this study showed that the history of dialysis was significantly longer in patients with the presence of anti-HCV vs. the negative ones (p<0.001). The mean history of dialysis was 8.8 ± 5.7 years in the group of anti-HCV positive patients, and 3.4 ± 2.9 years in the group of anti-HCV negative patients. The median history of dialysis in the group of infected patients was 8 years (interquartile range 4–12 years), and 3 years in the noninfected group (interquartile range 1–4 years) (Table 19).

Table 19. History of dialysis in anti-HCV positive and anti-HCV negative patients

Group	History of dialysis		
	mean ± SD	min – max	median (IQR)
Anti-HCV positive	8.8 ± 5.7	1 – 30	8 (4 – 12)
Anti-HCV negative	3.4 ± 2.9	1 – 22	3 (1 – 4)
	p<0.001		

p (Mann-Whitney U test)

Figure 7. Start of dialysis treatment



Eleven patients with terminal chronic kidney insufficiency were started on dialysis between 1986 and 1995, of whom 10 (90.9%) patients were found anti-HCV positive in this study. Between 1996 and 2005, 83 patients were started on dialysis, 69 (83.1%) of whom were anti-HCV positive. Most patients were started on dialysis between 2006 to 2015, among whom the prevalence of anti HCV antibodies was 31.5% (164/520). There was no information on the start of dialysis for 4 patients who agreed to participate in this study. Difference in the start of dialysis between HCV positive and HCV negative patients was shown to be statisticaly significant (Table 20a).

Table 20a. Distribution of anti-HCV positive and anti-HCV negative patients based on the year of dialysis initiation

Year of dialysis initiation	HCV positive n (%)	HCV negative n (%)	*p*-value
1986–1995 n = 11	10 (90.9)	1 (9.1)	0.0001
1996–2005 n = 83	69 (83.1)	14 (16.9)	
2006–2015 n = 520	164 (31.5)	356 (68.5)	
Total	**243**	**371**	**614**

p (Chi-square test)

Data on dialysis initiation were available for 144 HCV positive patients with the HCV genotype determined. Seven HCV positive samples of patients who were started on dialysis between 1986 and 1995 had HCV genotype determined, four of whom had subtype 1a, one had subtype 1b, and two had subtype 4d. Among 54 patients with determined HCV subtype who were started on dialysis between 1996 and 2005, 26 (48.1%) samples were of subtype 1a, one sample of subtype 1b, and 27 samples of subtype 4d. Among 83 patients with determined HCV subtype who were started on dialysis between 2006 and 2015, 62 (74.7%) had subtype 1a, 2 (2.4%) had subtype 1b, one sample was genotype 2, and 18 (21.7%) samples were subtype 4d (Table 20b).

Table 20b. HCV genotype based on the year of dialysis initiation

Region	HCV Genotype				
	Total n	Genotype 1a n (%)	Genotype 1b n (%)	Genotype 2 n (%)	Genotype 4d n (%)
1986–1995	7	4 (57.1)	1 (14.3)	0	2 (28.6)
1996–2005	54	26 (48.1)	1 (1.9)	0	27 (50.0)
2006–2015	83	62 (74.7)	2 (2.4)	1 (1.2)	18 (21.7)
Total	144	92	4	1	47

The anti-HCV positive patients underwent significantly more frequently dialysis than the HCV negative patients (p=0.03). There were no HCV positive patients who underwent dialysis once a week vs. 1.1% of HCV negative patients. There were 4.5% of HCV positive patients undergoing dialysis twice a week vs. 8.8% of HCV negative

patients, while 95.5% and 89.8% of HCV positive and negative patients, respectively, underwent dialysis three times a week (Table 21).

Table 21. Frequency of dialysis in HCV positive and HCV negative patients

Frequency of dialysis	**HCV positive n (%)**	**HCV negative n (%)**	***p*-value**
Once a week	0	4 (1.1)	0.03*
Twice a week	11 (4.5)	33 (8.8)	
Three times a week	233 (95.5)	336 (89.8)	
Missing	0	1 (0.3)	
Total	244	374	618

p (Chi-square test) $p<0.05$

Most patients on dialysis, both in the HCV infected and noninfected group, were started on dialysis at dialysis center in Prishtina (73.8% and 72.9%, respectively). Then, dialysis center in Peja followed, in which 7.0% of HCV positive and 10.4% of HCV negative patients were started on dialysis; 12.7% of HCV positive and 6.4% of HCV negative patients were started on dialysis outside Kosovo. In Mitrovica and Prizren, the greatest percentage of HCV positive patients (15.6% and 14.7%, respectively) continued to undergo dialysis, while at dialysis center in Prishtina the greatest percentage of HCV negative patients (34.5%) continued with their dialysis. At dialysis centers outside Kosovo, 15.6% of HCV positive and 8.6% of HCV negative patients continued to undergo dialysis.

In this analysis, a comparison was made between HCV positive and HCV negative patients in relation to the number of dialysis centers in which the dialysis was provided. Results showed that HCV positive patients were on dialysis significantly more frequently in more than one center than HCV negative patients (79.5% vs 49.3%, $p<0.0001$) (Table 22).

Table 22. Distribution of HCV positive and HCV negative patients based on dialysis center at which dialysis was started and continued

Variable	HCV positive n (%)	HCV negative n (%)
Dialysis center where dialysis was started		
Prishtina n = 454	178 (72.9)	276 (73.8)
Mitrovica n = 7	4 (1.6)	3 (0.8)
Peja n = 56	17 (7.0)	39 (10.4)
Gjakova n = 11	3 (1.2)	8 (2.1)
Prizren n = 28	8 (3.3)	20 (5.3)
Ferizaj n = 1	1 (0.4)	0
Gjilan n = 6	2 (0.8)	4 (1.1)
Other n = 55	31 (12.7)	24 (6.4)
Dialysis center where dialysis was continued		
Prishtina	29 (11.9)	129 (34.6)
Mitrovica	38 (15.6)	19 (5.1)
Peja	15 (6.1)	44 (11.8)
Gjakova	25 (10.2)	28 (7.5)
Prizren	36 (14.7)	43 (11.5)
Ferizaj	1 (0.4)	
Gjilan	1 (0.4)	4 (1.1)
Prishtina, Mitrovica	5 (2.1)	
Prishtina, Peja	7 (2.9)	5 (1.3)
Prishtina, Prizren	29 (11.9)	18 (4.8)
Prishtina, Ferizaj	4 (1.6)	29 (7.8)
Prishtina, Gjilan	10 (4.1)	22 (5.9)
Peja, Gjakova	5 (2.1)	
Prizren, Gjilan	1 (0.4)	
Other	38 (15.6)	32 (8.6)
Dialysis at more than one dialysis center		
No	50 (20.5)	189 (50.7)
Yes	194 (79.5)	184 (49.3)
	$p<0.0001$	

p (Chi-square test)

The association of the HCV infection with gender and age of the study patients, the centers where the dialysis was provided, the dialysis provided at more than one center, as well as the association of the HCV infection with blood transfusion, medical

interventions (dental, surgical), other high-risk behaviours, and with the level of knowledge of dialysis patients about HCV were analysed by Poisson analysis.

The unadjusted Poisson analysis for all dialysis centers of Kosovo showed that factors significantly associated with the anti-HCV positivity were the **age of patients** on dialysis (PR = 0.955, CI 0.972–0.998, *p*=0.021), **longer duration of dialysis** (PR = 1.032, CI 1.02–1.044, *p*<0.0001), **dialysis at more than one center** (PR = 1.251, CI 1.086–1.42, *p*=0.002), and **blood transfusion** (PR = 1.188, CI 1.006–1.404, *p*=0.043) (Table 23a).

Table 23a. Unadjusted Poisson analysis of factors associated with anti-HCV positivity for all Kosovo dialysis centers (n = 618)

Variable	**N (%)**	**PR**	**CI 95%**	***p*-value**
Gender				0.498
female	128 (42.8)	ref (1)		
male	116 (36.4)	0.955	0.836 – 1.91	
Age (years) mean=56.3 ± 12.7		0.985	0.972 – 0.998	0.021*
Duration of dialysis (years) mean=8.8 ± 5.7		1.032	1.02 – 1.044	< 0.0001
Region				0.06
Prishtina	30 (18.0)	ref (1)		
Mitrovica	44 (69.8)	1.44	1.138 – 1.822	
Peja	26 (33.3)	1.13	0.891 – 1.433	
Prizren	77 (50.3)	1.274	1.054 – 1.541	
Ferizaj	16 (35.6)	1.149	0.862 – 1.531	
Gjilan	17 (35.4)	1.148	0.867 – 1.519	
Gjakova	34 (53.1)	1.298	1.019 – 1.654	
Dialysis at more than one center				0.002**
No	50 (20.9)	ref (1)		
Yes	194 (51.3)	1.251	1.086 – 1.442	
Blood transfusion				0.043*
No	31 (21.8)	ref (1)		
Yes	213 (44.7)	1.188	1.006 – 1.404	
Dental services				0.704
No	35 (35.3)	ref (1)		

Yes	209 (40.3)	1.036	0.862 – 1.246	
Surgical intervention				0.991
No	156 (39.7)	ref (1)		
Yes	88 (39.1)	1.001	0.871 – 1.15	
Other risk behaviour				0.67
No	143 (36.8)	ref (1)		
Yes	101 (44.1)	1.054	0.918 – 1.209	
Knowledge about HCV infection				0.309
Yes	158 (45.4)	ref (1)		
No	79 (30.9)	0.9	0.784 – 1.033	
Don`t know	7 (50.0)	1.032	0.667 – 1.596	

PR (unadjusted prevalence rate) **p*<0.05 ***p*<0.01; p (Wald test)

Specifically for Prishtina dialysis center, the unadjusted Poisson analysis showed that factors significantly associated with the anti-HCV positivity were **longer duration of dialysis** (PR = 1.235 CI, 1.128 – 1.351, *p*<0.0001), and **blood transfusion** (PR = 3.762, CI 1.452 – 9.746, *p*=0.006) (Table 23b).

Table 23b. Unadjusted Poisson analysis of factors associated with anti-HCV positivity at Prishtina dialysis center (n = 167)

Variable	**N (%)**	**PR**	**CI 95%**	***p*-value**
Gender				
female	87 (52.1)	ref (1)		
male	80 (47.9)	1.009	0.462 – 2.2	0.983
Age (years) mean=57.4 ± 14.2		0.979	0.953 – 1.005	0.111
Duration of dialysis (years) mean=5.4 ± 4.5		1.235	1.128 – 1.351	< 0.0001*
Dialysis at more than one center				
No	152 (90.48)	ref (1)		
Yes	15 (8.93)	1.107	0.293 – 4.186	0.881
Blood transfusion				
No	71 (42.52)	ref (1)		
Yes	96 (57.48))	3.762	1.452 – 9.746	0.006*
Dental services				
No	25 (14.97)	ref (1)		

Yes	142 (85.03)	1.404	0.512 – 3.852	0.704
Surgical intervention No Yes	 103 (61.68) 64 (38.32)	 ref (1) 1.001	 0.871 – 1.15	 0.51
Other risk behaviour No Yes	 89 (53.29) 78 (46.71)	 ref (1) 1.049	 0.71 – 1.55	 0.809
Knowledge about HCV infection Yes No Don`t know	 54 (32.33) 113 (67.67)	 ref (1) 1.79	 0.719 – 4.461	 0.211

PR (unadjusted prevalence rate) **p*<0.01; p (Wald test)

In the multivariate analysis for all dialysis centers of Kosovo, **duration of dialysis** and **dialysis at more than one center** were shown as independent significant factors, associated with anti-HCV positivity (APR = 1.032, CI 1.017–1.042, *p*<0.0001; APR = 1.18, CI 1.02–1.365, *p*=0.026) (Table 24a).

Table 24a. Adjusted Poisson analysis of factors associated with anti-HCV positivity for all dialysis centers of Kosovo (n = 618)

Variable	**APR**	**CI 95%**	***p*-value**
Gender female male	 ref (1) 0.978	 0.818 – 1.17	 0.48
Age (years)	0.997	0.992 – 1.003	0.327
Duration of dialysis (years)	 1.032	 1.017 – 1.042	 < 0.0001*
Region Prishtina Mitrovica Peja Prizren Ferizaj Gjilan Gjakova	 ref (1) 1.436 1.134 1.271 1.163 1.151 1.302	 1.135 – 1.817 0.894 – 1.439 1.051 – 1.537 0.871 – 1.552 0.87 – 1.524 1.022 – 1.659	 0.065
Dialysis at more than one center No Yes	 ref (1) 1.18	 1.02 – 1.365	 0.026*

Blood transfusion No Yes	 ref (1) 0.92	 0.817 – 1.041	 0.341
Dental services No Yes	 ref (1) 1.039	 0.864 – 1.249	 0.685
Surgical intervention No Yes	 ref (1) 1.002	 0.872 – 1.151	 0.978
Other risk behaviour No Yes	 ref (1) 0.949	 0.827 – 1.088	 0.453
Knowledge about HCV infection Yes No Don`t know	 ref (1) 1.055 0.908	 0.679 – 1.639 0.853 – 1.156	 0.362

APR (Adjusted prevalence rate) *p<0.05; p (Wald test)

Analyzing data for Prishtina dialysis center specifically, the multivariate analysis showed the following as independent significant factors associated with the anti-HCV positivity: **age** (APR = 0.96, CI 0.926–0.994, p=0.023), **longer duration of dialysis** (APR = 1.258, CI 1.132–1.398, p<0.0001), **blood transfusion** (APR = 3.077 CI, 1.129–8.384, p=0.028), and **knowledge about HCV (**APR = 1.395 CI, 1.071–1.763, p=0.038) (Table 24b).

Table 24b. Adjusted Poisson analysis of factors associated with anti-HCV positivity for Prishtina dialysis center (n = 167)

Variable	**APR**	**CI 95%**	***p*-value**
Gender female male	 ref (1) 0.42	 0.089 – 1.98	 0.27
Age (years)	0.96	0.926 – 0.994	0.023*
Duration of dialysis (years)	1.258	1.132 – 1.398	< 0.0001
Dialysis at more than one center No Yes	 ref (1) 1.225	 0.255 – 5.886	 0.800

Blood transfusion No Yes	 ref (1) 3.077	 1.129 – 8.384	 0.028*
Dental services No Yes	 ref (1) 0.915	 0.245 – 3.413	 0.894
Surgical intervention No Yes	 ref (1) 1.46	 0.532 – 4.004	 0.462
Other risk behaviour No Yes	 ref (1) 0.953	 0.195 – 4.657	 0.953
Knowledge about HCV infection Yes No Don`t know	 ref (1) 1.395	 1.071 – 1.763	 0.038*

APR (Adjusted prevalence rate) *p<0.05; p (Wald test)

5.1.2.4 Treatment of HCV infection among dialysis patients

Only 18 out of 244 (7.4%) dialysis patients infected with HCV received therapy with pegylated interferon and ribavirin at the Clinic for Infectious Diseases, University Clinical Center of Kosovo. Most anti-HCV positive patients on dialysis (77.0%) did not know about any reasons for not being enrolled in the HCV treatment program.

The treatment was completed for 12 (66.6%) patients, one patient discontinued the treatment due to side effects (abdominal pain), while the reason for discontinuation of the therapy by other patients was not stated. Six (33.3%) of the treated patients had no side effects related to the treatment. The remaining anti-HCV positive dialysis patients who received treatment mentioned several complaints as presented in Table 25.

Table 25. Information related to the treatment of anti-HCV positive patients on dialysis

Variable		N (%)
Treated with interferon/ribavirin	Yes	18 (2.9)
	No	600 (97.1)
Treatment completed	Yes	12 (66.7)
	No	5 (27.8)
	Missing	1 (5.5)
Reason/s for not being enrolled in the treatment with interferon/ribavirin	I didn't want to receive treatment	0
	No place available in the treatment program	0
	No financial possibilities to personally finance the treatment	1 (0.4)
	Didn't have information that treatment for hepatitis C infection exists	2 (0.8)
	Don`t know	188 (77.0)
	Missing data	53 (21.7)
Complaints related to interferon/ribavirin treatment	No complaints	6 (33.3)
	Vomiting	1 (5.5)
	Fever	3 (16.7)
	Anemia	1 (5.5)
	Diabetes	1 (5.5)
	Fatigue	1 (5.5)
	Knee pain	1 (5.5)
	Frequent interventions	1 (5.5)
	Missing data	3 (16.7)

5.1.3 Management information from hemodialysis units

A total of 708 patients with chronic kidney insufficiency were on dialysis in the Republic of Kosovo in December 2015, with the greatest percentage at dialysis centers in Prishtina (26.1%) and Prizren (24.9%), and the lowest percentage at the dialysis center in Ferizaj (7.1%).

At dialysis centers of Kosovo, 18 doctors were employed, 5 of them at the dialysis center in Prishtina, and only one doctor at the centers of Mitrovica, Ferizaj, and Gjakova. On average, one doctor provided care for 39 patients, and per region, the dialysis center in Gjilan was shown the best staffed with doctors (16 patients per doctor), and the worst situation concerning the number of doctors was in Mitrovica and Gjakova, where the number of the patients per doctor was 70 and 69, respectively.

At dialysis centers in Kosovo, 129 nurses were employed, mostly at the dialysis center in Prishtina (32), with the lowest number at the center of Ferizaj (10). One nurse, on average, provided care for 5 to 6 patients on dialysis in Kosovo. One nurse provided care for the lowest number of patients (4) in Gjakova, whereas at dialysis centers in Prizren and Peja, one nurse provided care for 6 to 7 patients.

A total of 189 dialysis machines were operational, the greatest number at the center of Prishtina (56), the lowest number at the center of Mitrovica (18). On average, 4 patients were using one dialysis machine, or per region, the lowest number of patients (2) were using one machine at Ferizaj center, whereas at the center in Prizren 5 patients were on dialysis on one machine.

The surface area of premises reserved for dialysis ranged between 250–300 square meters in Gjakova, up to 1,000 in Prishtina. There was regular maintenance of dialysis machines, trained staff provided care for HCV positive patients only, and at the centers of Peja and Prizren the premises for HCV positive and negative patients were not separated as they were in other centers of Kosovo.

At the dialysis center in Prizren, there was insufficient supply of disposable single-use gloves, at the centers in Mitrovica and Gjilan the staff complained about the poor quality of gloves. There were no sterile gauzes for daily work at dialysis centers in Peja, Prizren and Gjakova, while the centers in Prizren, Gjilan and Gjakova did not have sufficient disinfection material for daily work.

Only dialysis centers in Ferizaj and Gjilan received regularly the anti-HCV test results before they placed the patient on dialysis machine. The most common sources of the anti-HCV test results were the National Institute of Public Health of Kosovo and different private biochemical laboratories.

