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Incidence of HIV, HBV and HCV infections detected by PCR method in BiH

Đemo Subašić,[1] Irma Salimović,[1] Rijad Konjhodžić,[1] Amira Pekuša-Redžić[2] and Mensura Šeremet[1]

Apstract.

- Direct monitoring by sensitive nucleic-acid tests would provide data accurately to measure the risk and to assess risk-reduction procedures. We adapted PCR method for investigation the incidence of HIV, hepatitis C virus and hepatitis B virus infections during last two (HBV, HCV) or three years (HIV) in Bosnia and Herzegovina.
- For this PCR testing individual plasma samples were analysed. Virus deoxyribonucleic acids were extracted by QIAamp nucleic acids extraction method. The HCV ribonucleic acid was extracted by High Pure Viral RNA Kit (Roche) also from plasma samples. We detected integrated form of HIV and HBV-viral DNA but in the case of HCV it was RNA from free viral particles present in the blood of patient. For HCV detection an additional reverse transcription step before PCR amplifications was performed. Amplified virus-specific sequences by specific complementary primers were detected by agarose-gel electrophoresis.
- Using this validated methodology routine we checked 184 persons on HIV infection from July 1998. up to the end of 2000. 8 (4.4%) of them were HIV-RNA positive. One of positive persons was a patient from Clinic of Infectious Diseases, other 7 HIV-RNA positive (6 injection drug users and 1 from promiscuity-risk group) were tested on their personal request. During 1999. and 2000 we performed 54 tests for identification HBV infected individuals and found 11 (20.4%) HBV positive persons. 7 of them were patients from Clinic of Gastroenterohaepathology, 1 from Paediatric Clinic and 3 from external medical institution. The HCV tests for the same period included 148 tested persons. 9 (6.1%) of them were HCV-RNA positive. It was 1 patient from Clinic of Infectious Diseases, 1 patient from Institute of Nephrology, 5 patients from Clinic of Gastroenterohaepathology and other 2 patients were from external medical institution. None of PCR-tested persons had HBV/HCV or other dual positivism.
- HIV, HBV and HCV have been the viruses most intensively subjected to PCR analysis. It has been necessary to have an overview of incidence of infectious diseases caused by these viruses for a transition-state country such as BiH. In

[1] Institute for Microbiology, Immunology and Parazitology, Clinical Centre University of Sarajevo, Bosnia and Herzegovina

[2] Medical faculty University of Sarajevo, Bosnia and Herzegovina

consideration of number of our population there is relatively high percentage of HIV, HCV and especially HBV positivism. It would be necessary to realize better transmission control preventing infections by these viruses in BiH. Those are the results of PCR diagnostics without serological investigations.

Introduction

HIV is a retrovirus, a member of the *Lentivirinae* subfamily, which have genes composed of *ribonucleic acid (RNA) molecules.* The outer shell of the virus is known as the *viral envelope.* Embedded in the viral envelope is a complex protein known as env, which consists of an outer protruding cap *glycoprotein* (gp) *120*, and a stem *gp41.* Within the viral envelope is an HIV protein called *p17* (matrix), and within this is the viral core or capsid, which is made of another viral protein *p24* (core antigen). The major elements contained within the viral core are two single strands of HIV RNA, a protein *p7* (nucleocapsid), and three enzyme proteins, *p51* (reverse transcriptase), *p11* (protease) and *p32* (integrase).

Retroviruses, like all viruses, can only replicate within a living host cell because they contain only RNA and they do not contain DNA. In addition, retroviruses use RNA as a template to make DNA. Infection begins when an HIV particle encounters a cell with a surface molecule called CD4. The virus particle uses gp120 to attach itself to the cell membrane and then enters the cell. Within the cell the virus particle releases its RNA, and the enzyme *reverse transcriptase* then converts the viral RNA into DNA. This new HIV DNA then moves into the cell's nucleus where with the help of the enzyme *integrase* it is then inserted into the host cells DNA. Once it is in the cell's genes HIV DNA is called a provirus (Singleton et al., 1993). The HIV provirus is then replicated by the host cell, which can then release new infectious virus particles.

The integrated provirus remains associated with the cellular chromosomal DNA for the life of the infected cell. Further, the integrated provirus can either actively transcribe the genes for the structural proteins of the virus, or, by selective transcription of only the complex array of viral regulatory genes, remain transcriptionally constrained and thereby not release viral particles. The latter condition is frequently referred to as the "latent state" (Cohen et al., 1994). Because proviral DNA is present regardless of the transcriptional state of the cell, early efforts targeted to direct detection of the virus used proviral DNA as template.