Table 26. Human resources and infrastructure at dialysis centers of Kosovo in December 2015

Dialysis center	No. of MDs	No. of nurses	No. of patients	No. of dialysis machines	Surface area (m^2)	Regular maintenance	Separate dialysis machines for HCV positive patients	Separate premises for HCV positive patients	Staff dedicated for HCV positive patients only
Prishtina	5/37	32/5.8	185 (26.1%)	56/3.3	1000	yes	yes	yes	yes
Mitrovica	1/70	12/5.8	70 (9. 9%)	18/3.9	350 – 400	yes	yes	yes	yes
Peja	2/47	15/6.3	94 (13.3%)	22/4.3	600	yes	yes	no	yes
Prizren	4/44	27/6.5	176 (24.9%)	37/4.8	800	yes	yes	no	yes
Ferizaj	1/50	10/5	50 (7.1%)	30/1.7	300	yes	yes	yes	yes
Gjilan	4/16	15/4.3	64 (9.0%)	20/3.2	280	yes	yes	yes	yes
Gjakova	1/69	18/3.8	69 (9.7%)	22/3.1	250 – 300	yes	yes	yes	yes
Total	18/39 [a]	129/5.5 [b]	708	205/3.5 [c]					

[a] Number of patients per doctor [b] Number of patients per nurse [c] Number of patients per machine

Table 27. Availability of consumables and anti-HCV testing at dialysis centers of Kosovo in December 2015

Dialysis center	Sufficient supply of gloves	Sufficient supply of sterile gauzes	Sufficient supply of disinfection material	Anti-HCV test result before patient is admited to dialysis	Laboratory that performs testing for anti-HCV	Manager opinion – reasons for high prevalence of HCV infection in your dialysis unit
Prishtina	yes	yes	yes	no	National Institute of Public Health of Kosovo, private laboratories	-
Mitrovica	yes in last year, poor quality	yes	yes	no	Private laboratories	Human factor, surgical interventions, many transfusions
Peja	yes	no	yes	no	Private laboratories	Human factor, surgical interventions, many transfusions
Prizren	no	no	no	no	National Institute of Public Health of Kosovo, private laboratories	Lack of appropriate premises; after the war a situation when even gloves were missing
Ferizaj	yes	yes	yes	yes	National Institute of Public Health of Kosovo, private laboratories	Lack of on-time detection of HCV infection
Gjilan	yes	yes	no	yes	National Institute of Public Health of Kosovo, private laboratories	Lack of HCV testing capacities; human mistakes; machine not properly disinfected
Gjakova	yes in last year, poor quality	no	no	no	National Institute of Public Health of Kosovo, private laboratories	Inherited problem; lack of appropriate premises; in the past all patients were in the same premises; no regular testing

5.2 HCV INFECTION AMONG INJECTING DRUG USERS IN PRISHTINA, KOSOVO

5.2.1 Laboratory data

Ninety nine (48.3%) out of a total of 205 IDUs participating in the study were anti-HCV positive. There were no HIV positive cases among the study population of IDUs. In four subjects, the presence of antibodies against *Treponema pallidum* (2.0%) was detected, and 18 (8.8%) were HBsAg positive.

The presence of HCV RNA was detected in 70 (70.7%) anti-HCV positive IDUs. HCV genotyping was possible among all HCV RNA positive IDUs (70), and the following subtypes were determined: subtype 1a (45/70; 64.3%), subtype 3a (24/70; 34.3%), and subtype 2k (1/70; 1.4%).

IL28 genotype was determined among 98 out of 99 anti-HCV positive IDUs. Allele CC was the most prevalent, determined in 47 (47.9%), followed by CT and TT genotypes with 37 and 14 samples (37.8%, 14.3%), respectively (Table 28).

Table 28. Results of laboratory tests on IDUs in Prishtina Region of Kosovo

Variable	N (%)
Anti-HIV	0
Anti-*Treponema pallidum*	4 (2.0)
HBsAg	18 (8. 8)
Anti-HCV	99 (48.3)
HCV core region sequenced	70 (70.7)
HCV subtype 1a 3a 2k	70 45 (64.3) 24 (34.3) 1 (1.4)
IL28B CT CC TT	98 37 (37.8) 47 (47.9) 14 (14.3)

Figure 8. Presence of antibodies against HCV among IDUs in Prishtina Region, Kosovo

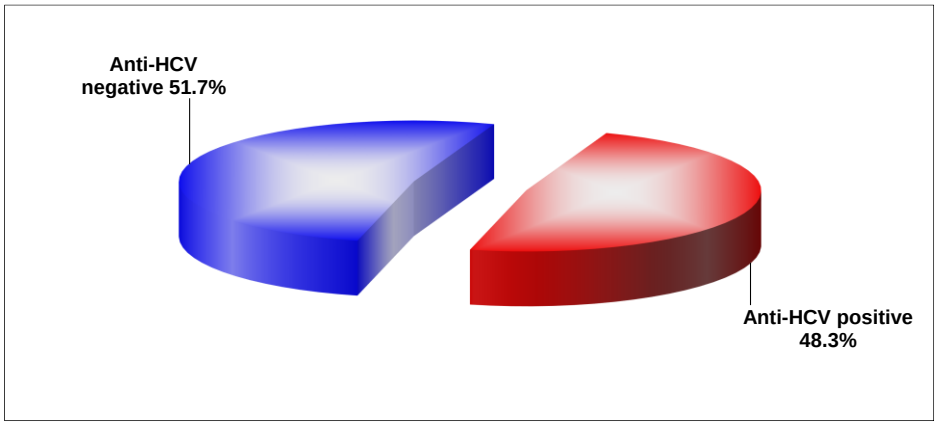


Figure 9. Distribution of HCV genotypes among IDUs in Prishtina Region, Kosovo

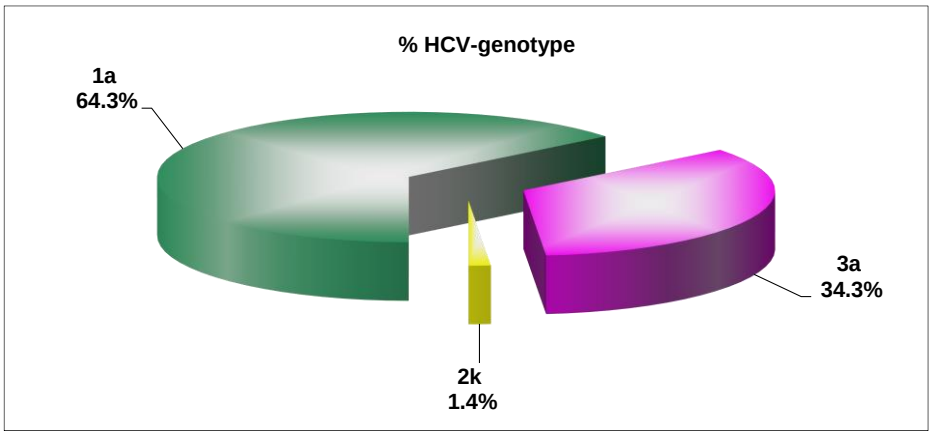
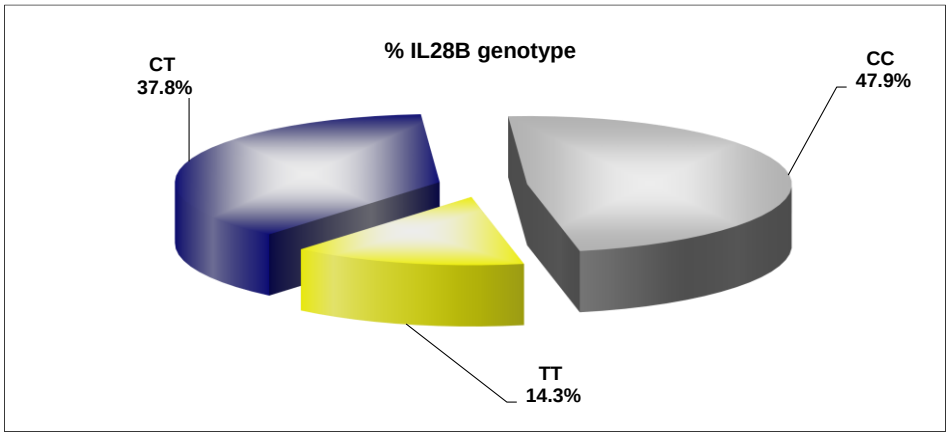


Figure 10. Distribution of IL28 polymorphism among anti-HCV positive IDUs in Prishtina Region, Kosovo



IL28 polymorphism CC was present more frequently than CT and TT in anti-HCV positive IDUs, either being HCV RNA positive (43.5%, 39.1%, and 17.4%, respectively) or negative (58.6%, 34.5%, and 6.9%, respectively). However, those differences were not statistically significant (p=0.26) (Table 29).

Table 29. IL28B polymorphism among anti-HCV positive IDUs in Prishtina Region of Kosovo

IL28B polymorphism	**HCV RNA**		***p*-value**
	negative	**positive**	
	n (%)	**n (%)**	
CT	10 (34.5)	27 (39.1)	0.26
CC	17 (58.6)	30 (43.5)	
TT	2 (6.9)	12 (17.4)	

p (Chi-square test)

Significant differences in the distribution of CT, CC and TT of the IL28B among anti-HCV positive IDUs infected with HCV genotypes 1a and 3a (p=0.74) were not found (Table 30).

Table 30. IL28B polymorphism among anti-HCV positive IDUs in Prishtina Region of Kosovo with HCV genotype 1a and 3a

IL28B polymorphism among anti-HCV positive	**HCV genotype**		***p*-value**
	1a	**3a**	
	n (%)	**n (%)**	
CT	16 (59.3)	11 (40.7)	0.74
CC	20 (69.0)	9 (31.0)	
TT	8 (66.7)	4 (33.3)	

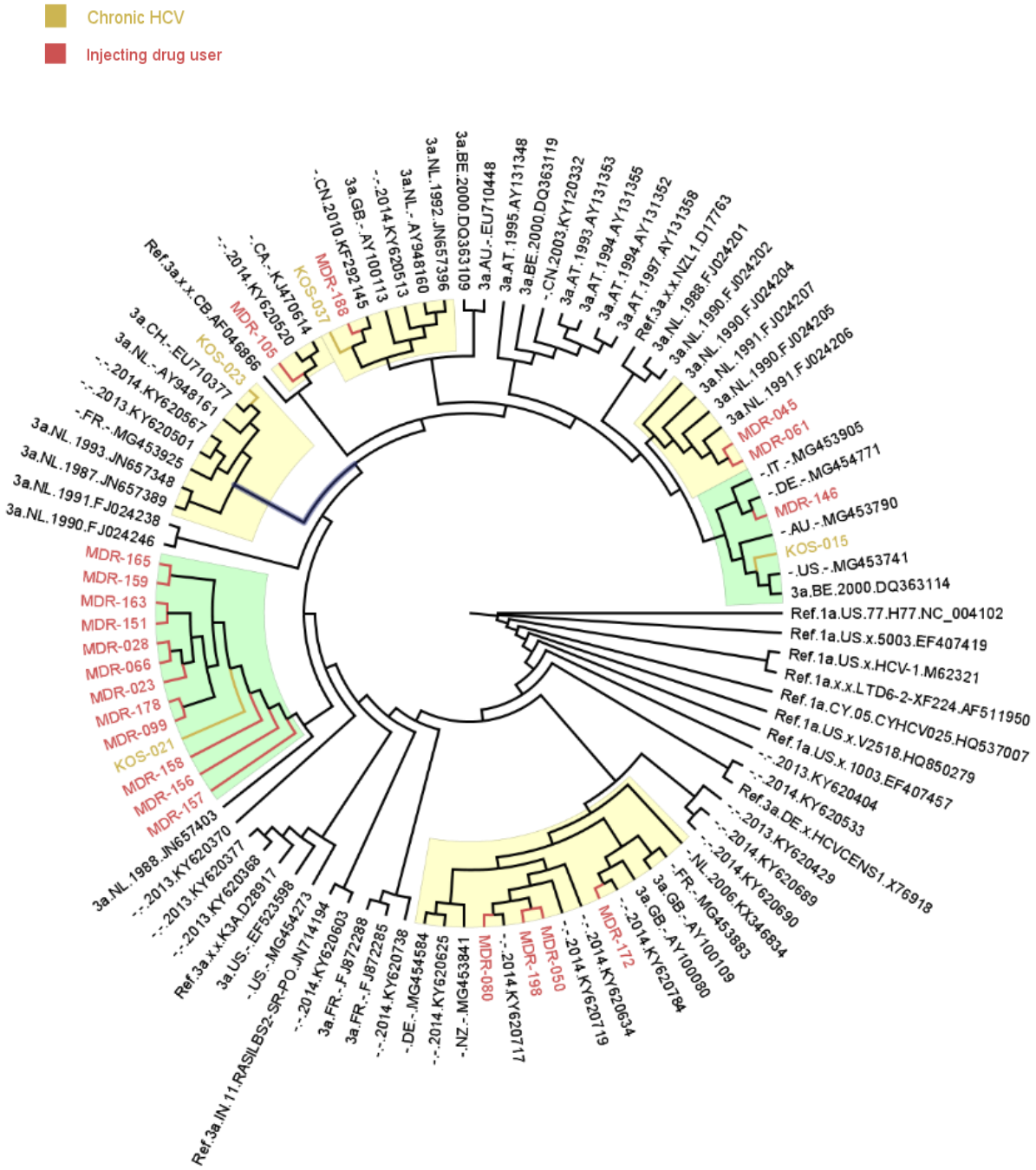
p (Chi-square test)

Cladogram phylogenetic trees for HCV subtypes 1a and 3a were constructed with sequences of the NS5B region isolated from IDUs (Figures 1 and 11, respectively).

Among the cladogram phylogenetic tree consisted of 39 sequences of HCV subtypes 1a isolated from IDUs in Prishtina, two clusters were revealed with aLRT>0.9, one with 5 sequences and another with 3 sequences. Those two clusters were part of a larger cluster with aLRT>0.8 consisted in total of 26 sample sequences of IDUs. These findings show that among IDU samples belonging to HCV subtype 1a isolated in this study, 66.7% (26/39) were observed with a phylogenetic link (Fig. 1).

With regard to cladogram phylogenetic tree constructed with sequences of the HCV subtype 3a (n=21), there was a presence of one large cluster with aLRT>0.9 with 12 sequences, and another cluster with aLRT>0.8 with 3 samples from IDUs and one transmission pair. In total, 71.4% (15/21) of samples belonging to the HCV subtype 3a isolated from IDUs in Prishtina Region of Kosovo showed phylogenetic clustering (Fig. 11).

Figure 11. The maximum likelihood phylogenetic tree of HCV subtype 3a. Sequences from IDUs and chronic HCV pateints are colored differently. Clusters with aLRT>0.9 are highlighted in green and clusters with aLRT>0.8 are highlighted in yellow.



5.2.2 Demographic characteristics of IDUs

Among 205 IDUs from the Prishtina Region of Kosovo, most were men (183/204 – 89.7%), with a mean age of 36.2 years (range 24–59 years), and in the age group 30–39 (50.7%). Male IDUs were found older than the female but the difference was not significant (36.4 ± 7.2 vs 34.4 ± 6.1). Almost half of the recruited IDUs had completed secondary education (101/205; 49.3%). During study implementation, most subjects were single (49.8%; 102/205), while 30.7% (63/205) were married. Most IDUs, in the past three months, lived in their own house/apartment (42.9%; 88/205) or lived together with their parents (32.7%; 67/205). Four subjects responded they were in prison at that time. About 9% of the IDUs had no income in the past month, most of them were financially supported by their family (39.5%), and approximately one third was permanently employed (29.8%) (Table 31).

Table 31. Demographic characteristics of IDUs in Prishtina Region of Kosovo

Variable		N (%)
Gender	Male	183 (89.3)
	Female	21 (10.2)
	Missing	1 (0.5)
Age group (years)	19 – 29	37 (18.1)
	30 – 39	104 (50.7)
	≥ 40	64 (31.2)
Education	No formal education	2 (1.0)
	Some primary	5 (2.4)
	Primary	43 (21.0)
	Some secondary	19 (9.3)
	Secondary	101 (49.3)
	Some college	29 (14.1)
	College / university	6 (2.9)
Marital status	Married	63 (30.7)
	Divorced	28 (13.6)
	Widowed	3 (1.5)
	In a steady relationship	9 (4.4)
	Single	102 (49.8)
Place of residence in the past three months	In your house or apartment	88 (42.9)
	In a rented house or apartment	38 (18.5)
	In your parents' house or apartment	67 (32.7)

	In someone else's house or apartment	4 (1.9)
	No permanent location	1 (0.5)
	Prison	4 (1.9)
	Other	3 (1.5)
Main source of income in the past month	No income in the past month	19 (9.3)
	Permanent employment	61 (29.8)
	Temporary job/part time job	23 (11.2)
	Family support	81 (39.5)
	Selling drugs	/
	Stealing and/or begging	5 (2.4)
	Other	16 (7.8)

Results from the survey showed that the anti-HCV positive IDUs were most frequently men (92.9%), and mainly aged from 30 to 39 years (48.5%). As for marital status, single and married were the most frequent categories of anti-HCV positive IDUs (41.4% and 37.4%, respectively). Most of them (34.3% and 56.6%, respectively) had completed primary and secondary education. In the past three months, the anti-HCV positive IDUs lived most frequently in their own house/apartment (43.4%), or in the house of their parents (28.3%), and three of them were in prison.

There were 7 (7.1%) IDUs infected with hepatitis C virus without any income in the past month, 25 (25.3%) were permanently employed, most of them were financially supported by their families – 40 (40.4%), three subjects said they were stealing or begging in order to provide for their living. None of them reported to be engaged in selling drugs.

In the group of male IDUs, the prevalence of antibodies against HCV was non-significantly higher compared to the female IDUs (49.7% vs 33.3%, p=0.15).
HCV prevalence among IDUs correlated significantly with their age (t=5.07 p=0.000001). The age of the IDUs infected with hepatitis C virus was, on average, 38.6 ± 6.9 years, and of the noninfected 33.9 ± 6.5 years. When age was stratified, the greatest percentage of the infected subjects belonged to the age group older than 40 years (65.6%), followed by the age group 30–39 years (46.2%), and the age group 19–29 years, in which 24.3% had antibodies against HCV (p=0.00027).

When comparing married to nonmarried IDUs, a significantly different prevalence of the antibodies against HCV (58.7% and 43.7%, respectively; p=0.046) was observed. In the group with different marital status, the highest HCV prevalence was found of IDUs being divorced, followed by those being married, and without a partner (64.3%, 58.7%, and 40.2%, respectively). The difference in HCV prevalence among the analyzed categories of marital status was also statistically significant (p=0.02).

The participants' educational level had a significant impact on HCV prevalence (p=0.0005). Anti-HCV positive IDUs had more frequently primary or lower education compared to anti-HCV negative IDUs.

The prevalence of antibodies against HCV depended neither on where the IDUs lived (p=0.58) nor on their employment status (p=0.17). Infection with HCV was present in three out of four IDUs that were in prison in the past three months. Unemployed IDUs were non-significantly more frequently anti-HCV positive (51.4% vs 41.0%) than the employed IDUs (Table 32).