Viral hepatitis is a systematic disease primarily involving the liver. HBV, the cause of serum hepatitis, is classified as a *hepadnavirus* (Brooks et al., 1998). HBV establishes chronic infections, especially in those infected as infants; it is a major factor in the eventual development of liver disease and

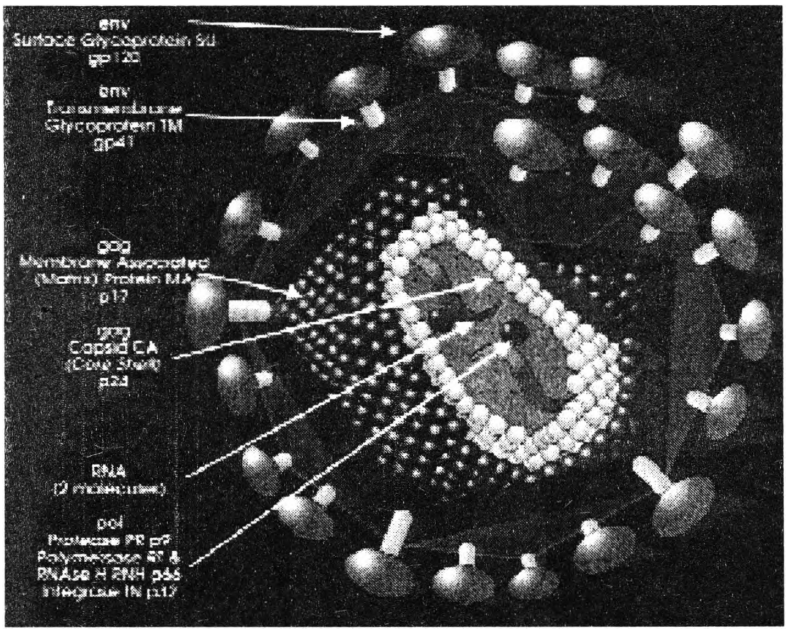

Figure 1. *Complete **HIV** viral particle (structure and components)*

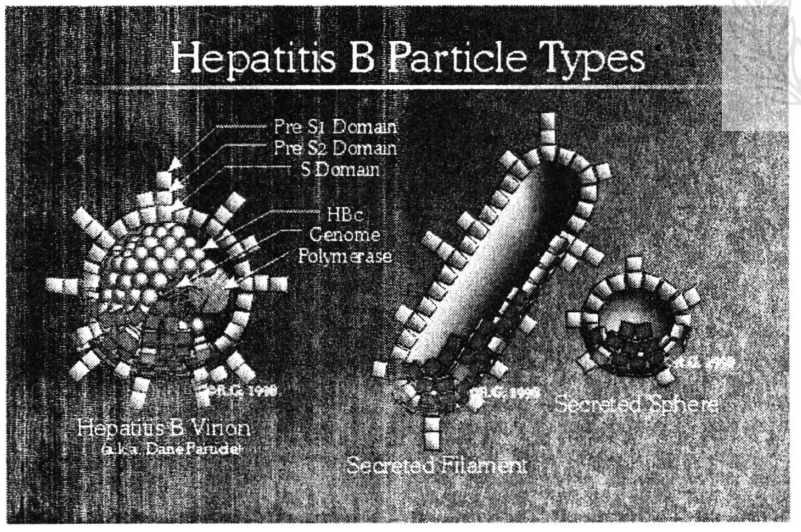

Figure 2. *Hepatitis **B** particle types (Hepatitis **B** virion, secreted filament and secreted sphere)*

hepatocellular carcinoma in those individuals. The viral genome consists of partially double-stranded circular *DNA*, 3200 bp in length. Different HBV isolates share 90-98% nucleotide sequence homology. In ca. 5-10% of individuals infected with HBV, HBsAg persists in the circulation for >6 months, and a carrier state is established which may persist for life. The carrier state may be asymptomatic (latent infection) or symptomatic (with e.g. chronic active hepatitis, cirhosis, and/or HCC-human hepatocellular carcinoma), and is most likely to develop in e.g. perinatally-infected or immunodeficient individuals. In HBV carriers, viral DNA may integrate at one or (apparently random) sites in the host cell genome; the integrated sequences may be complete genomes or subgenomic fragments. Integrated viral DNA may continue to be expressed, resulting in the continued synthesis of e.g. HBsAg; however, in HCC cells virus replication and gene expression appear generally to be absent. Integrated HBV-DNA has been found in the majority of HCC cells examined. The full-length DNA minus strand is complementary to all HBV mRNAs; the positive strand is variable and between 50 and 80% of unit length.