Table 32. Demographic data of IDUs in Prishtina Region by anti-HCV status

Variable		Total	Anti-HCV positive n (%)	Anti-HCV negative n (%)	*p*-value
Gender	Male	183	91 (49.7)	92 (50.3)	[a]0.15
	Female	21	7 (33.3)	14 (66.7)	
Age group	19–29	37	9 (24.3)	28 (75.7)	[a]0.00027*
	30–39	104	48 (46.2)	56 (53.8)	
	≥ 40	64	42 (65.6)	22 (34.4)	
Marital status	Married	63	37 (58.7)	26 (41.3)	[b]0.02*
	Divorced	28	18 (64.3)	10 (35.7)	
	Widowed	3	1 (33.3)	2 (66.7)	
	In a steady relationship	9	2 (22.2)	7 (77.8)	
	Single	102	41 (40.2)	61 (59.8)	
Married/not married	Married	63	37 (58.7)	26 (41.3)	[a]0.046*
	Not married	142	62 (43.7)	80 (56.3)	
Education	≤ Primary	50	34 (68.0)	16 (32.0)	

	Secondary	120	56 (46.7)	64 (53.3)	[a]0.0005**
	Higher	35	9 (25.7)	26 (74.3)	
Place of living	In own house	88	43 (48.9)	45 (51.1)	
	In a rented house or apartment	38	17 (44.7)	21 (55.3)	
	In your parents' house or apartment	67	28 (41.8%)	39 (58.2)	
	In someone else's house or apartment	4	4(100%)	0	
	No permanent location	1	1 (100%)	0	
	In someone else's house	3	3 (100%)	0)	[b]0.58
	Prison	4	3 (75.0)	1 (25.0)	
Employment	Employed	61	25 (41.0)	36 (59.0)	
	Unemployed	144	74 (51.4)	70 (48.6)	[a]0.17

[a](Chi-square test) [b](Fisher's exact test) *p<0.05 **p<0.01

Figure 12. Prevalence of anti-HCV among IDUs by age groups

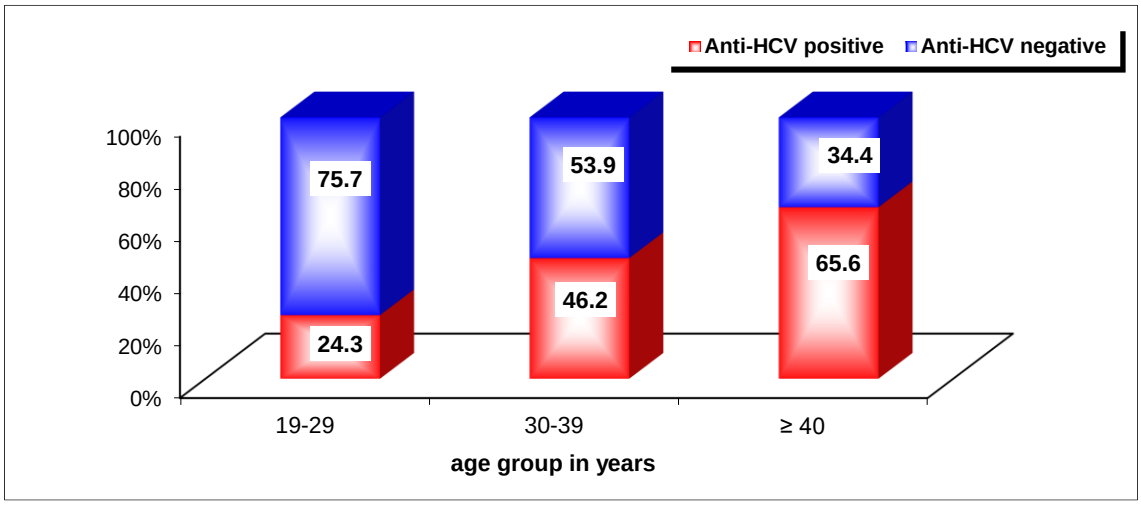


Figure 13. Prevalence of anti-HCV among IDUs by marital status

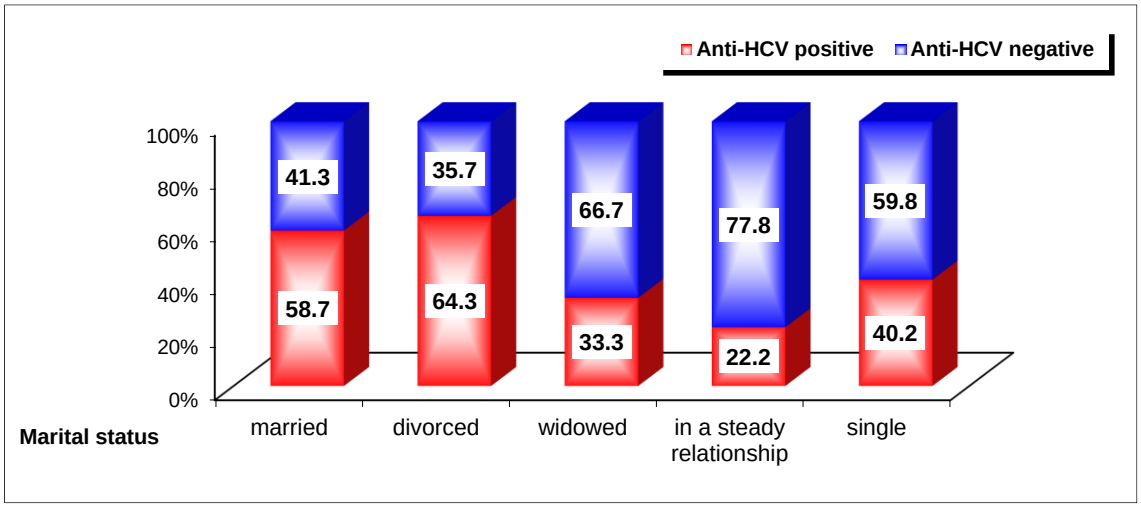
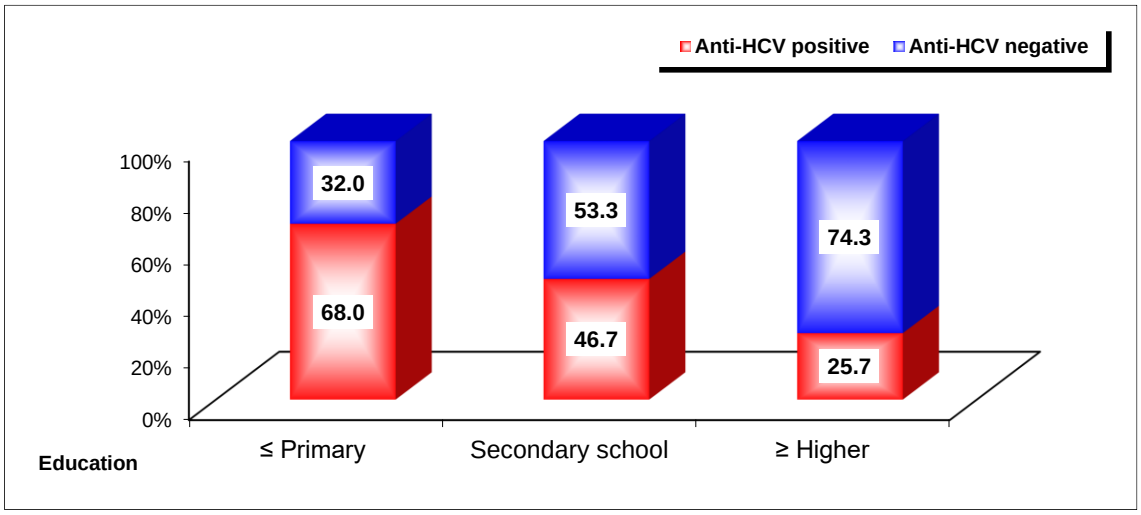


Figure 14. Prevalence of anti-HCV among IDUs by educational level



5.2.3 Drug use practices

Significant difference in duration of injection drug use was observed between anti-HCV positive and anti-HCV negative IDUs (p=0.0016). Longer duration of drug injection was observed in the group of anti-HCV positive IDUs compared to anti-HCV negative IDUs (15.2 ± 6.3 vs 12.3 ± 6.7 years) (Table 33).

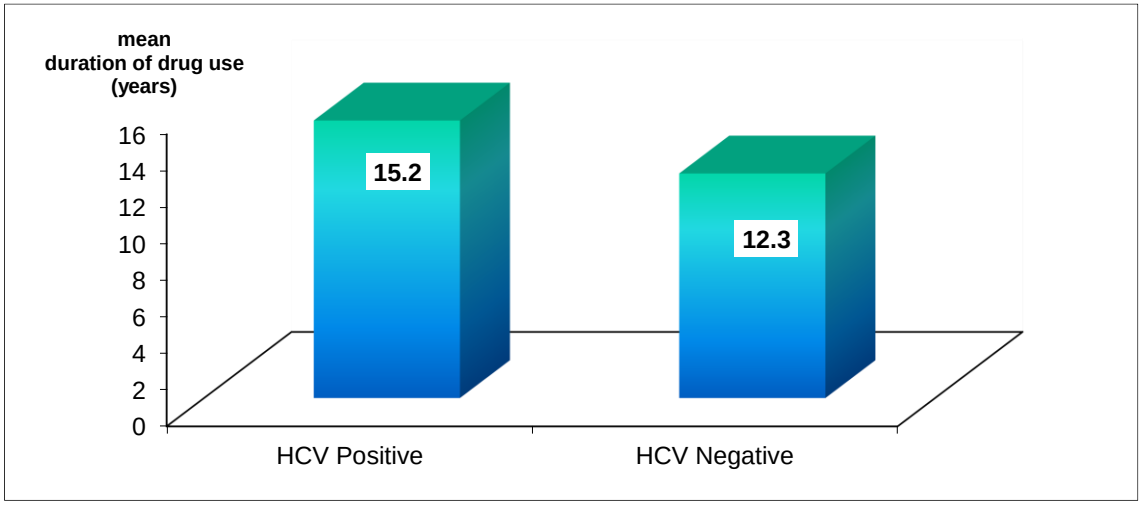
However, in the group of IDUs who were anti-HCV positive, there was no significant difference in the duration of drug injection between 1a and 3a HCV genotypes (p=0.22), and among CT, CC and TT genotypes of IL28B (p=0.32).

Table 33. Duration of injection drug use in relation to anti-HCV presence, HCV genotype and IL28B polymorphism of IDUs in Prishtina Region of Kosovo

Variable	Result	Duration of drug use in years		*p*-value
		mean ± SD	min – max	
HCV	Positive	15.2 ± 6.3	5 – 35	[c]0.0016**
	Negative	12.3 ± 6.7	5 – 37	
HCV GN	1a	16 ± 6.7	5 – 35	[c]0.22
	3a	13.9 ± 6.6	6 – 37	
	2k	11		
IL28B	CT	16.5± 7.2	6 – 37	[d]0.32
	CC	14.3 ± 6.2	6 – 31	
	TT	15.9 ± 6.9	8 – 32	

[c] (Student's t test) [d] (Analysis of Variance) **p<0.01

Figure 15. Mean duration of injection drug use among anti-HCV positive and negative IDUs



HCV prevalence among IDUs correlated significantly with the duration of the drug use (p=0.031). When duration of injection drug use was stratified, the highest prevalence of the antibodies against HCV was found in the group of IDUs who injected drugs for 25 to 29 years (75.0%), followed by lower anti-HCV prevalence in the groups of IDUs having shorter history of drug injection (Table 34).

The frequency of drug injection had a significant impact on the HCV prevalence among IDUs (p<0.0001). The IDUs who injected drugs more frequently had a higher prevalence of antibodies against HCV. The HCV infection was detected in all 10 IDUs who reported drug injection four or more times a day. The lowest prevalence of infection was found among IDUs who injected drugs only once, or 2–3 times per month (31.3% and 27.8%, respectively).

As for the impact of the place of drug injection on HCV prevalence, the results showed a significant impact of the so called 'shooting galleries' or similar places where IDUs gather (p=0.0006). Two thirds (67.2%) of anti-HCV positive IDUs had injected drugs in shooting galleries (or similar places where IDUs gather) compared to 32.8% of anti-HCV negative IDUs.

As for the type of drugs injected, anti-HCV positive IDUs were using statistically

significantly more heroin and cocaine combined (p=0.011), amphetamine (p=0.002), morphine (p=0.007), and methadone (p<0.001), compared to anti-HCV negative IDUs.

There was no significant difference between anti-HCV positive and anti-HCV negative IDUs with regard to injection with already used needles and syringes (p=0.055), use of sterile needles and syringes (p=0.056), frequency of using sterile needles and syringes, and applied method for cleaning needles (p=0.23).

The anti-HCV positive IDUs significantly more commonly obtained sterile syringes and needles from the non-governmental organizations than the HCV negative IDUs (58.8% vs 41.2% p=0.00002) (Table 34).

Table 34. Drug use practices of IDUs in Prishtina Region of Kosovo

Variable		Total	Anti-HCV positive n(%)	Anti-HCV negative n (%)	*p*-value
Duration of drug use (years)	5–9	69	23 (33.3)	46 (66.7)	[b]0.031*
	10–14	52	25 (48.1)	27 (51.9)	
	15–19	45	27 (60.0)	18 (40.0)	
	20–24	28	17 (60.7)	11 (39.3)	
	25–29	4	3 (75.0)	1 (25.0)	
	30>	7	4 (57.1)	3 (42.9)	
Duration of drug use (years)	= 5 years	5	1 (20.0)	4 (80.0)	[a]0.2
	> 5 years	200	98 (49.0)	102 (51.0)	
Frequency of injection drug use	Once a month	67	21 (31.3)	46 (68.7)	[b]< 0.001
	2–3 times a month	36	10 (27.8)	26 (72.2)	
	1–3 times a week	43	24 (55.8)	19 (44.2)	
	4–6 times a week	6	5 (83.3)	1 (16.7)	
	1–3 times a day	43	29 (67.4)	14 (32.6)	
	4 or more times a day	10	10 (100)	0	
	At home	161	77 (47.8)	84 (52.2)	[a]0.8
	In a private house or	4	2 (50.0)	2 (50.0)	[b]1.0

Place of drug use	apartment				
	In a public place, e.g. a bar, shop, toilet	5	3 (60.0)	2 (40.0)	[b]0.67
	In a dealer's house or apartment	4	1 (25.0)	3 (75.0)	[b]0.62
	On the street or in the park	17	7 (41.2)	10 (58.8)	[a]0.54
	In a shooting gallery or in another place where IDUs gather	58	39 (67.2)	19 (32.8)	[a]0.0006**
	In prison	1	1	0	
	Other	18	9 (50.0)	9 (50.0)	[a]0.88
Type of drugs injected	Heroin	201	98 (48.8)	103 (51.2)	[a]0.35
	Cocaine	55	30 (54.5)	25 (45.5)	[a]0.28
	Heroin and cocaine combined	52	33 (63.5)	19 (36.5)	[a]0.011*
	Amphetamine	12	11 (91.7)	1 (8.3)	[a]0.002**
	Morphine	21	16 (76.2)	5 (23.8)	[a]0.007**
	Opium	11	8 (72.7)	3 (27.3)	[a]0.095
	Methadone	143	89 (62.2)	54 (37.8)	[a]< 0.0001
Use of used needles/syringes	Yes	24	16 (66.7)	8 (33.3)	[a]0.055
	No	181	83 (45.9)	98 (54.1)	
Use of sterile needles/syringes	Yes	202	98 (48.5)	104 (51.5)	[b]0.052
	No	3	1 (33.3)	2 (66.7)	
Somebody else used your needle/syringe afterwards	Yes	190	94 (49.5)	96 (50.5)	[a]0.13
	No	14	4 (28.6)	10 (71.4)	
Frequency of using sterile needles/syringes	Always (100%)	163	79 (48.5)	84 (51.5)	[b]1.0
	Most of the time (75%)	39	19 (48.7)	20 (51.3)	
	About every second time (50%)	2	1 (50.0)	1 (50.0)	
	Sometimes (25%)		/	/	
	Rarely (about		/	/	

	10%)				
	Never or almost never		/	/	
Method of cleaning of used needle/syringes	With cold water	20	12 (60.0)	8 (40.0)	[b]0.23
	With warm water	3	1 (33.3)	2 (66.7)	
	With hot water	14	11 (78.6)	3 (21.4)	
	With boiling water	30	14 (46.7)	16 (53.3)	
	With soap or detergent		/	/	
	With bleach		/	/	
	With alcohol	15	6 (40.0)	9 (60.0)	
	Other	4	3 (75.0)	1 (25.0)	
Place of obtaining sterile needles/syringes	Pharmacy or hospital	204	99 (48.5)	105 (51.5)	[b]1.0
	I buy them in the street	1	0	1	
	From a non-governmental organization	136	80 (58.8)	56 (41.2)	[a]0.00002**

[a](Chi-square test) [b](Fisher's exact test) *p<0.05 **p<0.01

Figure 16. Prevalence of anti-HCV among IDUs by duration of drug use

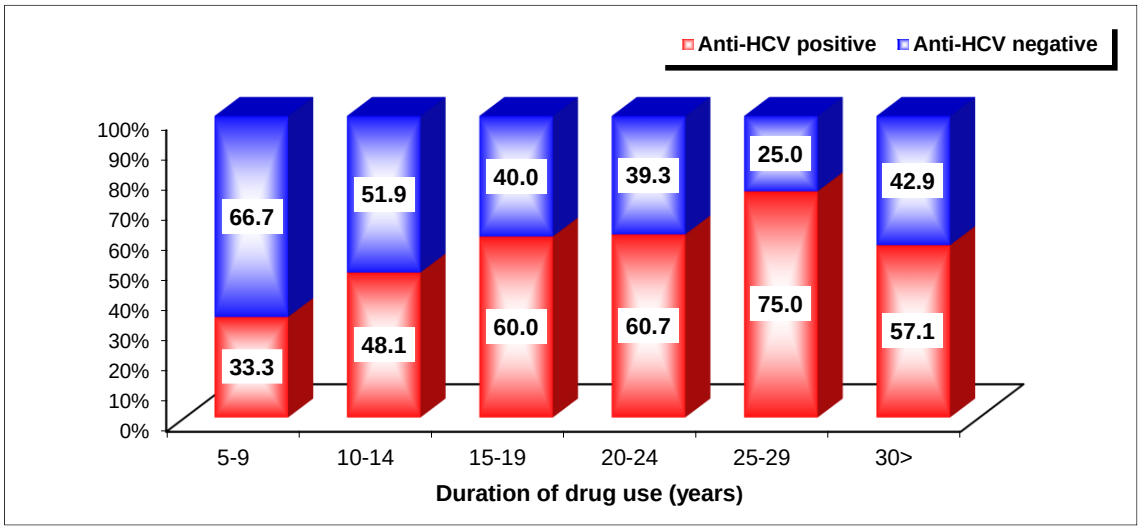


Figure 17. Prevalence of anti-HCV among IDUs by frequency of drug injection

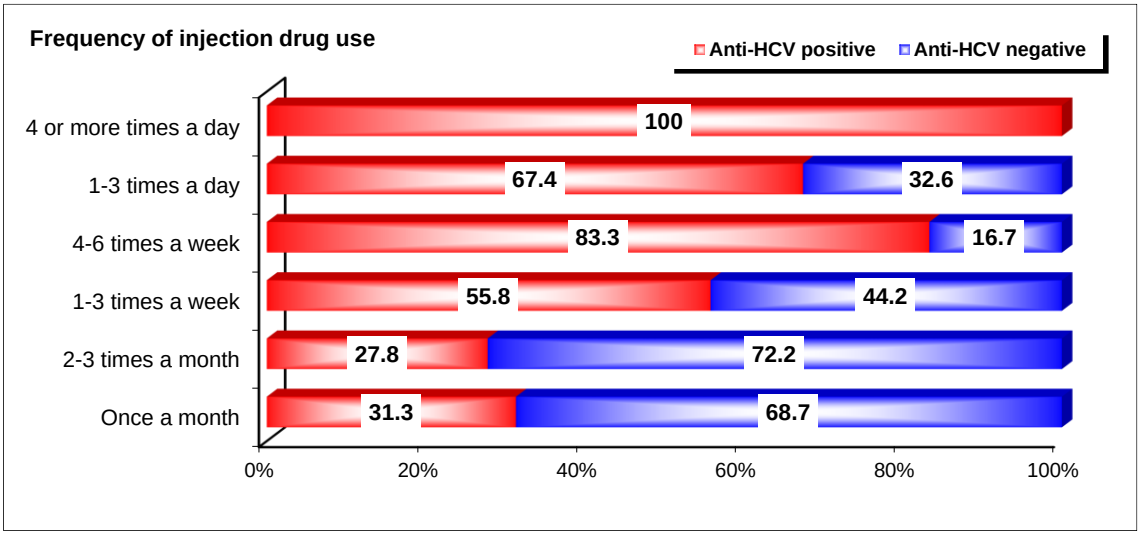


Table 35 shows the distribution of HCV genotypes by duration of drug use, frequency of injection, place of injection, type of drug, usage of used or sterile needles and syringes. All these characteristics related to the usage of drugs differed non-significantly between 1a and 3a HCV subtypes of IDUs.

Table 35. HCV Genotypes and drug use practices among IDUs in Prishtina Region of Kosovo

Variable		HCV Genotype			*p*-value
		1a n (%)	3a n (%)	2k n (%)	
Duration of drug use (years)	5–9	7 (50.0)	7 (50.0)	0	[b]0.24
	10–14	12 (66.7)	5 (27.8)	1 (5.5)	
	15–19	13 (56.5)	10 (43.5)	0	
	20–24	10 (90.9)	1 (9.1)	0	
	25–29	1	0	0	
	30>	2	1	0	
	= 5 years	1	0	0	[b]1.0
	> 5 years	44 (63.8)	24 (34.8)	1 (1.45)	
Frequency of injection drug use	Once	8 (61.5)	5 (38.5)	0	[b]0.16
	2–3 times	4 (80.0)	1 (20.0)	0	
	1–3 times a week	16 (80.0)	4 (20.0)	0	
	4–6 times a week	1	2	0	
	1–3 times a day	14 (66.7)	7 (33.3)	0	
	4 or more times a	2 (25.0)	5 (62.5)	1 (12.5)	

	day				
Place of drug use	At home	38 (65.5)	19 (32.8)	1 (1.7)	[b]0.74
	In a private house or apartment	2	0	0	
	In a public place, e.g. a bar, shop, toilet	2	0	0	
	In a dealer's house or apartment	0	0	0	
	On the street or in the park	3	0	0	
	In a shooting gallery or in another place where IDUs gather	16 (64.0)	8 (32.0)	1(4.0)	[a]0.85
	In prison	0	1	0	
	Other	3	2	0	[b]1.0
Type of drugs injected	Heroin	45 (65.2)	23 (33.3)	1 (1.5)	[b]0.35
	Cocaine	15 (68.2)	7 (31.8)	0	[a]0.72
	Heroin and cocaine combined	18 (72.0)	7 (28.0)	0	[a]0.37
	Amphetamine	6 (100)	0	0	
	Morphine	11 (73.3)	4 (26.7)	0	[a]0.46
	Opium	3	2	0	[b]1.0
	Methadone	38 (60.3)	24 (38.1)	1 (1.6)	[b]0.087
Use of used needles/syringes	Yes	5 (45.5)	5 (45.4)	1 (9.1)	[b]0.3
	No	40 (67.8)	19 (32.2)	0	
Use of sterile needles/syringes	Yes	44 (63.8)	24 (34.8)	1 (1.4)	[b]1.0
	No	1	0	0	
Somebody else used your needle/syringe afterwards	Yes	3	0	0	[b]0.55
	No	41 (62.1)	24 (36.4)	1 (1.5)	
Frequency of use of sterile needles/syringes	Always (100%)	37 (66.1)	19 (33.9)	0	[b]0.56
	Most of the time (75%)	8 (61.5)	4 (30.8)	1 (7.7)	
	About every second time (50%)	0	1	0	
	With cold water	5 (55.6)	3 (33.3)	1 (11.1)	

Method of cleaning used needles/syringes	With warm water	1			
	With hot water	6 (75.0)	2 (25.0)	0	
	With boiling water	8 (72.7)	3 (27.3)	0	
	With soap or detergent	/			
	With bleach	/			
	With alcohol	3	1	0	
	Other	1	1	0	
Place of obtaining sterile needles/syringes	A pharmacy or hospital	10 (76.9)	3 (23.1)	0	[b]0.6
	Non-governmental organization	35 (61.4)	21 (36.8)	1 (1.8)	

[a](Chi-square test) [b](Fisher's exact test)

5.2.4 Experience with the police and prison

Results of the analysis about the experience of IDUs with the police showed that 103 (50.2%) of them were arrested for drug use. Significantly more anti-HCV positive IDUs were arested (p=0.02). More than half (110/205; 53.7%) of IDUs reported ever being imprisoned. Anti-HCV positive IDUs significantly more frequently reported ever being in prison (60.9%, p=0.0001). However, there was no significant difference among anti-HCV positive and negative IDUs with regard to injecting drugs during imprisonment (Table 36).

Table 36. Experience with police and prison of IDUs in Prishtina Region of Kosovo

Variable		Total	Anti-HCV positive	Anti-HCV negative	*p*-value
Arrested for drug use	Yes	103	58 (56.3)	45 (43.7)	[a]0.02*
	No	102	41 (40.2)	61 (59.8)	
Imprisoned	Yes	110	67 (60.9)	43 (39.1)	[a]0.0001**
	No	95	32 (33.7)	63 (66.3)	
Injected drugs during imprisonment	Yes	6	4 (66.7)	2 (33.3)	[b]1.0
	No	104	63 (60.58)	41 (39.42)	

[a](Chi-square test) [b](Fisher's exact test) *p<0.05 **p<0.01

Figure 18. Prevalence of anti-HCV among IDUs with regard to being ever arrested

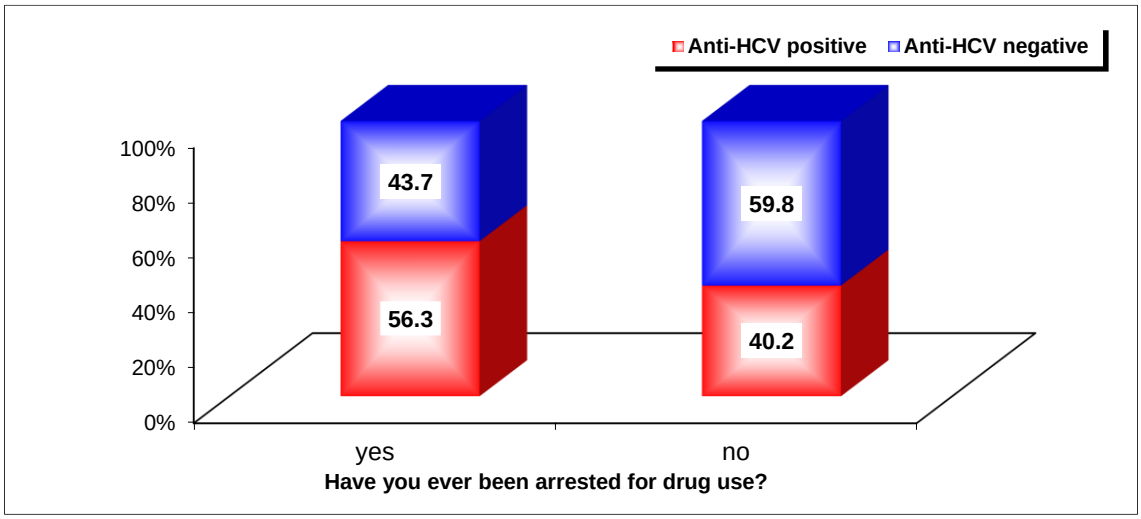
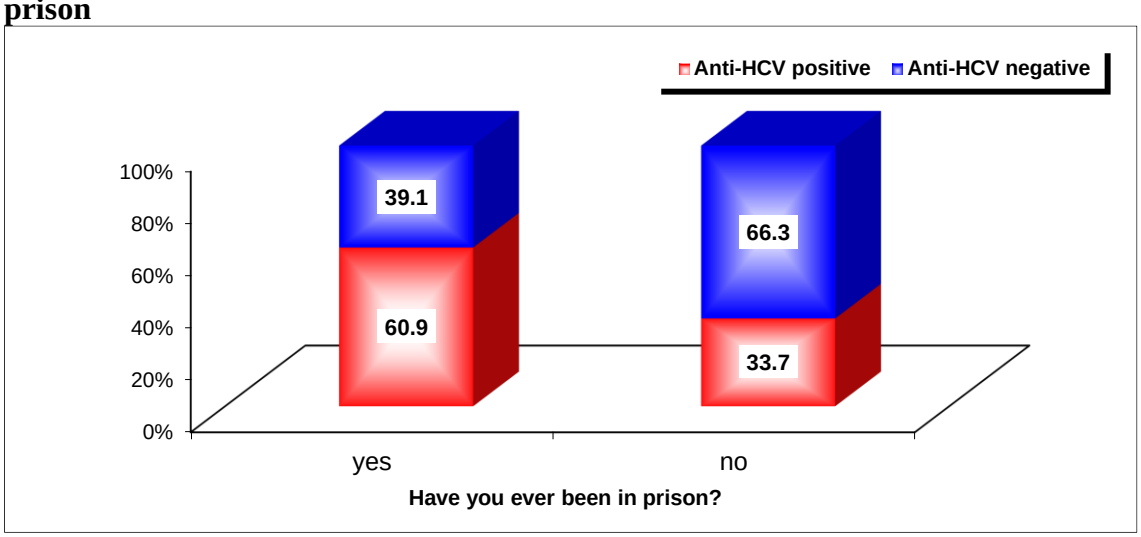


Figure 19. Prevalence of anti-HCV among IDUs with regard to ever being in prison



5.2.5 Sexual practices

A total of 149 (72.7%) IDUs had a sexual intercourse within the month prior to participation in the study. IDUs who had sexual intercourse within the past month were infected by HCV non-significantly more frequently than the IDUs who denied this practice (50.3% vs 44.4%; p=0.46).

Most IDUs who had sexual intercourse in the past month (71.8%) had one sexual partner within the past month. All the three IDUs who had sexual intercourse with 4 different persons in the past month were HCV positive. The prevalence of antibodies against HCV was also high in the group of IDUs who, within the period of the past month, had sexual intercourse with three different individuals (64.3%). Difference in the number of sexual partners in the past month between HCV positive and HCV negative IDUs was not statistically significant (p=0.23).

Forty-eight IDUs that had sexual intercourse in the past month reported always using condoms (31.8%). The prevalence of HCV infection was not statistically significantly different among the IDUs who used condoms all the time when they had a sexual intercourse in the past month and those who did not (p=0.26). The HCV positive and the HCV negative IDUs differed significantly by the use of condoms during their last sexual intercourse with their marital partner or permanent partner (p=0.037), i.e. 58.3% of IDUs who used condoms during their last sexual intercourse with their marital or permanent partner were anti-HCV positive vs 41.7% who were anti-HCV negative.
In this study population of IDUs, 18 (9.1%) had a regular sexual partner who injected drugs, eight of which (44.4%) also had hepatitis C; four IDUs had a regular sexual partner who previously injected drugs, two of whom were HCV positive. There were no statistically significant differences in the prevalence of antibodies against HCV among IDUs with a permanent sexual partner who injected drugs, IDUs with a permanent sexual partner who did not inject drugs, and IDUs with a permanent sexual partner who used to inject drugs previously (p=0.93).

A total of 120 (61.9%) IDUs used a condom during their last sexual intercourse with a casual partner. Among them, 60 (50.0%) had HCV infection, while in the group of 74 IDUs who did not use a condom, 47.3% had HCV infection (p=0.7).

Twelve (6.0%) IDUs had sexual intercourse in the past month with a non-regular partner who injected drugs, six of whom were HCV positive. No significant difference was observed between anti-HCV positive and anti-HCV negative patients related to

having sexual intercourse with a non-regular partner who injected drugs in the past month (p=0.9).

The prevalence of antibodies against HCV differed non-significantly among the groups who used a condom the last time they had sexual intercourse with a casual partner injecting drugs and who did not use a condom or did not remember using a condom (p=0.7).

Statistically significant difference was not found for the infection frequency with HCV among the IDUs who had anal sex (47.7%), had no anal sex (52.9%), and those who refused to answer this question (41.2%) (p=0.47). Also, non-significant difference was found in the frequency of HCV infection among the IDUs who used or did not use a condom during anal sex (48.5% vs 45.5%) (p=0.8) (Table 37).

Table 37. Sexual practices and HCV infection among IDUs in Prishtina Region of Kosovo

Variable		Total n	Anti-HCV positive n (%)	Anti-HCV negatives n (%)	*p*-value
Sexual intercourse in the past month	Yes	149	75 (50.3)	74 (49.7)	[a]0.46
	No	54	24 (44.4)	30 (55.6)	
Number of sexual partners in the past month	1	107	52 (48.6)	55 (51.4)	[b]0.23
	2	25	11 (44.0)	14 (56.0)	
	3	14	9 (64.3)	5 (35.7)	
	4	3	3 (100)	0	
Condom use during each sexual intercourse in the past month	Yes	48	28 (58.3)	20 (41.7)	[b]0.2
	No	100	47 (47)	53 (53)	
	Don't remember	1	0	1	
Condom use during each sexual intercourse with regular partner or spouse	Yes	72	42 (58.3)	30 (41.7)	[a]0.037*
	No	128	55 (43.0)	73 (57.0)	
Regular sex	Yes	18	8 (44.4)	10 (55.6)	

partner injects drugs	No	179	88 (49.2)	91 (50.8)	[b]0.93
	No, but he/she used to inject	4	2	2	
Condom use during the last sexual intercourse with casual partner	Yes	120	60 (50.0)	60 (50.0)	[a]0.7
	No	74	35 (47.3)	39 (52.7)	
Sexual intercourse in the past month with a non-regular partner who also injected drugs	Yes	12	6 (50.0)	6 (50.0)	[a]0.9
	No	188	92 (48.9)	96 (51.1)	
Condom use during the last sexual intercourse with casual partner who injected drugs	Yes	45	23 (51.1)	22 (48.9)	[a]0.7
	No	38	20 (52.6)	18 (47.4)	
	Don't remember	25	11 (44.0)	14 (56.0)	
Anal sex	Yes	65	31 (47.7)	34 (52.3)	[a]0.47
	No	102	54 (52.9)	48 (47.1)	
	Refused to answer	34	14 (41.2)	20 (58.8)	
Anal sex with:	Only women	64	30 (46.9)	34 (53.1)	
	Only men	1	0	1	[b]1.0
Condom use during the last anal sex	Yes	33	16 (48.5)	17 (51.5)	[a]0.8
	No	33	15 (45.5)	18 (54.5)	

[a](Chi-square test) [b](Fisher's exact test) *$p<0.05$

Distribution of HCV genotypes was not significantly correlated with any of the IDUs sexual practices, in the Prishtina Region (Table 38).

Table 38. HCV genotypes among IDUs in Prishtina Region of Kosovo and their sexual practices

Variable		**HCV Genotype**			***p*-value**
		1**a** **n (%)**	3**a** **n (%)**	**2k** **n (%)**	
Sexual intercourse in the past month	Yes	37 (68.5)	16 (29.6)	1 (1.9)	[a]0.8
	No	8 (50)	8 (50)	0	
Number of sexual	1	27 (77.1)	8 (22.9)	0	

partners in the past month	2	6 (66.7)	2 (22.2)	1 (11.1)	[b]0.105
	3	4 (44.4)	5 (55.6)	0	
	4	0	1	0	
Condom use during each sexual intercourse in the past month	Yes	13 (65)	7 (35)	0	[a]0.79
	No	24 (66.7)	11 (30.5)	1 (2.8)	
Condom use during the last sexual intercourse with regular partner	yes	20 (71.4)	8 (28.6)	0	[a]0.53
	no	25 (62.5)	14 (35)	1 (2.5)	
Regular sex partner injects drugs	Yes	0	2 (66.7)	1 (33.3)	[b]0.21
	No	43 (67.2)	21 (32.8)	0	
	No, but he/she used to inject	2	0	0	
Condom use during the last sexual intercourse with casual partner	Yes	27 (69.2)	12 (30.8)	0	[a]0.31
	No	16 (55.2)	12 (41.4)	1 (3.4)	
Sexual intercourse in the past month with non-regular partner who also injected drugs	Yes	0	3	0	[a]0.015*
	No	45 (67.2)	21 (31.3)	1 (1.5)	
Condom use during the last sexual intercourse with casual partner who injected drugs	Yes	13 (72.2)	5 (27.8)	0	[b]0.5
	No	8 (50)	7 (43.8)	1 (6.2)	
	Don't remember	4 (57.1)	3 (42)	0	
Anal sex	Yes	11 (52.4)	9 (42.9)	1 (4.7)	[a]0.41
	No	28 (71.8)	11 (28.2)	0	
	Refused to answer	6 (60)	4 (40)	0	
Anal sex with:	Women only	11 (55)	9 (45)		
Condom use during the last anal sex	Yes	6 (50)	6 (50)	0	[a]0.58
	No	5 (55.6)	3 (33.3)	1 (11.1)	

[a](Chi-square test) [b](Fisher's exact test) *$p<0.05$

5.2.6 Sex work

Eleven (5.4%) IDUs reported getting paid for sex in the past month. The prevalence of antibodies against HCV in those IDUs was 63.6%, vs. 48.2% in the group of IDUs who did not get paid for sexual intercourse in the past month. Association of sexual intercourse for money and HCV infection was not statistically significant (p=0.32). A similar situation was observed with regard to the use of condom during the last paid sexual intecourse.

In the past 12 months, 9 (4.5%) IDUs paid or gave drugs for sex, and seven (70.0%) reported using a condom the last time they were paying for sex. Nevertheless, none of those sexual behaviors was significantly different between anti-HCV positive and anti-HCV negative IDUs (Table 39).

Table 39. Sex work and HCV infection among IDUs in Prishtina Region of Kosovo

Variable		Total n	Anti-HCV positive n (%)	Anti-HCV negative n (%)	*p*-value
Got paid for sexual intercourse in the past month	Yes	11	7 (63.6)	4 (36.4)	[a]0.32
	No	191	92 (48.2)	99 (51.8)	
Condom use during the last sexual intercourse that got paid for	Yes	4	3 (75)	1 (25)	
	No	4	3 (75)	1 (25)	
Paid/gave goods/drugs to have sexual intercourse in the past 12 months	Yes	9	5 (55.6)	4 (44.4)	[b]0.74
	No	193	94 (48.7)	99 (51.3)	
Condom use during the last paid (for money or drugs) sexual intercourse	Yes	7	4 (57.1)	3 (42.9)	[b]0.2
	No	2	0	2 (100)	

[a](Chi-square test) [b](Fisher's exact test)

5.2.7 Treatment for drug addiction

A total of 161 (78.5%) IDUs used NGO Labyrinth services in the previous year, 71 (44.1%) of whom were anti-HCV positive. The statistical analysis showed that the IDUs

who were not using Labyrinth services were significantly more commonly infected with HCV (79.1% vs 44.1%; *p*=0.000046).

Half of IDUs (50.1%) entered the treatment program in order to reduce or quit drug use, with statistically significantly more anti-HCV positive IDUs in the treatment program (57.7% vs 38.0%; *p*=0.000048).

Information about the type of treatment was obtained from 105 IDUs, and 73 (69.5%) of them most frequently visited the rehabilitation program in a medical treatment facility. The type of treatment received had no significant impact on the frequency of infection with HCV (*p*=0.5) (Table 40).