Replication of Hepatitis B Virus: HBV attachement to a receptor on the surface of hepatocytes occurs via a portion of the pre-S region of HBsAg. After uncoating of the virus, unidentified cellular enzymes convert the partially double-stranded viral DNA to *cccDNA (covalently closed circular double-stranded DNA*, in the nucleus. The cccDNA serves as template for the production of all HBV transcripts, including a 3.5-kb pregenome RNA. The pregenome RNA becomes encapsidated by ta packaging signal located near the 5' end of the RNA into newly synthesized core particles, where it serves as template for the HBV *reverse transcriptase* encoded within the polymerase gene. An Rnase H activity of the polymerase removes the RNA template as the negative-strand DNA is being synthesized. Positive-strand DNA synthesis does not proceed to completion within the core, resulting in replicative intermediates consisting of full-length minus-strand DNA plus variable length (20-80%) positive-strand DNA. Core particles containing these DNA replicative intermediates bud from pre-Golgi membranes (acquiring HBsAg in the process) and may either exit the cell or reenter the intracellular infection cycle.

HCV is a positive-stranded *RNA* virus, classified as a *flavivirus*. The genome is 9.5 kb in size and encodes a core protein, two envelope glycoproteins, and several nonstructural proteins (e.g., Ser protease/helicase, RNA-dependent RNA polymerase). The expression of cDNA clones of HCV in yeast led to the development of serologic tests for antibodies to HCV. Most new infections with HCV are subclinical. Over 50% of HCV patients develop chronic hepatitis, and many are at risk of progressing to cirhosis. In some countries, as in Japan, HCV infection often leads to hepatocellular carcinoma.

About 25,000 individuals die annually of chronic liver disease and cirhosis in the USA; HCV appears to be a major contributor to this burden.

HCV displays genomic diversity, with different genotypes predominating in different parts of the world. The virus undergoes sequence variation during chronic infections. This genetic diversity is not correlated with differences in clinical disease.

The diagnosis of HCV infection can be made by qualitatively detecting HCV RNA using gene amplification techniques (e.g., RT-PCR) in serum or plasma of patient within 1-2 weeks after exposure to the virus and weeks before the onset of alanine aminotransferase (ALT) elevations or the appearance of anti-HCV.

Material and methods

I Extraction of genomic DNA (integrated form of viral DNA) from plasma or serum sample - (QIAGEN-QIAamp Blood Mini Kit) - HIV, HBV integrated form

1. 200 μl sample is added to the 20 μl QIAGEN Protease (or Proteinase K) into the 1.5-ml microcentrifuge tube
2. 200 μl Buffer AL is added to the sample and mixed by pulse-vortexing for 15 s.
3. Mix is incubated at 56°C for 10 min
4. Briefly centrifugation the 1.5-ml microcentrifuge tube is performed to remove drops from the inside of the lid.
5. 200 μl ethanol (96-100 %) is added to the sample, and mixed again by pulse-vortexing for 15 s. After mixing, briefly centrifugation the 1.5-ml microcentrifuge tube is performed to remove drops from the inside of the lid.
6. The mixture from step 5 is carefully applied to the QIAamp spin column (in a 2-ml collection tube) and centrifuged at 6500 rpm for 1 min 20 s. The QIAamp spin column is placed in a clean 2-ml collection tube, and the tube containing the filtrate is discarded.
7. 500 μl Buffer AW1 is added to the QIAamp spin column and centrifugation at 6500 for 1 min 20 s is performed. The QIAamp spin column is placed in a clean 2-ml collection tube, and the tube containing the filtrate is discarded.
8. 500 μl Buffer AW2 is added to the QIAamp spin column and centrifugation at 13 000 rpm for 1 min 10 s is performed. The QIAamp spin column is placed in a clean 1.5-ml microcentrifuge tube, and the collection tube containing the filtrate is discarded.
9. For elution of extracted DNA 200 μl Buffer AE or distilled water is added to the QIAamp spin column. Incubation at room temperature for 1 min is performed, and then centrifugation at 6500 rpm for 1 min 10 s.

II Direct purification of viral RNA from serum or plasma with High Pure Viral RNA Kit (Roche) - HCV

1. 200 μl serum or plasma mixed well with 400 μl working solution (binding buffer supplemented with poly(A) carrier RNA).
2. The High Pure filter tube and the collection tube are combined and pipetted the sample in the upper reservoir.
3. Centrifugation for 15 s at 10 000 rpm (ca. 8000 $\times$ g) in a standard table top centrifuge.
4. The flowthrough is discarded and the filter tube is combined with a new collection tube.
5. To the upper reservoir is added 450 μl wash buffer and centrifugation is performed as in step 4.
6. The flowthrough is discarded and the filter tube is combined with a new collection tube. 450 μl wash buffer was added to the upper reservoir and centrifugation is performed as in steps 4,5. Finally, centrifugation for 10 s at max. speed (ca. 13 000 x g) is performed to remove residual wash buffer.
7. The collection tube is discarded and the filter is inserted in a clean, nuclease-free 1.5 ml reaction tube.
8. For the elution of the viral RNA is used 50 μl of elution buffer. After elution buffer was added to the filter tube centrifugation is performed for 1 min at 10 000 rpm (ca. 8000 $\times$ g).
9. The RNA is stable and can be used directly or stored at -80°C for later analysis.