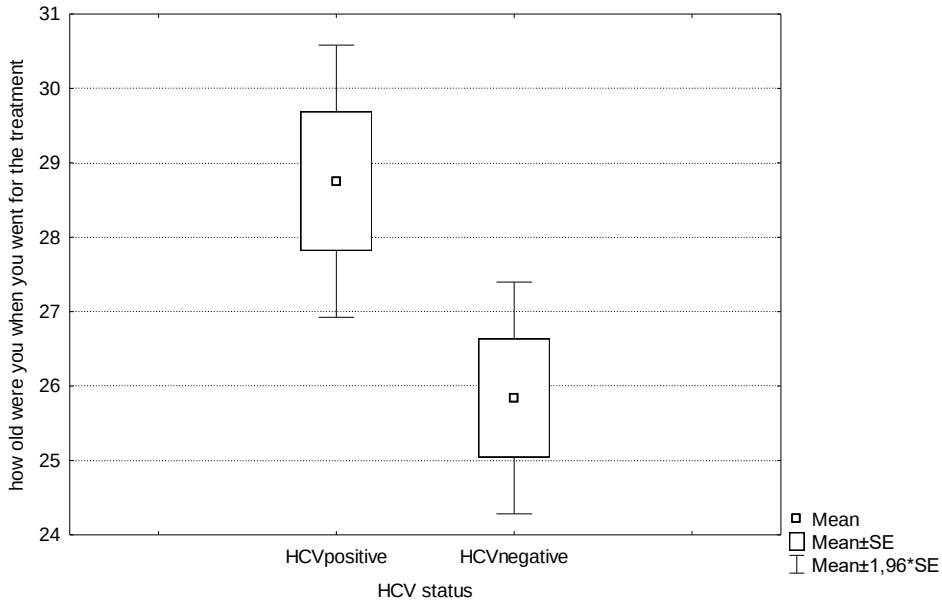
Table 40. Treatment for drug addiction and HCV infection among IDUs in Prishtina Region of Kosovo

Variable		Total	Anti-HCV positive	Anti-HCV negative	*p*-value
Have you used Labirint services in the past year	Yes	161	71 (44.1)	90 (55.9)	[a]0.000046**
	No	43	34 (79.1)	9 (20.9)	
Treatment to reduce or quit use of drugs	Yes	104	60 (57.7)	44 (42.3)	[a]0.000048**
	No	100	38 (38.0)	62 (62)	
Type of treatment	Rehabilitation program run by an NGO	13	7 (53.9)	6 (46.1)	[b]0.5
	Rehabilitation program in a medical treatment facility	73	46 (63)	27 (37)	
	Rehabilitation program in prison	6	4 (66.7)	2 (33.3)	
	Detoxication treatment by my family	2	1	1	
	Self-help	5	1 (20)	4 (80)	
	Other	6	3 (50)	3 (50)	

[a](Chi-square test) [b](Fisher's exact test); **$p<0.01$

The average age at which HCV positive IDUs received treatment was 28.7±7.3 years, versus the average age of HCV negative IDUs – 25.8 ± 5.3 (p=0.026).

Figure 20. Average IDUs age at treatment initiation in Prishtina, Kosovo



5.2.8 Logistic regression analysis

Logistic regression analysis was used in order to analyze the factors being associated with the HCV infection and to predict the presence of antibodies against HCV among IDUs.

Univariate logistic regression analysis showed that factors significantly associated with anti-HCV positivity were:

- The age of IDUs (OR: 1.111, 95% CI 1.062 – 1.163; $p<0.001$), more precisely the age group from 30 to 39 years (OR: 2.667, 95% CI 1.146 – 6.203; $p=0.023$), and 40 years and older (OR: 5.939, 95% CI 2.388 – 14.772; $p<0.001$);
- IDUs being married (OR: 1.836, 95% CI 1.006 – 3.350; $p=0.048$);
- With completed primary education or no formal primary education compared to the higher education (OR: 6.139, 95% CI 2.343 – 16.083; $p<0.001$), and completed secondary education compared to higher education (OR: 2.528, 95% CI 1.093 – 5.747; $p=0.03$);
- Had a longer history of drug use (OR: 1.384, 95% CI 1.245 – 1.538; $p=0.001$);
- Drug injection of 1 to 3 times per week compared to once (OR: 2.267, 95% CI 1.252 – 6.114; $p=0.012$), drug injection 4 to 6 times per week compared to once (OR: 10.952, 95% CI 1.204 – 9.660; $p=0.034$), and drug injection of 1 to 3 times per day compared to once (OR: 4.537, 95% CI 1.997 – 10.308; $p<0.001$);
- Drug injection in the so called ‘shooting galleries’ where IDUs gather (OR: 2.882, 95% CI 1.499 – 5.540; $p=0.002$);
- IDUs who were ever arrested for drug use (OR: 1.918, 95% CI 1.101 – 3.341; $p=0.022$);
- IDUs who were ever in prison (OR: 3.068, 95% CI 1.713 – 5.438; $p<0.001$).

Table 41. Univariate logistic regression model to predict HCV infection among IDUs in Prishtina, Kosovo					
Variable	**N**	**Anti-HCV positive (%)**	**Unadjusted OR**	**CI 95%**	***p*-value**
Gender					
Female	21	33.3	ref (1)		
Male	183	49.7	1.978	0.763 – 5.128	0.16
Missing	1				
Age (years)			1.111	1.062 – 1.163	<0.001
Age (years)					
19–29	37	24.3	ref (1)		
30–39	104	46.2	2.667	1.146 – 6.203	0.023*
≥ 40	64	65.6	5.939	2.388 – 14.772	<0.001
Marital status					
Not married	142	43.7	ref (1)		
Married	63	58.7	1.836	1.006 – 3.350	0.048*
Education					
Higher	35	25.7	ref (1)		
Primary	30	68	6.139	2.343 – 16.083	<0.001
Secondary	120	46.7	2.528	1.093 – 5.747	0.030*
Employment					
Employed	61	41	ref (1)		
Unemployed	144	51.4	1.522	0.831 – 2.790	0.174
Duration of drug use (years)			1,384	1.245 – 1.538	0.001**
Duration of drug use (years)					
5–9	69	33.3	ref(1)		
10–14	52	48.1	1.852	0.884 – 3.878	0.102
15–19	45	60	3	1.377 – 6.535	0.006**
20–24	28	60.7	3.091	1.246 – 7.669	0.015*
25–29	4	75	6	0.591 – 20.924	0.13
>30	7	57.1	2.667	0.55 – 12.926	0.223
Frequency of injection					

Once	67	31.3	ref (1)		
2–3 times	36	27.8	0.842	0.347 – 2.059	0.707
1–3 times a week	43	55.8	2.267	1.252 – 6.114	0.012*
4–6 times a week	6	83.3	10.952	1.204 – 9.660	0.034*
1–3 times a day	43	67.4	4.537	1.997 – 10.308	0.001**
4 or more times a day	10	100	4.00E+09	0	0.999
Place of injecting drugs					
At home	161	47.8	ref(1)		
On the street or in the park	17	41.2	0.778	0.281 – 2.150	0.628
In a shooting gallery or in another place where IDUs gather	58	67.2	2.882	1.499 – 5.540	0.002**
Other	18	50	1.091	0.560 – 2.125	0.798
Drugs					
Heroin					
No	4	25	ref(1)		
Yes	201	48.8	2.854	0.292 – 27.906	0.367
Cocaine					
No	150	45.3	ref(1)		
Yes	55	54.5	1.409	0.758 – 2.620	0.279
Heroin and cocaine					
No	153	43.1	ref(1)		
Yes	52	63.5	2.289	1.197 – 4.380	0.012*
Morphine					
No	184	44.6	ref(1)		
Yes	21	76.7	3.894	1.369 – 11.076	0.011*
Opium					
No	194	47.4	ref(1)		
Yes	11	72.7	3.018	0.777 – 18.262	0.110
Methadone					
No	62	16.1	ref(1)		
Yes	143	62.2	8.57	4.022 – 18.262	0.001**
Use of used needles/syringes					

No	181	45.9	ref (1)		
Yes	24	66.7	2.361	0.962 – 5.795	0.061
Arrested for drug use					
No	102	40.2	ref (1)		
Yes	103	56.3	1.918	1.101 – 3.341	0.022*
Imprisonment					
No	95	33.7	ref (1)		
Yes	110	60.9	3.068	1.713 – 5.438	0.001**
Sexual intercourse					
No	54	44.4	ref (1)		
Yes	149	50.3	1.09	0.582 – 2.043	0.788
Missing	3				
Sex for money					
No	190	48.2	ref (1)		
Yes	11	63.6	1.265	0.373 – 4.288	0.706
Missing	4				
Treatment for quitting drugs					
No	100	38.9	ref (1)		
Yes	104	57.7	1.272	0.732-2.211	0.394
Missing	1				

*p<0.05; **p<0.01; OR=odds ratio; CI=confidence interval;

Multivariate logistic regression analysis confirmed that independent significant predictors for the presence of anti-HCV among IDUs were: the age of IDUs (p=0.009), duration of drug use (p=0.002), the lowest educational level (p=0.039), drug injection in the so called 'shooting galleries' where the individuals gather for drug injection (p=0.009), and being in prison (p=0.01).

With increase of age by one year, the risk for anti-HCV positivity increased by 8.6% (OR: 1.086, 95% CI 1.021 – 1.156);

The drug addicts with completed primary education or informal primary education compared to higher education had a 2.8 times higher risk for anti-HCV positivity (OR: 2.886, 95% CI 1.199 – 8.33);

Increase of the duration of drug injection by one year increased the risk for anti-HCV positivity by 7.2% (OR: 1.072, 95% CI 1.025 – 1.121);

Drug users who injected drugs in the so-called 'shooting galleries' had a 2.5 times greater risk for anti-HCV positivity compared to IDUs injecting at other places (OR: 3.011, 95% CI 1.542 – 5.878);

Being in prison increased the risk of the IDUs for anti-HCV positivity 2.26 times (OR: 2.26, 95% CI 1.215 – 4.204).

Table 42. Multivariate logistic regression model to predict HCV infection among IDUs in Prishtina

Variable	N	Anti-HCV positive (%)	Adjusted OR	CI 95%	*p*-value
Gender					
Female	21	33.3	ref (1)		
Male	183	49.7	1.77	0.653 – 4.800	0.260
Missing	1				
Age (years)					
			1.086	1.021 – 1.156	0.009**
Age (years)					
19–29	37	24.3	ref (1)		
30–39	104	46.2	2.297	0.873 – 5.331	0.096
≥ 40	64	65.6	3.221	0.949 – 5.563	0.065
Marital status					
Not married	142	43.7	ref (1)		
Married	63	58.7	1.173	0.472 – 2.914	0.730
Education					
higher	35	25.7	ref (1)		
primary	30	68	2.886	1.199 – 8.330	0.039*
secondary	120	46.7	1.710	0.682 – 4.289	0.253

Employment					
Employed	61	41	ref (1)		
Unemployed	144	51.4	1.324	0.668 – 2.621	0.420
Duration of drug use (years)			1.072	1.025 – 1.121	0.002**
Duration of drug use (years)					
5–9	69	33.3	ref (1)		
10–14	52	48.1	1.777	0.810 – 3.898	0-151
15–19	45	60	2.119	0.924 – 4.863	0.076
20–24	28	60.7	1.342	0.487 – 3.699	0.569
25–29	4	75	1.746	0.157 – 19.417	0.650
>30	7	57.1	0.362	0.056 – 2.344	0.286
Frequency of injection					
Once	67	31.3	ref (1)		
2–3 times	36	27.8	0.991	0.393 – 2.499	0.985
1–3 times a week	43	55.8	1.370	0.942 – 5.393	0.620
4–6 times a week	6	83.3	1.070	0.411 – 2.788	0.890
1–3 times a day	43	67.4	1.676	0.997 – 9.660	0.820
4 or more times a day	10	100	3.00E+09	0	***
Place of injecting drugs					
At home	161	47.8	ref (1)		
On the street or in the park	17	41.2	0.764	0.273-2.142	0.609
In a shooting gallery or in another place where IDUs gather	58	67.2	3.011	1.542-5.878	0.001**

Other places	18	50	1.098	0.552-2.186	0.820
Drugs					
Heroin					
No	4	25.0	ref (1)		
Yes	201	48.8	1.711	0.171- 17.158	0.648
Cocaine					
No	150	45.3	ref (1)		
Yes	55	54.5	1.407	0.728 – 2.719	0.310
Heroin and cocaine					
No	153	43.1	ref (1)		
Yes	52	63.5	1.825	0.920 – 3.619	0.085
Morphine					
No	184	44.6	ref (1)		
Yes	21	76.7	2.604	0.802 – 8.456	0.110
Opium					
No	194	47.4	ref (1)		
Yes	11	72.7	1.428	0.318 – 6.405	0.640
Methadone					
No	62	16.1	ref (1)		
Yes	143	62.2	3.72	0.911 – 10.560	0.140
Use of used needles/syringes					
No	181	45.9	ref (1)		
Yes	24	66.7	2.307	0.905 – 5.881	0.080
Arrested for drug use					
No	102	40.2	ref (1)		
Yes	103	56.3	1.679	0.917 – 3.075	0.093
Imprisonment					
No	95	33.7	ref (1)		
Yes	110	60.9	2.26	1.215 – 4.204	0.010*
Sexual intercourse					
No	54	44.4	ref (1)		
Yes	149	50.3	1.117	0.966 – 1.171	0.737
Missing	2				
Sex for money					
No	190	48.2	ref (1)		
Yes	11	63.6	1.033	0.294 – 3.625	0.959

No	4				
Treatment for quitting drugs					
No	100	38.9	ref (1)		
Yes	104	57.7	1.132	0.639 – 2.006	0.672
Missing	1				

*p<0.05; **p<0.01; *** Not able to be calculated; OR=odds ratio; CI=confidence interval

5.3 HCV INFECTION AMONG CHRONIC PATIENTS AND BLOOD DONORS

Samples of 59 individuals infected with HCV were included in the study, 50 of them were from HCV chronic patients at the Infectious Disease Clinic of the University Clinical Center of Kosovo, and 9 were from blood donors. Through cross checking of cases, it was noticed that three chronic HCV patients were included in the study population of dialysis patients.

HCV core region was detected among 31 (62.0 %) HCV chronic patients, and among all of them HCV genotype was determined, with the highest frequency of HCV subtype 1b – 13/31 (41.9%), followed by subtype 1a – 8/31 (25.8%), subtype 3a – 4/31 (12.9%), subtype 4d in 4/31 (12.9%) patients, and subtype 2c in 2/31 (6.5%) chronic HCV patients.

In the group of 10 HCV positive blood donors, the HCV genotype was determined in 6 (66.7%) patients, among whom there were four (66.7%) with subtype 1b and two (33.3%) with subtype 1a (Table 43).

Table 43. Distribution of HCV genotypes in HCV chronic patients and blood donors

HCV Genotype	Chronic HCV patients	HCV blood donors
	n (%)	n (%)
1a	8 (25.8)	2 (33.3)
1b	13 (41.9)	4 (66.7)
3a	4 (12.9)	
4d	4 (12.9)	
2c	2 (6.5)	
Total	31	6

IL28B gene was examined among all 50 HCV chronic patients, in which structure the most frequent expression was of the CT genotype – 28/50 (56.0%), followed by CC genotype – 19/50 (38.0%), and TT genotype – 3/50 (6.0%).

In the group of 9 HCV positive blood donors, the polymorphism of IL28B could be determined in 5 (55.6%) of them, whereas the expression of CC genotype was detected in one and the expression of CT genotype in four (Table 44).

Table 44. Expression of IL28B in chronic patients and blood donors

IL28B	Chronic HCV patients	HCV blood donors
	n (%)	n (%)
CC	19 (38.0)	1 (20.0)
CT	28 (56.0)	4 (80.0)
TT	3 (6.0)	
Total	50	5

Through cladogram phylogenetic trees of HCV subtypes 1a, 1b, 3a, and 4d, it is possible to notice that most sample sequences from the study chronic HCV patients are not clustered with each other, with a few minor exceptions.

In fact, out of a total of nine sequences from chronic HCV patients with HCV subtype 1a, four of them (separately) are inside three different clusters consisted of samples from different dialysis centers. Three other sequences from the same group and HCV subtype are located inside a cluster of HCV samples from IDUs (Fig.1).

There were 13 sequences of samples with HCV subtype 1b from chronic HCV patients for which a cladogram phylogenetic tree shows two triplets inside two clusters with aLRT>0.9. Among one of these clusters are also two samples from Gjilan dialysis center and two samples from blood donors (Fig.2).

Four sequences from the study chronic HCV patients with HCV subtype 3a are not clustered with each other. One sample from this group is located inside a cluster of 12 samples from IDUs with HCV subtype 3a (Fig.11).

Four sample sequences of chronic HCV patients with HCV subtype 4d are not clustered with each other and they are placed separately in three different clusters consisted of samples from different dialysis centers of Kosovo (Prizren, Prishtina, Mitrovica, Peja, Gjakova, Gjilan) (Fig.3).

With regard to sequences from HCV infected blood donors in this study, four samples with HCV subtype 1b are placed as paired samples in two different clusters. In one cluster with aLRT >0.8 inside cladogram of HCV subtype 1b are placed two sequences from two samples from blood donors together with a few control sequences. The second pair of samples of blood donors is placed in the cluster with aLRT>0.9 together with three samples from chronic HCV patients and two samples from Gjilan dialysis center.

Of two samples of HCV infected blood donors with HCV subtype 1a, one of them is placed inside a large cluster of HCV sequences from IDUs.

5.3.1 Socio-demographic data of chronic HCV patients and anti-HCV positive blood donors

A questionnaire on socio-demographic data and possible high risk activities related to HCV infection was completed through face-to-face interviews with 42 subjects, 35 of them HCV chronic patients and 7 anti-HCV positive blood donors. Among chronic HCV patients and blood donors enrolled in the study in 2013 at the Infectious Disease Clinic in Prishtina, University Clinical Center of Kosovo (UCCK), 13 (31.0%) were female and 29 (69.0%) male, with a mean age of 48.9 ± 13.2 years (minimum age 23 years, maximum age 69 years). Most subjects were 60–69 years old (13; 31.0%), followed by the age group of 39 and younger with 12 (28.6%) subjects.

One third of subjects (12/42; 28.6%) originated from Prishtina, followed by Mitrovica with 8 (8/42; 19.0%) subjects, Gjilan with 7 (16.7%), Ferizaj with 6 (14.3%), and Prizren and Gjakova with 2 (4.8%) subjects each. As for marital status, most subjects were married (35; 83.3%). As for educational level, 16 (38.1%) subjects completed

primary education, 15 (35.7%) secondary education, and the lowest percentage of subjects had higher level education (11/26.2%). Eighteen (42.9%) subjects were unemployed, 11 (26.2%) were housewives, and one was retired (Table 45).

Table 45. Demographic characteristics of chronic HCV patients and anti-HCV blood donors

Variable		**N (%)**
Gender	Female	13 (31.0)
	Male	29 (69.0)
Age group	≤ 39	12 (28.6)
	40 – 49	8 (19.0)
	50 – 59	9 (21.4)
	60 – 69	13 (31.0)
Region	Prishtina	12 (28.6)
	Mitrovica	8 (19.0)
	Peja	4 (9.5)
	Prizren	2 (4.8)
	Ferizaj	6 (14.3)
	Gjilan	7 (16.7)
	Gjakova	2 (4.8)
	Missing	1 (2.4)
Marital status	Single	3 (7.1)
	In a relationship	3 (7.1)
	Married	35 (83.3)
	Widow/er	1 (2.4)
Education	Primary	16 (38.1)
	Secondary	15 (35.7)
	Higher	11 (26.2)
Occupation	Housewife	11 (26.2)
	Farmer	2 (4.8)
	Physical worker	2 (4.8)
	Engineer	2 (4.8)
	Economist	3 (7.1)
	Health worker	1 (2.4)
	Other	20 (47.6)
	Missing	1 (2.4)
Employment	Unemployment	18 (42.9)
	Employed in public sector	4 (9.5)
	Employed in private sector	18 (42.9)

	Retired	1 (2.4)
	Missing	1 (2.4)

5.3.2 Knowledge about HCV

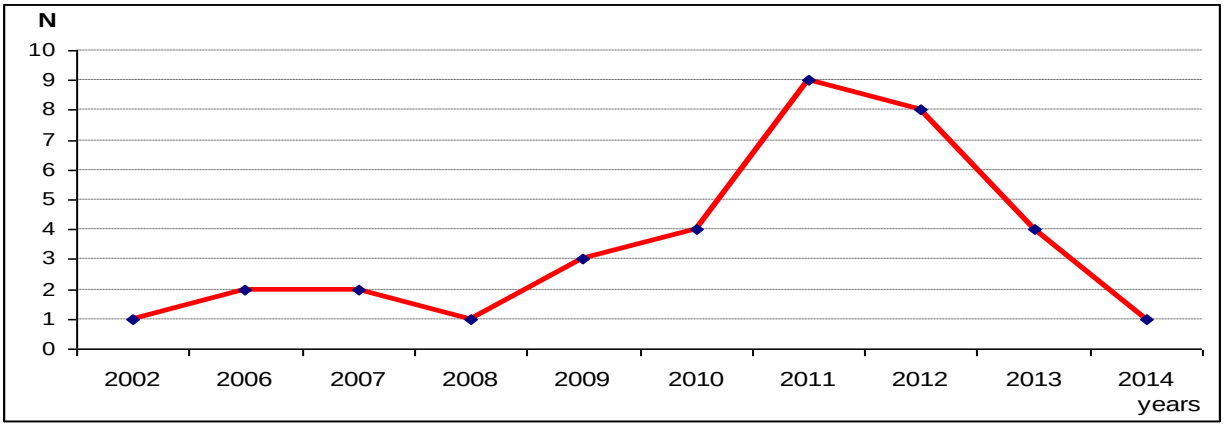
Results of this study showed that only 16.7% of chronic HCV patients and blood donors infected with HCV had heard about hepatitis C virus prior to their infection. At the time of the study, they had more information about the ways of HCV transmission – 33 (78.6%) responded affirmatively to the question 'Do you know how HCV is transmitted?'. Only 4 (9.5%) subjects reported ever having skin jaundice. Five (11.9%) HCV positive patients gave family history information about their family members being infected with HCV (Table 46).

Table 46. Chronic HCV patients'/Anti-HCV blood donor's knowledge about HCV

Variable		**N (%)**
Ever heard about hepatitis C virus	Yes	7 (16.7)
	No	34 (81.0)
	Don't know	1 (2.4)
Knew how hepatitis C virus is spread	Yes	33 (78.6)
	No	8 (19.0)
	Missing	1 (2.4)
Diagnosed with hepatitis C infection	Yes	36 (85.7)
	No	4 (9.5)
	Don't know	1 (2.4)
	Missing	1 (2.4)
Ever had skin jaundice	Yes	4 (9.5)
	No	37 (88.1)
	Missing	1 (2.4)
Family members having HCV infection	Yes	5 (11.9)
	No	34 (81.0)
	Don't know	1 (2.4)
	Missing	2 (4.8)

On average, those individuals were diagnosed with HCV 6.6 ± 2.5 years before the interviews (data obtained for 35 individuals). The shortest time of having the information on diagnosis with HCV infection was 3 years earlier, and the longest 15 years earlier (Figure 20).

Figure 20. Time of being diagnosed with HCV infection (year)



5.3.3 Risk factors

5.3.3.1 Blood transfusion

Fifteen (35.7%) patients in the study groups received blood transfusion. The University Clinical Center of Kosovo in Prishtina was the most frequent medical institution where those patients received blood transfusion. Table 47 shows the distribution of patients by the number of blood transfusions. The time when those anti-HCV positive patients received blood transfusion ranged between 3 and 42 years earlier, the first blood transfusion was received, on average, 13 ± 10.8 years earlier, the median time of the first transfusion was 10.5 years (range 5–31).

Table 47. Blood transfusions among chronic HCV positive patients

Variable		N (%)
Received blood transfusion	Yes	15 (35.7)
	No	26 (61.9)
	Missing	1 (2.4)
Health institution(s) where blood transfusions were received	University Clinical Center of Kosovo	7 (46.7)
	Regional hospital	5 (33.3)
	Other	2 (13.3)
	Missing	1 (6.7)
Number of blood transfusions	One time	5 (33.3)
	Two times	1 (6.7)
	Three times	1 (6.7)
	Four times	1 (6.7)
	3–4 times	1 (6.7)
	Few times	3 (20.0)
	Over ten times	2 (13.3)
	Missing	1 (6.7)

5.3.3.2 Dental services

Most patients used dental services (36/42; 85.7%). The type of the dental services and the medical institution where those services were provided are given in Table 48.

Table 48. Distribution of dental services among chronic HCV positive patients

Variable		N (%)
Ever utilized dental services	Yes	36 (85.7)
	No	5 (11.9)
	Missing	1 (2.4)
Type of dental service	Filling	3 (8.3)
	Extraction	7 (19.4)
	Filling, Extraction	20 (55.6)
	Prosthetics	2 (5.6)
	Other	3 (8.3)

	Missing	1 (2.8)
Medical institution where those services were provided	Family medicine center	3 (8.3)
	Private dentistry center	26 (72.2)
	Family medicine center, private dentistry center	3 (8.3)
	University Clinical Center for Dentistry of Kosovo	2 (5.6)
	Other, Germany	1 (2.8)
	Missing	1 (2.8)

5.3.3.3 Surgical interventions

A great part of chronic HCV positive patients underwent surgical interventions (30; 71.4%). The type of surgical interventions those patients were subjected to and the name/type of the medical institution where the surgical interventions took place are given in Table 49. Abdominal surgical interventions were most frequent, and most of them took place at the University Clinical Center of Kosovo in Prishtina.

Table 49. Distribution of surgical interventions among chronic HCV positive patients

Variable		**N (%)**
Ever had surgical intervention	Yes	30 (71.4)
	No	11 (26.2)
	Missing	1 (2.4)
Type of surgical intervention	Abdominal	9 (30)
	Urological	6 (20)
	Gynecological	1 (3.3)
	Kidney transplantation	1 (3.3)
	Throat	1 (3.3)
	Venous problem	2 (6.7)
	Hydrocele	1 (3.3)
	Extraction of one lung	1 (3.3)
	Nose operation	1 (3.3)
	Biopsy of the neck	1 (3.3)
	Abdominal, Gynecological	1 (3.3)
	Operation on the leg	1 (3.3)

	Missing	4 (13.3)
Medical institution where the intervention took place	University Clinical Center of Kosovo	10 (33.3)
	Regional hospital	7 (23.3)
	Private hospital	1 (3.3)
	Other	7 (23.3)
	University Clinical Center of Kosovo, Other	1 (3.3)

5.3.3.4 Dialysis

Five patients (11.9%) within the HCV treatment program were also on dialysis, all of them started their dialysis in Prishtina, one continued to utilize dialysis services in this center, one patient continued dialysis services in Ferizaj and Gjilan, one patient used dialysis services in Mitrovica, one continued in Prizren and one in Gjakova (Table 50). It needs to be emphasized that three HCV chronic patients that were enrolled in the study within the HCV treatment program were also included in the study on dialysis patients. One of them was a patient from Prizren with code KOS-006 within the treatment program and with code KOS-303 at dialysis center in Prizren. This patient's samples were processed separately at the laboratory, being both of HCV genotype 1a and, at the phylogenetic tree, of HCV genotype 1a (Fig. 1), and placed in the same sub-cluster of samples from Prizren dialysis center. The same situation was with a patient from Ferizaj with code KOS-014 within the HCV treatment program and code KOS-393 at dialysis center of Ferizaj, and finally both samples were placed next to each other at the phylogenetic tree of HCV genotype 1 (Fig. 1) at the sub-cluster of samples from Ferizaj, Gjilan and Prishtina dialysis center. The third patient, included in the study in both groups – within the HCV treatment program and at dialysis centers, was a patient with sample code KOS-042 within the treatment program and with code KOS-226 at a dialysis center in Mitrovica. In this case, the sample KOS-226 was without detection of HCV RNA, but the sample with code KOS-042 was of HCV genotype 1a and, at the phylogenetic tree, of HCV genotype 1a (Fig. 1), and was placed inside the cluster of samples collected at Mitrovica dialysis center.

Table 50. Distribution of chronic HCV positive patients in relation to dialysis

Variable		N (%)
Ever undergone dialysis	Yes	5 (11.9)
	No	35 (83.3)
	Missing	2 (4.8)

5.3.3.5 Other risk factors

About half of the patients practiced some of the following risk behaviors: tattoo, ear or other body piercing, injected drugs, used shaving kit belonging to somebody else, or used somebody else's tooth brush. It needs to be emphasized that all patients responding affirmatively to the question about ear/body piercing were females, which corresponds to the local custom of having an ear pierced. Two patients within the HCV treatment program were placed in the cluster of samples belonging to IDUs within cladogram of HCV genotype 1a (Fig. 1). Of those two patients, one with code KOS-027 was interviewed and mentioned having injected drugs. Unfortunately, the second patient with code KOS-049 was not reachable for interview. Four (9.5%) HCV positive patients had experience as prisoners, and one had hemophilia (Table 51).

Table 51. Distribution of risk behaviors among chronic HCV positive patients

Variable		N (%)
Ever had a high risk behavior	Yes	22 (52.4)
	No	20 (47.6)
Type of high risk behaviour	Tattoo	1 (4.5)
	Ear or other body piercing	12 (54.5)
	Injected drugs	1 (4.5)
	Used somebody else's shaving kit	1 (4.5)
	Used somebody else's tooth brush	1 (4.5)
	Organ transplantation	2 (9.1)
	Having a sexual partner (other than regular/wife/husband)	1 (4.5)
	Tattoo, ear or other body piercing, injected drugs, needlestick injury at work, used same toilet with family	2 (9.1)

	member	
	Tattoo, ear or other body piercing, used shaving kit belonging to somebody else, etc.	1 (4.5)
Ever imprisoned	Yes	4 (9.5)
	No	37 (88.1)
	Missing	1 (2.4)
Diagnosed with hemophilia	Yes	1 (2.4)
	No	40 (95.2)
	Missing	1 (2.4)

5.3.4 Treatment

Thirty-four (80.1%) chronic HCV patients received therapy with pegylated interferon and ribavirin, 32 (94.12%) patients were administered this therapy at the Clinic for Infectious Diseases, University Clinical Center of Kosovo in Prishtina, one was treated in Albania and France. Twenty-nine (85.3%) patients completed the treatment, for 3 patients (8.3%) the treatment was discontinued due to adverse events, for the remaining 2 patients (5.9%) the reason why this treatment was not completed was not stated. Nine (24.1%) of the treated patients had no complaints concerning treatment with pegylated interferon/ribavirin. Among the rest of chronic HCV patients undergoing treatment, the main complaints are shown in Table 52.

Table 52. Information associated with treatment of chronic HCV positive patients

Variable		**N (%)**
Treated with interferon/ribavirin	Yes	34 (80.9)
	No	8 (19.1)
Place of treatment with interferon/ribavirin	Clinic for Infection Diseases, UCCK, Prishtina	32 (94.1)
	Albania (2002); France (2005); UCCK Prishtina	1 (2.9)
	Missing	1 (2.9)
Treatment course with interferon/ribavirin completed	Yes	29 (85.3)
	No	3 (8.8)
	Missing	2 (5.9)
Main complaints related to	No complaints	9 (26.5)

treatment with interferon/ribavirin	Tiredness	1 (2.9)
	Fever, tiredness	4 (11.8)
	Fever, tiredness, anemia, vomiting	1 (2.9)
	Fever, tiredness, weakness, anemia	1 (2.9)
	Fever, tiredness, itching, exanthemas	1 (2.9)
	Tiredness, weakness, headache, lack of sleep, anxiety, hypotension	1 (2.9)
	Headache, tiredness, pain in legs	1 (2.9)
	Fever, body pain	1 (2.9)
	Fever, thrombocytopenia	1 (2.9)
	Fever, fatigue, thrombocytopenia	1 (2.9)
	Fever, tiredness, body aches	1 (2.9)
	Anemia, leukopenia, sepsis	1 (2.9)
	Fever, pain in legs, headache, tiredness	1 (2.9)
	Tiredness, fever, stomach aches	1 (2.9)
	Cough	1 (2.9)
	Fatigue, weight loss, appetite loss, body aches	1 (2.9)
	Headache, tiredness, dizziness, body pain	1 (2.9)
	Weight loss after treatment	1 (2.9)
	Pain in legs, itching, leukopenia	1 (2.9)
	Missing	3 (8.8)

The distribution of HCV genotypes did not differ significantly between chronic patients with and without family history of HCV infection (p=0.13), between chronic patients who did not have or had dental interventions (p=0.55), between chronic patients who did not have or had a surgical intervention (p=0.89) and risk behaviors such as: tattoo, ear or other body piercing, injected drugs, needlestick injury at work, used somebody else`s shaving kit, used somebody else`s tooth brush or used the same toilet with a family member (p=0.16), with or without experience of being in prison (p=0.13), whereas it significantly differed among chronic patients who did not receive and received blood transfusion (p=0.039), and did not undergo hemodialysis (p=0.023). In the group of two patients who had an HCV positive family member, HCV subtype 1a was found in one patient, and HCV subtype 1b in another.

In the group of 10 patients who received blood transfusion, HCV subtype 1a was found in four, HCV subtype 1b in four, HCV subtype 3a in one, and HCV subtype 2c in one patient.

The 1a and 1b HCV subtypes were non-significantly more frequently present in patients who used dental services, compared to those who did not – 4/4 (100%); 9/10 (90%) vs 1/10 (10%), respectively.

In the group of 16 patients who had surgical interventions, HCV subtype 1b was detected in 7 (43.7%) patients.

In the group of four patients who underwent dialysis, HCV subtype 1a was found in three patients and HCV subtype 4d in one patient. HCV subtype 1a was detected in two patients who were imprisoned (Table 53).

Table 53. HCV genotypes in chronic HCV patients by risk factor

Variable		**HCV Genotype**					***p*-value**
		1**a** **n (%)**	1b **n (%)**	3a **n (%)**	**4d n (%)**	**2c n (%)**	
Family members with hepatitis C virus	Yes	1 (50)	1 (50)	0	0	0	0.13
	No	3 (17.6)	8 (47.1)	1 (5.9)	3 (17.6)	2 (11.8)	
Ever received blood transfusion	Yes	4 (40)	4 (40)	1 (10)	0	1(10)	0.039*
	No	0	6 (60)	0	3 (30)	1 (10)	
Ever utilized dental services	Yes	4 (22.2)	9 (50)	1 (5.6)	2 (11.1)	2 (11.1)	0.55
	No	0	1 (50)	0	1(50)	0	
Ever had surgical intervention	Yes	3 (18.8)	7 (43.7)	1 (6.3)	3 (18.7)	2 (12.5)	0.89
	No	1 (25)	3 (75)	0	0	0	
Ever undergone dialysis	Yes	3 (75)	0	0	1 (25)	0	0.023*
	No	1 (6.3)	10 (62.5)	1 (6.2)	2 (12.5)	2 (12.5)	
Ever had: tattoo, ear or other body piercing, injected drugs, needlestick injury at work, used somebody else`s shaving kit or	Yes	1 (11.1)	3 (33.3)	1 (11.1)	2 (22.2)	2 (22.2)	0.16
	No	7(31.8)	10 (45.5)	3 (13.6)	2 (9.1)	0	

tooth brush							
Ever been imprisoned	Yes	2 (100)	0	0	0	0	0.13
	No	2 (11.1)	10 (55.6)	1 (5.6)	3 (16.7)	2 (11.1)	

p (Fisher's exact test, two-tailed test) *p<0.05

6. Discussion

6.1 HCV infection among dialysis patients in Prishtina and overall in Kosovo

6.1.1. Anti-HCV prevalence, HCV genotypes and phylogenetic analysis

This is the first study conducted in Kosovo among patients in all seven dialysis centers of Kosovo that determined the overall prevalence of HCV infection, the presence of HCV genotypes, phylogenetic clustering of HCV viral isolates according to genotype and sequencing of HCV NS5B region, and possible association of HCV infection with demographic and high risk behaviors/activities.

Results of the study showed a high percentage of anti-HCV positive patients in dialysis centers of Kosovo, ranging from 22% at Prishtina dialysis center and going up to 91% in Gjakova. In fact, dialysis centers of Kosovo have already been facing high levels of HCV infection for many years. Data from this study show the situation in this respect has deteriorated (from 42.9% in 2008 to 53.0% in 2013; χ^2, p=0.0004) (85).

Up to two decades ago, many European countries have been facing high levels of HCV infection in hemodialysis units but a progressive reduction of blood transfusions (due to the availability of erythropoietin in the late 1980s), followed in the early 1990s by the screening of blood donors with increasingly sensitive anti-HCV tests and strict application of infection control practices, resulted in a decline of HCV seroprevalence among dialysis patients. From 1991 to 2000, anti-HCV prevalence decreased in France from 42% to 30%, in Sweden from 16% to 9%, in Italy from 28% to 16%, in Hungary from 26% to 15%, in Belgium from 13.5% to 6.8%, and also in the United Kingdom from 7% to 3% (86–88). In Croatia, HCV seroprevalence declined from 44% in 1992 (89) to 23% in 2003 (90). Studies by Atanasova et al. showed a decrease in anti-HCV

prevalence from 48% to 14.7% in a hemodialysis unit in Plovdiv, Bulgaria from 2008 to 2014 (91, 92).

The HCV infection continues to be an issue also in a number of countries neighboring Kosovo. High rates of HCV infection have been registered in Skopje, Macedonia, with anti-HCV prevalence of 32% (93), and in Bosnia and Herzegovina with 59% (94), followed by 26% in Greece (95). Somewhat lower HCV prevalence was noted at Tirana dialysis center, Albania, where anti-HCV prevalence was 16.7% (96), followed by 12.7% in Serbia (97), 7.9% in Turkey in 2011 (98), and from 2.3% to 3.4% at a number of hemodialysis centers in Croatia (99, 100).

Sequencing of specific regions of HCV genome for genotyping purposes and phylogenetic identification of isolates in clusters as a tool for assisting in identifying health care associated HCV infections have been used widely (101, 102).

This study showed that HCV subtypes 1a, 4d, 1b, and 2 were circulating throughout Kosovo dialysis centers. The most prevalent was HCV subtype 1a (61.6%), followed by subtype 4d (33.2%), 1b (3.6%), and the lowest presence of HCV genotype 2 with only 1.4%.

In all dialysis centers, including Prishtina, HCV subtype 1a was most frequent, except in Peja, at which dialysis center HCV subtype 4d was most prevalent. This distribution of HCV subtypes is specific for dialysis centers of Kosovo, since according to results of this study, HCV subtypes 1a and 3a were the most prevalent among injecting drug users in Prishtina, and HCV subtype 1b was most prevalent among chronic HCV patients and blood donors in Kosovo.

Contrary to Kosovo, most studies from the region are placing HCV subtype 1b as most frequent among dialysis patients. Among anti-HCV positive hemodialysis patients in northwest Croatia, subtype 1b has been detected in 75% of patients and genotype 3 in 20.8% of patients (103). In a study with approximately 1,000 Turkish patients undergoing dialysis in three different regions of Turkey, HCV genotype 1b was most

prevalent with 73% (104). Among 61 anti-HCV positive patients at a dialysis unit in Tirana, Albania, the predominant HCV subtype was 1b in 25 patients (50%), followed by subtypes 2c, 4a, 3a, and 1a in 18%, 14%, 8%, and 6% of cases, respectively (105). HCV subtype 1b seems to be most frequent in the Netherlands and France, whereas in Italian hemodialysis patients subtypes 2a and 3a predominate (106). HCV genotype 1 was shown to be most prevalent in hemodialysis patients (89.0%) in Macedonia (107).

Similarly to Kosovo with regard to HCV subtype 1a, in a number of studies in Brazil, HCV genotype 1 was shown to be most prevalent (46.7% – 64.9%) among hemodialysis patients, followed by genotype 3 (19.0% – 30.6%), and genotype 2 (4.6% – 5.3%). HCV genotypes 4, 5, and 6 have not been identified in those studies. Within genotype 1, subtype 1a was shown to be most prevalent (74.1%), followed by subtype 1b (11.1%) (108, 109).

The final confirmation that HCV infection among dialysis patients in Kosovo should be attributed to dialysis services was obtained through phylogenetic analysis of HCV isolates of HCV subtypes 1a, 1b, and 4d of dialysis patients. A high degree of phylogenetic clustering of HCV subtypes 1a (100%), 1b (63.6%), and 4d (96.4%) among dialysis sample sequences from Kosovo, including samples from Prishtina dialysis center, clearly indicates that many HCV infections originate within dialysis centers.

6.1.2 Contributing factors for HCV spreading in dialysis centers of Kosovo

This study tried to gain an insight into the underlining conditions that might contribute to the spread of HCV infection within dialysis centers of Kosovo. Therefore, we analyzed different factors, including duration of dialysis, use of blood transfusion and issues related to the management of dialysis units, such as staffing with a nephrologist and nurses, availability of dialysis machines, premises, anti-HCV testing, supply with consumables needed for daily work, etc.

Initially, different anti-HCV prevalences among seven dialysis units in Kosovo suggested that specific factors within dialysis centers could have impacted this situation.

Multivariate data analysis showed that the main factors contributing to anti-HCV positivity were duration of dialysis and utilizing dialysis services in more than one dialysis center in Kosovo. Also, patients who had longer history of utilization of dialysis services had higher anti-HCV prevalence, ranging from 32% for those with less than 9 years of utilization of dialysis services in Kosovo and going up to 91% for those with 20–30 years of experience on dialysis.

According to the staff at dialysis units in Kosovo, the need for transfused blood for dialysis patients in Kosovo has also decreased in recent years due to usage of erythropoietin. In addition, donated blood used in case of need is mandatorily screened for the presence of anti-HCV and HCV antigen at the National Blood Transfusion Center of Kosovo (NBTCK). Data from the NBTCK show that in the period 2011–2013 there were 73,295 blood donations, out of which only 29 (0.04%) resulted as anti-HCV positive. These data indicate that blood donation could not be taken as a contributing factor in recent HCV infections at dialysis units in Kosovo. Nevertheless, the unadjusted Poisson analysis for dialysis patients in Kosovo revealed blood transfusion as one of the factors associated with anti-HCV positivity. In addition, both unadjusted and adjusted Poisson analyses revealed blood transfusion as one of the significant factors associated with anti-HCV positivity among dialysis patients at Prishtina dialysis center. Therefore, this issue needs to be further investigated.

Exploring data on the management of dialysis centers, a number of issues could be seen as contributing to high levels of HCV infection at dialysis units in Kosovo.

Staffing is one of the issues. It is an important issue because it is directly related to the quality of service provision at dialysis units (110). It was observed that in almost all dialysis centers of Kosovo the number of nephrologists (internal disease specialists with sub-specialty in nephrology) and nurses was lower than needed in comparison to the number of patients/dialysis. Nephrologists in Gjakova, Mitrovica, Peja, and Ferizaj are not full-time engaged only at hemodialysis units but they also have to work at other internal disease units of the regional hospitals. While in Croatia the regulations for

management of dialysis units require having one doctor per 35 dialysis patients (or for 10–12 dialyses per day), and one nurse per 4 dialysis patients, in Kosovo there is one doctor on average per 43 patients (or one doctor per 20 dialyses per day) and 5.5 dialysis patients per one nurse (111). In addition, there is no infection control nurse and renal dialysis technician as working positions in either of the dialysis centers of Kosovo (112).

Additionally, sufficient working premises are lacking in dialysis centers and, consequently, in a number of them anti-HCV positive and HBsAg positive patients are located in the same room with anti-HCV and HBsAg negative patients. An exception to this situation is Prishtina dialysis center since it is currently located in the totally new premises with sufficient space and staff allocations for HBsAg and anti-HCV positive patients.

In most centers in Kosovo, new dialysis patients do not have anti-HCV test results prior to admission for dialysis. In those centers where anti-HCV results are available at the admission of patients, the quality of testing is unknown. Again, an exception is the dialysis center of Prishtina, since in the past the anti-HCV testing was provided to this center by the National Centre for Blood Transfusion, located just a few meters from Prishtina dialysis center. Furthermore, in recent years the anti-HCV testing has been provided to Prishtina dialysis center by the Department of Microbiology at the National Institute of Public Health of Kosovo, again located approximately 100 meters from the Prishtina dialysis center.

The disinfection materials were not available at all times during a daily work in some dialysis centers (e.g. Gjakova, Prizren, and Gjilan) and when they were available, their content (type and concentration) was unknown. There was no written protocol for disinfection at dialysis units. It is well known that HCV can remain viable in the environment (on equipment, clothing, etc.) for at least 16 hours (113). A more recent study found that HCV could maintain infectivity for up to 6 weeks at 4°C and 22°C (114). This means that an accidental contact with HCV-contaminated fomites and other hospital equipment could be linked with health care associated HCV infections even

after prolonged periods following their deposition. Moreover, the HCV infectivity is influenced by HCV viral titer, temperature and humidity of the environment. It is already known that commercially available antiseptics can reduce the infectivity of HCV on surfaces only when used at the recommended concentrations but not when further diluted (115, 116).

Most dialysis patients in Kosovo in this study were either unemployed or retired, meaning they are a vulnerable group of population also from economic and social aspect. The study results showed that dialysis patients did not have accurate knowledge about their anti-HCV status or they were reluctant to present verbally their serostatus to study investigators. Multivariable analysis among Prishtina dialysis patients revealed that lack of knowledge about HCV was significantly associated with anti-HCV positivity. In direct interviews with patients, we observed that dialysis patients were very much concerned about the risks of HCV infection at dialysis units but since they depended on dialysis service, they did not have sufficient 'courage' to bring this issue forward to managers of dialysis units or higher health authorities in Kosovo.

Other possible factors, such as possible transmission among family members, surgical and dental interventions, tattoos, injecting drugs, piercing, using others' shaving kits, imprisonment and having hemophilia, were not found to have played any role in the transmission of HCV at dialysis centers of Kosovo.

As for the treatment of HCV infection, only 7.4% of anti-HCV positive dialysis patients received treatment. In this group, 66.6% completed the entire course of treatment, which is still based only on administration of pegylated interferon and ribavirin. Direct acting antivirals for HCV treatment are not available through public health system in Kosovo.

6.2 HCV infection among injecting drug users in Prishtina

6.2.1 Anti-HCV prevalence, HCV genotypes and phylogenetic analysis

Almost half (48.3%) of the IDUs in Prishtina Region of Kosovo had antibodies against HCV in this study. On the other hand, a situation with HIV infection in this group is very good since there were no HIV positive cases, the presence of antibodies against *Treponema pallidum* (cause of syphilis) was very low (2%), and the prevalence of HBsAg was 8.8%.

IDUs are the group of population which is affected in high prevalence with HCV infection. This problem is especially evident in Eastern and South-Eastern Europe where the rate of injecting drug use is almost five times higher than global average (117).

HCV antibody levels among national samples of IDUs in 2011–2012 varied from 19 to 84%, among seven of the 11 European countries with national data reporting a prevalence rate over 50% (118).

According to the European Monitoring Center for Drugs and Drug Addiction, report from 2016 among European countries with national trend data (2008–2014), statistically significant ($p<0.05$) increases of HCV infection were reported in the following five countries: Greece, Latvia, Hungary, Slovenia, and Turkey (119).

High prevalence of HCV infection among IDUs is also present in the countries neighboring to Kosovo. The 2014 bio-behavioral study among IDUs in Skopje, Macedonia, found that 289 out of 401 IDUs tested had the presence of antibodies against HCV, resulting in anti-HCV prevalence of 72.1% (120). According to the Behavioral and Biological Surveillance study in Albania conducted in 2011, the prevalence of anti-HCV among 200 IDUs in Tirana was estimated to be 28.8% (121). In

Serbia, the second cycle of integrated cross-sectional bio-behavioral surveillance survey was carried in 2010 among IDUs using respondent-driven sampling methodology. A total of 571 IDUs were included in the survey (371 in Belgrade and 200 in Niš). The prevalence of hepatitis C infection was 77.4 % in Belgrade and 60.5 % in Niš (122). A Behavioral and Biological Surveillance study among IDUs in Bosnia and Herzegovina from 2012 revealed anti-HCV prevalence of 43.4% among IDUs in Sarajevo, 22.5% in Zenica, 34.9% in Banja Luka, 43.4% in Mostar, and 12% in Bijeljina, with an average of 31.2% for the entire Bosnia and Herzegovina (123). A bio-behavioral survey using respondent-driven sampling (RDS) in IDUs was conducted in Podgorica, Montenegro, in 2013. A total of 402 IDUs were recruited. According to results of that study, HIV prevalence was 1.1%, while the prevalence of HCV and HBsAg was 53% and 1.4%, respectively (124).

In addition to European countries, and Kosovo neighboring countries specifically, high prevalences of HCV infection are recorded worldwide.

A study by Li et al. conducted in a China-Myanmar border city in 2012 found anti-HCV prevalence to be 41.5% among 370 recruited IDUs. Participants with HCV infection were more likely to be HIV positive, have injected more types of drugs, have shared other injection equipment and have unprotected sex with regular sex partners (125).

A respondent-driven sampling (RDS) study was conducted during the period January–December 2013 among 14,481 IDUs in 15 cities from 11 states in India. Overall weighted anti-HCV prevalence was 37.2%. Correlates of HCV infection included higher lifetime injection frequency, HIV positivity, and a higher prevalence of persons with HIV RNA > 1,000 copies/ml in the community (126).

Among the 407 recruited IDUs in Ottawa, Canada, through a chain-referral method (behavioral-biological surveillance study), the HCV prevalence was 60.6% (127).

In addition to showing the prevalence of antibodies to HCV, the present study, for the first time in Kosovo and also in the neighboring countries including the Behavioral and

Biological Surveillance studies among IDUs, provides information on the distribution of HCV genotypes among IDUs and their clustering through phylogenetic analysis.

Almost 2/3 (64.3%) of isolated HCV genotypes in this study belonged to subtype 1a, followed by 1/3 (34.3%) belonging to subtype 3a, and only one isolate (1.4%) was identified as HCV subtype 2k. There were no other HCV genotypes isolated among IDUs in Prishtina Region of Kosovo during the study. Since these are the first data on the distribution of HCV genotypes among IDUs in Kosovo, we are not in the position to compare data with other in-country similar studies. Nevertheless, it is interesting to compare HCV genotypes distributed among IDUs with HCV genotypes among HCV positive patients at dialysis units of Kosovo in 2013, among whom also HCV subtype 1a was more present (with 2/3), followed by subtype 4d (1/3), subtype 1b (3.18%), and only one sample with genotype 2 (0.64%). There was no HCV subtype 3a isolated among dialysis patients in Kosovo. In addition, among 29 chronic HCV patients at the Infectious Disease of the University Clinical Center, Prishtina, Kosovo, in the period 2012–2013, 44.8% (13/29) had HCV subtype 1b, 27.6% (8/29) subtype 1a, and 13.8% (4/29) subtypes 3a and 4d. Among six HCV positive samples taken from blood donors in Prishtina, Kosovo, for which HCV genotype was determined, four (66.7%) had HCV subtype 1b, and the remaining two (33.3) had subtype 1a.

The study results did not find any significant difference in HCV genotype distribution among IDUs in Prishtina Region in relation to drug use and sexual practices.

Many studies worldwide on HCV genotype distribution among IDUs have shown subtypes 1a and 3a to be most prevalent.

The molecular epidemiology of HCV was studied among 108 IDUs (who also had HIV infection) from 7 European countries (Austria, Italy, the Netherlands, Scotland, Spain, Switzerland, and France) by van Asten et al. The most prevalent HCV subtypes were 1a (36%) and 3a (33%), but the percentage of genotype 4 was also relatively high, ranging from 7% in northern Europe to 24% in southern Europe (78). The distribution of HCV genotypes among IDUs in Albania showed that HCV genotype 3 was predominant

(85.7%), followed by genotype 1 (9.5%) (128). Genotype 3a was the most prevalent type among IDUs in Bulgaria (129). Among IDUs in Hungary, HCV genotype 1 was most prevalent with 74.2%, followed by subtype 3a with 22.7%, and genotype 4 with 3%. This genotype distribution among IDUs was different from the one from general population in which HCV subtype 1b is more prevalent and subtype 3a is rarely seen (130). The molecular epidemiology study of HCV isolates in Lisbon, Portugal, obtained from 124 individuals with HCV presence belonging to IDUs showed that the most prevalent subtypes were 1a and 3a; however, genotype 4 viruses (subtypes 4a and 4d) were present among 24.6% of IDUs (131).

HCV subtype 1a was most prevalent among 407 IDUs of Ottawa, Canada, with 65.9%, followed by subtype 3a (22.5%), 1b and 2b (both with 4.9%), and subtype 2a with 1.6% (127).

As part of a comprehensive health care program for people who use drugs in Nepal, among 401 IDUs in three regions of Nepal, HCV genotypes were determined in 164 of the 168 HCV-RNA positive participants. Out of this number, 66 had HCV genotype 1 (40.2%) and 98 had genotype 3 (59.8%) (132).

The most prevalent HCV genotypes among IDUs in Croatia are subtype 3a (60.5%) and 1a (23.7%) (133).

Phylogenetic analysis of HCV subtypes 1a and 3a obtained from IDUs in Prishtina Region has revealed significant clustering of samples. High degree phylogenetic clustering of HCV subtypes 1a (66.7%) and 3a (71.4%) isolates among IDUs in Prishtina Region indicates that many HCV infections originate within networks of this group. The fact that this study was implemented using respondent-driven sampling could also have contributed to this high percentage of HCV clustering.

Since we have not been in the position to find similar HCV phylogenetic clustering information from respondent-driven sampling in the region, a comparison with similar studies worldwide has been made.

In a study by Jacka et al., among 501 IDUs with HCV subtypes 1a and 3a in Vancouver, Canada, 31% (n=156) were in pairs/clusters. This was a longstanding longitudinal prospective study covering the period 1996–2012. Factors independently associated with phylogenetic clustering included: age <40, human immunodeficiency virus (HIV) infection, HCV seroconversion, and recent syringe borrowing (134).

A study by Pilon et al., among 407 recruited IDUs in Ottawa, Canada, found that 38% of HCV sequences obtained were associated with clusters. According to the same study, the most susceptible IDUs (most at risk by behavior) would have been exposed and infected very early in their injecting history leading to the relatively low level of HCV phylogenetic clustering observed in the study (124).

According to a longitudinal study by Sacks-Davis et al., with data collected between July 2005 and August 2008 in Melbourne, Australia, newly acquired infections (primary and reinfection) and new viral strains are more than twice as likely to be in phylogenetic clusters than infections present at enrolment that are not classified as newly acquired (135).

It needs to be emphasized that the goal of phylogenetic analysis of HCV isolates from IDUs in Prishtina Region of Kosovo was not to analyze factors associated with clustering. However, this component of analysis is recommended to be taken into consideration in further biological and behavioral surveillance studies with IDUs in Kosovo.

The single nucleotide polymorphism (SNP) located on chromosome 19, rs12979860, located 3 kb upstream of interleukin 28B (IL28B) gene encoding interferon-λ3, has been found to be strongly associated with treatment-induced viral clearance in HCV infected patients with HCV genotype 1, and has also been found to be associated with a three-fold increase in spontaneous clearance of HCV infection independent of ethnic/racial background (136–137).

In this study, we found that among anti-HCV positive IDUs who were HCV RNA negative, the CC IL28B polymorphism was present among 58.6% of them and was higher than CT and TT genotype. Among anti-HCV positive IDUs with the presence of HCV RNA, the CC IL28B was also higher (43.5%) compared to CT or TT. This frequency is in line with the European pattern of this allele distribution (138). However, differences in the prevalence of IL28B polymorphism among anti-HCV positive IDUs in Prishtina, Kosovo, were not significant, being either HCV RNA positive or negative, and between the ones infected with either HCV subtypes 1a or 3a.

6.2.2 Factors contributing to HCV spreading among IDUs

Results of this study showed that IDUs of older age had the highest anti-HCV prevalence. This is probably due to the fact that older IDUs were injecting drugs for a longer period of time and had a higher frequency of drug injection; both of those drug use practices were shown to be significant factors for anti-HCV positivity. On the other hand, the lowest (24.3%) anti-HCV prevalence was observed among younger IDUs, which could be seen as a window of opportunity for prevention of HCV spreading among younger IDUs if harm reduction activities are to be implemented. In the BIOBSS study on IDUs conducted in Skopje, Macedonia, just like in the Kosovo study, specific prevalence by age groups indicated that the risk of HCV infection increases with age, where in those aged 18–24 years, the prevalence of HCV was 47.1% compared to the age group of 35–39 where the prevalence was 83.6%, and the age group of 40–49 years, where the prevalence was 85.9% (120). Similar findings were found also in Podgorica, in the Montenegro study with IDUs, where HCV prevalence was 31.5% in those younger than 25 years of age and 57.4% among those older than 26 years (124).

In addition to age and duration of drug use, IDUs with lower level of education and those ever imprisoned in their lifetime had more chances to be HCV infected. Having lower level of education means also limited opportunities for employment for this group of IDUs, and if we add to this a high unemployment rate among general population of

Kosovo, it makes this situation even worse for IDUs. It is well known that sharing drug injecting equipment, and the associated social and economic situation, are factors that contribute to HCV transmission among IDUs (139-141).

The issue of imprisonment and HCV infection has to be further investigated in order to gain more information if this is related to drug use inside the prison system or increased possibilities for imprisonment due to longer drug use and out of law behavior related to drug use.

Study results highlighted the importance the so-called 'shooting galleries' have on the transmission of HCV infection among IDUs. Anti-HCV positive IDUs who were injecting drugs in shooting galleries or other places where IDUs gather were statistically significantly more numerous than HCV negative IDUs. It is well known that contaminated needles are not the only vehicle for blood-borne HCV transmission; the virus remains infective in a liquid, syringes, and on inanimate surfaces for weeks (142, 143). Therefore, injecting at shooting galleries is often associated with an increased risk of infection by blood-borne pathogens (144–146).

Results of this study showed that almost all IDUs reported using a sterile needle/syringe for drug injection. On the other hand, 92.6% of IDUs reported that somebody else used their needle/syringe. This is an interesting finding since 79.9% of IDUs reported that they always (in 100% of drug injections) used a sterile needle/syringe. This could imply that the study missed most IDUs injecting drugs with already used needles/syringes or it could indicate that IDUs participating in the study were not giving true information. The latter could have some rationale based on the fact that most study participants used harm reduction services from the NGO Labyrinth, staff of which organization conducted the interviews, and IDUs would not like to be seen in front of them as they are not following their instructions. Compared to other similar studies in which needle/syringe sharing has been associated with a higher risk for HCV infection, in the present study this type of drug use pattern did not show any significant difference between anti-HCV positive and anti-HCV negative IDUs. For example, in a study by Hagan et al., any syringe sharing has been associated with a 3-fold higher risk of HCV

infection (145). Needle sharing frequency has been the most important risk factor for hepatitis C among IDUs in Croatia. The HCV seroprevalence rate has ranged from 27.3% in IDUs who answered that they shared needles occasionally to 100% in IDUs who always shared needles ($p<0.001$) (147).

Almost all IDUs reported buying sterile needles/syringes from pharmacies and 2/3 of IDUs reported receiving sterile/needles from NGOs, this last figure being significantly higher than observed for anti-HCV positive IDUs. In Tallinn, Estonia, those IDUs using pharmacies as the main source of sterile needles were shown to have lower odds for being infected with HCV, and pharmacy users were more likely to have a regular or temporary employment, and were also more likely to have health insurance compared to IDUs using NGOs needle/syringe exchange program (148). Considering the fact that 70% of IDUs in the present study were unemployed, buying sterile needles/syringes for this group of people is a real challenge; therefore, a needle/syringe exchange program implemented through organizations working on harm reduction is highly important. In fact, the needle/syringe exchange program and opioid substitution treatment (OST) should be the core interventions for prevention and control of infectious diseases among IDUs.

Unfortunately, the harm reduction program for IDUs in Kosovo is limited in its experience and scope of coverage. The needle and syringe exchange program was initiated in 2009 through a local non-governmental organization Labyrinth by drop-in center in Prishtina, followed by two other similar centers in Prizren (second largest region in Kosovo) and Gjilan. In 2010, approximately 45,000 needles/syringes were distributed by the Labyrinth (149). The average number of needles/syringes distributed yearly per injecting drug user through a specialized program was five in the period 2011/2012. On the other hand, coverage with opioid substitution treatment (with methadone) was only 2.1% of IDUs in 2012, when OST was initiated (150).

If we wanted to estimate the number of needed sterile needles/syringes for IDUs, this could be calculated based on the number of IDUs in Kosovo (which is approx. 3,000–5,000) and the number of injections per specific period of time. Based on the data from

the present study, approximately half of the IDUs injected drugs at a frequency once a day. If we take the number of 3,000 IDUs living in Kosovo (which is the lowest estimated number), then we arrive at the number of 547,500 needles/syringes needed for one year in case we wanted to cover 100% injections with sterile needles/syringes. The average frequency of drug injections for the second half of IDUs in this study was four times per month; therefore, similar calculations as above would give us a number of 72,000 needles/syringes. The total estimated number of needed sterile needles/syringes, in order to be always used during each injection of drugs among the lowest estimated number of IDUs in Kosovo, per one year, would be 619,500.

Unfortunately, there is no treatment program for HCV infected IDUs in Kosovo at this moment; therefore, there are no data on the relationship between IL28B polymorphism among IDUs and their treatment outcome. Implementing the HCV treatment program for HCV infected IDUs in Kosovo would be a highly challenging activity considering the limited financial resources within the health system of Kosovo and the issues related to IDUs, such as socio-economic and living conditions, stigmatization and marginalization.

Nevertheless, some studies suggest that treatment of chronic HCV infection in the current and former IDUs could be successful, and additional benefits of such a treatment delivered in a community-based, multidisciplinary, primary care model may extend beyond narrowly defined virological outcomes (151).

Some HCV treatment programs among IDUs have shown that the HCV treatment is not associated with drug use or used needle and syringe borrowing during follow-up, but is associated with decreased ancillary injecting equipment sharing (152).

6.3 HCV infection among chronic patients and blood donors in Prishtina

6.3.1 HCV genotypes and phylogenetic analysis among chronic HCV patients and blood donors

Results of this study showed that the most prevalent HCV genotype among chronic HCV patients in Kosovo was subtype 1b with 41.9%, followed by subtype 1a with 25.8%, and by subtypes 3a and 4d with 12.9% each. Similarly, HCV subtype 1b was most prevalent among blood donors with 66.6%, followed by subtype 1a with 33.3%. This could indicate that, among general population infected with HCV in Kosovo who do not belong to dialysis patients or drug users, the most common is HCV genotype 1 (67.7% among HCV chronic patients and 100% among blood donors), with HCV subtype 1b being more present. To this contributes also the fact that among chronic HCV patients from Infectious Disease Clinic, University Clinical Center of Kosovo, at the time of this study implementation, there was a small portion of dialysis patients and IDUs. This finding is similar to the HCV genotype distribution in Europe, estimated to have predominant HCV genotype 1 (64.4%), followed by genotype 3 (25.5%), genotype 2 (5.5%), and genotype 4 (3.7%) (153).

Through cladogram phylogenetic trees of HCV subtypes 1a, 1b, 3a and 4d, it was possible to notice that most sample sequences from the study chronic HCV patients and blood donors were not clustered with each other, with a few minor exceptions of limited number of samples included in clusters with dialysis patients or IDUs, which could indicate that those chronic HCV patients were either dialysis patients or IDUs.

6.3.2 Factors contributing to HCV spreading

Since most chronic HCV patients denied having any risk factor attributable to HCV infection (excluding ear piercing, which is very common among females in Kosovo), were not utilizing dialysis services, or did not have any family members living with

HCV, then it could be speculated that most probably they could have been infected through medical interventions such as surgical or dental services, since 71–85% of chronic HCV patients used those services.

The HCV treatment program with pegylated interferon and ribavirin started in Kosovo in 2011, with very limited number of HCV patients enrolled per year. In fact, for many years, worldwide, pegylated interferon and ribavirin have been the main drugs for treatment of HCV infection. Through administration of pegylated interferon-alpha combined with ribavirin in the treatment of chronic hepatitis C among patients with genotypes 1 up to 46% of patients' experienced sustained virological response, and those infected with HCV genotype 2 and 3 experienced even higher responses, with rates in excess of 80% (154).

The HCV treatment program at the Infectious Disease Clinic, UCCK, Prishtina, is funded by the public funds of Kosovo and is free of charge. Unfortunately, there are no published data on treatment success or HCV genotype distribution among this group of patients in Kosovo. Results of this study showed that the CC genotype was present among 38% of chronic HCV patients in Kosovo. Unfortunately, during the study, we did not have the opportunity to cross-check the presence of CC genotype among chronic HCV patients with treatment induced viral clearance or spontaneous clearance of HCV infection due to lack of data from the Infectious Disease Clinic, UCCK. Furthermore, due to insufficient volume of samples (and due to frequent handling of samples), we were not in the position to measure the viral load of those patients enrolled in the study.

DAAs are not available yet through public HCV treatment program in Kosovo (not part of essential list of drugs of public health system) due to high costs of these drugs and a limited budget for pharmaceuticals within the budget of the Ministry of Health.

7. Conclusion

This study, for the first time in Kosovo, provides comprehensive data on HCV prevalence among dialysis patients and IDUs, combined with HCV genotype distribution among these groups of population, adding genotype information of chronic HCV patients and blood donors, and their genetic relatedness of HCV isolates through phylogenetic analyses and socio-demographic data.

The study reveals that most prevalent HCV genotype among all studied groups (dialysis patients in Prishtina and overall in Kosovo, IDUs, chronic HCV patients and blood donors) is genotype 1, with subtype 1a being most prevalent among dialysis patients and IDUs, and subtype 1b being most prevalent among chronic HCV patients and blood donors. HCV subtype 3a is the second most prevalent among IDUs and it is not found among dialysis patients or blood donors. The subtype 4d is the second most prevalent among dialysis patients in Prishtina and overall in Kosovo, and this subtype is not found among IDUs or blood donors.

The phylogenetic trees of HCV subtypes 1a, 1b, 3a, and 4d developed from sample sequences of studied groups have provided a significant number of phylogenetic clusters, which are mainly related to specific dialysis centers or drug injection.

7.1 Hemodialysis

This study highlights high levels of HCV infection at dialysis units in Prishtina and overall of Kosovo. Different percentages of anti-HCV prevalence and specific HCV subtype distribution in regional dialysis centers of Kosovo, genetic relatedness of HCV isolates presented in phylogenetic clusters according to subtype and dialysis centers, association of HCV infection with age of dialysis patients, duration of dialysis, change

of dialysis centers and blood transfusion, are strong indicators of large HCV transmission at dialysis centers of Kosovo.

Prishtina dialysis center is the largest dialysis center in Kosovo, and at the same time has the lowest prevalence (22%) of anti-HCV positivity among its patients. This situation is probably due to the fact that this dialysis center has larger premises, with structural division of these premises and staff specifically for HbsAg and anti-HCV positive patients. Furthermore, being located inside the capital of the country and being part of the University Clinical Center of Kosovo, it has possibilities to have better access to decision makers, financial and human resources as well as medical and laboratory services.

HCV subtypes 1a and 4d are most prevalent among dialysis patients in Prishtina, with subtype 1a being very slightly higher. Longer history of dialysis is also one of the main factors associated with anti-HCV positivity, similar to all dialysis patients in Kosovo; nevertheless, blood transfusion, age and having less knowledge about HCV have been shown as factors associated with anti-HCV positivity among dialysis patients in Prishtina.

High level of anti-HCV prevalence among dialysis patients in Kosovo in this study is an indicator that factors that have impacted decrease of HCV infection at dialysis units in Europe and other developed countries in the 1990s have not been implemented entirely and/or properly even to date. In contrast to most European countries that have achieved to decrease infection with HCV at dialysis units over the past 20–25 years, Kosovo has failed in this endeavor. Infection control practices are very much limited at dialysis units of Kosovo, the staff, especially nurses, are not educated on infection prevention practices. There is lack of laboratory support with regard to HCV testing of patients undergoing dialysis, and the one which is provided is not offered at the time needed or lacks information on quality control. Except for Prishtina, other dialysis units are suffering from lack of sufficient premises and continual supply with daily consumables that would be needed for infection prevention. Lack of sufficient number of nephrologists, especially in Mitrovica, Gjakova, Ferizaj and Peja, is another important

pitfall in this system. Many dialysis patients are not aware of their anti-HCV status and, generally, of their rights as patients. Currently, there is a very small percentage of HCV infected dialysis patients within the treatment program, which is provided for a limited number of people free of charge by the Ministry of Health.

This situation makes as imperative the development and implementation of a special HCV prevention and treatment program for dialysis patients in Kosovo. In order to initiate the HCV prevention program at dialysis units of Kosovo, including Prishtina dialysis center, the following steps could be recommended to be undertaken by the Ministry of Health, Hospital and University Service of Kosovo (University Clinical Center of Kosovo in Prishtina and Regional Hospital where dialysis centers are placed):

- Infection control practices should be established and strongly implemented in all dialysis centers of Kosovo; dialysis staff has to be trained on the appropriate infection control practices; sufficient quantities of needed consumables should be made available at all times at dialysis units;
- Regular and timely screening for the presence of antibodies against HCV should be available at all times and in all regions of Kosovo. In addition, qualitative RT-PCR testing should be introduced in order to detect HCV infected cases in which anti-HCV testing would result in a negative test result due to initial stage of infection;
- Sufficient number of health staff should be employed, especially specialists in nephrology in Gjakova, Mitrovica, Ferizaj and Peja;
- There should be infrastructural investments by the government in building appropriate and sufficient hemodialysis premises in Kosovo;
- In addition, the possibility of introducing peritoneal dialysis should be investigated;
- Special attention should be given to education of dialysis patients on HCV infection prevention and their rights as patients;
- The HCV treatment program of Kosovo should expand and include HCV positive dialysis patients within the program;

- The blood transfusion service should screen donated blood with qualitative molecular tests for HCV, replacing current serological testing based on antigen/antibody detection, in order to prevent possible transmission of HCV to dialysis patients through blood transfusion.

Implementation of these recommendations would require the development of a more detailed action plan, with specific timeframe, people to be engaged, and sufficient budget. Even in the best case scenario of implementation of above mentioned recommendations, it would take considerable time to improve the situation. Nevertheless, Kosovo should address this serious health problem, which in fact should be one of the main priorities of the health system in Kosovo. Further delays would result in additional HCV infections in dialysis patients, increased morbidity and mortality among dialysis patients, and increased HCV transmission risk.

7.2. Injecting drug users

Almost half of the IDUs in Prishtina Region of Kosovo are infected with HCV. This prevalence is within the estimated global HCV prevalence among IDUs. Age of IDUs, having longer history of drug injection, lower level of education, drug injection in so-called ‘shooting galleries’, and ever being in prison are shown to be the main predictors of HCV infection among IDUs in Prishtina study.

While HCV subtype 1a is present also among other groups of population with HCV infection in Kosovo, genotype 3a is currently associated only with drug use. High percentages of HCV isolates from IDUs belonging to either HCV subtypes 1a or 3a have shown phylogenetic clustering presenting information on HCV transmission within the community of IDUs who live/inject drugs in Prishtina.

Among IDUs who are anti-HCV positive and HCV RNA negative, almost 2/3 have presented with IL28B polymorphism CC. In addition, less than half of anti-HCV positive IDUs with HCV RNA presence have CC genotype of IL28B polymorphism, which in case of inclusion in the current HCV treatment program (with pegylated

interferferon and ribavirin) could lead to rapid and sustained virological response and impact decreased possibilities of HCV transmission.

In order to prevent further spreading of HCV infection among IDUs in Prishtina and overall in Kosovo, there is a need for implementation of a harm reduction program, with sufficient quality and scope of coverage, which should include needle exchange program, methadone maintenance program and peer education through outreach work. Since 'shooting galleries' have been shown as one of the sites that contribute to the spread of HCV infection, these locations should be targeted as a priority. There should be a special focus of HCV prevention activities among younger IDUs, since they have a lower rate of HCV infection compared to older groups of IDUs, and this could present a window of opportunity. The treatment of HCV infection among IDUs in Kosovo is an activity that should be taken into consideration since, in addition to possible health benefits from virus clearance, it is also one of the main components of prevention of HCV spreading.

7.3 Chronic HCV patients and blood donors

The HCV treatment program within the public health system provides treatment to a low number of chronic HCV patients due to limited budget. Current treatment protocols are based only on pegylated interferon and ribavirin, since DAAs are not available through public health funds. Different to dialysis patients and IDUs, HCV subtype 1b is shown to be most present among chronic HCV patients and blood donors, providing the basis for possibilities of having this genotype more present among general population. Since most chronic HCV patients and blood donors deny having any high risk behavior that could result in HCV infection, the most possible route of infection could be considered to be a medical intervention (mainly dental or surgical). This situation brings to attention once again the importance of strict adherence to aseptic techniques in the provision of medical services that could pose a risk in transmission of blood-borne pathogens.

The Kosovo health system should include into the HCV treatment program the

availability of DAAs and should expand in scope of coverage with inclusion of a larger number of chronic HCV patients, anti-HCV positive dialysis patients and IDUs.

The WHO Global Health Sector Strategy on viral hepatitis 2016–2021 aims to contribute to elimination of viral hepatitis (including HCV) as a major public health issue by 2030 through 90% reduction of new infections, 100% of blood donations screened in quality assured manner, 90% of injections administered safely, 300 sterile needles and syringes provided to each IDU per year, 90% of diagnosed HCV infections and 80% of HCV infections treated. The Kosovo health system needs to make tremendous efforts in achieving these targets. Nevertheless, with higher commitment of health staff, sufficient support from key governmental stakeholders, and involvement of positive HCV patients through non-governmental organizations, this situation could be improved with activities primarily targeting dialysis patients and IDUs.

8. Abstract in Croatian

Molekularna epidemiologija HCV-a u Regiji Priština na Kosovu, Xhevat Jakupi, 2018

Cilj: Utvrditi genetsku raznolikost HCV-a među različitim skupinama populacije u Prištini, na Kosovu.

Metode: Učinjena je genotipizacija i sekvenciranje HCV-a na anti-HCV pozitivnim uzorcima krvi bolesnika na hemodijalizi, korisnicima droga injektiranjem, darivatelja krvi i kroničnih pacijenata zaraženih virusom hepatitisa C. Za bolje razumijevanje čimbenika povezanih s HCV infekcijom, korišteni su intervjui kod svih ispitivanih skupina.

Rezultati: Pozitivni anti-HCV nalazi dobiveni su kod 22% bolesnika na hemodijalizi u Prištini, kao prosjek u 53% bolesnika u centrima za hemodijalizu na Kosovu, kao i kod 48% korisnika droga injektiranjem. HCV genotip 1a bio je najzastupljeniji kod bolesnika na hemodijalizi u Prištini (47%) i sveukupno na Kosovu (62%) te među korisnicima droga injektiranjem (64%), dok je genotip 1b bio najzastupljeniji među kroničnim bolesnicima zaraženim virusom hepatitisa C (42%) i darivateljima krvi (67%). Drugi po redu najzastupljeniji genotipovi bili su 3a među korisnicima droga injektiranjem (34%) te 4d među bolesnicima na hemodijalizi u Prištini (44%) te sveukupno u centrima za hemodijalizu na Kosovu (33%). Filogenetska stabla HCV genotipova 1a, 1b, 3a i 4d pokazala su filogenetsko grupiranje uzoraka u klastere s obzirom na centre za hemodijalizu i korištenje droga. Dužina perioda na hemodijalizi i dobivanje hemodijalize u različitim centrima uočeni su kao čimbenici koji su povezani s anti-HCV pozitivnim nalazima među bolesnicima na hemodijalizi na Kosovu, dok su specifično za Prištinu, pored već spomenutih, ti čimbenici uključivali i transfuziju krvi, dob i znanje o HCV-u među bolesnicima na hemodijalizi, a među korisnicima droga injektiranjem to su bili dob, trajanje korištenja droga ubrizgivanjem, niža razina obrazovanja, ubrizgavanje droga u nadziranim prostorima (tzv. 'shooting galleries') te boravak u zatvoru.

Zaključak: Na Kosovu postoji visoka razina HCV infekcija u centru za hemodijalizu u Prištini i općenito u centrima za hemodijalizu na Kosovu te kod korisnika droga injektiranjem, a najzastupljeniji je HCV genotip 1a. Zbog ovakve situacije ponovno se naglašava potreba za strogim pridržavanjem mjera za suzbijanje HCV infekcije u centrima za hemodijalizu te provedbu aktivnosti za smanjivanje štete kod korisnika droga injektiranjem.
Ključne riječi: HCV, prevalencija, genotip, hemodijaliza, korisnici droga injektiranjem.

9. Abstract in English

Molecular epidemiology of HCV in Prishtina region of Kosovo, Xhevat Jakupi, 2018

Aim: To determine genetic diversity of HCV among different population groups in Prishtina, Kosovo.

Methods: Anti-HCV positive samples of dialysis patients, IDUs, blood donors, and chronic HCV patients were HCV genotyped and sequenced. To better understand the underlying conditions of HCV infection, interviews were used with all groups.

Results: Anti-HCV positive findings were observed in 22% of dialysis patients in Prishtina, and as average among 53% of patients at dialysis centers of Kosovo, as well as in 48% of IDUs. HCV subtype 1a was most prevalent among dialysis patients in Prishtina (47%) and overall in Kosovo (62%), and among IDUs (64%), whereas genotype 1b was most prevalent among chronic HCV patients (42%) and blood donors (67%). The second most prevalent genotypes were 3a among IDUs (34%) and 4d among dialysis patients in Prishtina (44%) and overall at dialysis centers of Kosovo (33%). Phylogenetic trees of HCV subtypes 1a, 1b, 3a, and 4d showed phylogenetic clusters of samples related to dialysis centers or drug use. Duration of dialysis and receiving dialysis in different centers were factors associated with anti-HCV positivity among Kosovo dialysis patients, whereas specifically for Prishtina, in addition to the above, those factors included blood transfusion, age and knowledge about HCV among dialysis patients, while among IDUs those were age, duration of drug injection, lower educational level, drug injection in shooting galleries, and being in prison.

Conclusion: High levels of HCV infection at dialysis center in Prishtina and overall at Kosovo dialysis centers and among IDUs are present in Kosovo, with HCV subtype 1a being most prevalent. This situation reiterates the need for strict adherence to infection control practices at dialysis centers and implementation of harm reduction activities among IDUs.

Keywords: HCV, prevalence, genotype, dialysis, IDUs.

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11. Brief curriculum vitae

Xhevat Jakupi was born in 1973 in Prishtina, Kosovo. He graduated from the Medical Faculty, University of Prishtina in 1999. Until 2002, he worked at the WHO Liason Office in Prishtina on HIV/AIDS Program. In 2002, he was appointed a teaching assistant for the Medical Microbiology course of the Medical Faculty, University of Prishtina. He completed specialization in Microbiology in 2006, and gained Master degree in 2008 at the Medical Faculty, University of Prishtina. In 2009, he was employed as a microbiologist at the National Institute of Public Health of Kosovo. Currently he is working as the Director of the Department of Microbiology, at the National Institute of Public Health of Kosovo, the position held since 2010.

During the years of work at the National Institute of Public Health of Kosovo, he established and made functional a laboratory for molecular diagnostics of important public health pathogens, including testing for influenza, Crimean Congo hemorrhagic fever, and hemorrhagic fever with renal syndrome. He also increased laboratory capacities for HIV, HBV and HCV testing.