III Guanidine isothiocyanate (GTC)/silica gel extraction of nucleic acids

(in consideration of Addenbrooke's Hospital Cambridge - Public Health and Clinical Microbiology, Boom et al., 1990) was performed just several times when above mentioned kits were not available.

IV Oligonucletide primers

Primers for HIV PCR: amplification region-gag

1. rnd.:	GAG1 primer: 5'-GCG AGA GCG TCA GTA TTA AGC GG-3'	23 nt
	GAG4 primer: 5'-TCT GAT AAT GCT GAA AAC ATG GG-3'	23 nt
2. rnd.:	GAG2 primer: 5'-GGG AAA AAA TTC GGT TAA GGC C-3'	22 nt
	GAG3 primer: 5'-CTT CTA CTA CTT TTA CCC ATG C-3'	22 nt

Final fragment length of PCR product after second round amplification is 412 bp.

Primers for HBV-PCR: amplification region-HBS gene

HBS1 primer: 5'-CAA GGT ATG TTG CCC GTT TG-3'	20 nt
HBS2 primer: 5'-AAA GCC CTG CGA ACC ACT GA-3'	20 nt

Fragment length of PCR product is 259 bp.

Primers for In house HCV nested RT-PCR: region of amplification - 5' UTR

RT-PCR: random primers	6 nt-hexamers
HCV detection:	
1. rnd: NCR2 primer: 5'-ATA CTC GAG GTG CAC GGT CTA CGA CT-3'	26 nt
OKA3 primer: 5'-CTG TGA GGA ACT ACT GTC TT-3'	20 nt
(outer)	
2. rnd: NCR4 primer: 5'-CAC TCT CGA GCA CCC TAT CAG GCA GT-3'	26 nt
OKA1 primer: 5'-TTC ACG CAG AAA GCG TCT AG-3'	20 nt
(outer)	

Fragment length of PCR product after first round amplification is 299 bp and final fragment length after second round amplification is 235 bp.

V PCR reactions (in consideration of Addenbrooke's Hospital Cambridge - Public Health and Clinical Microbiology)

HIV: *two rounds of amplification*

- We used 20 μl of First round mix (10 x buffer II, 75 mM $MgCl_2$, dNTPs, Taq polymerase 5 U/μl, GAG1 and GAG2 primers 20 pmol/μl, RNase free water) and 5 μl *of template* (extracted DNA or positive control). The total volume of reaction mix is 25 μl.
- *Positive control:* HIV positive plasma sample.
- *PCR cycle:* The pre-heating of sample at 94°C for 2.5 min is followed by *25* PCR cycles (denaturation at 94°C for 25 s, primer annealing at 50°C for 35 s and primer extension at 68°C for 2.5 min), finishing of polymerization reaction is performed at 68°C for 9.5 min and at the end, cooling at 4°C. All reactions are performed in PCR thermocycler machine (Perkin Elmer GeneAmp PCR system 2400).
- *20 μl of Second round mix* (10 x buffer II, 75 mM MgCl2, dNTPs, Taq polymerase 5 U/μl, GAG2 and GAG3 primers 20 pmol/μl, RNase free water) and *2 μl of the first round PCR product.* The total volume of reaction mix is 22 μl.
- PCR cycle: is the same as in the first round amplification.

HBV: *one round amplification*

- We used *40 µl of First round mix* (10 x buffer II, 75 mM MgCl2, dNTPs, Taq polymerase 5 U/µl, HBS1 and HBS2 primers 20 pmol/µl, RNase free water) and *10 µl of template* (extracted DNA or positive control). The total volume of reaction mix is 50 µl.
- *Positive control*: HBsAg positive plasma sample.
- *PCR cycle:* The pre-heating of sample at 95°C for 5 min is followed by *30* PCR cycles (denaturation at 95°C for 2 min, primer annealing at 55°C for 2 min and primer extension at 70°C for 2 min), finishing of polymerization reaction is performed at 70°C for 5 min and at the end, cooling at 4°C. All reactions are performed in PCR thermocycler machine (Perkin Elmer GeneAmp PCR system 2400).

HCV: *RT-PCR*
Step one: Reverse transcription

- 1 µl of random primer mixed to 20 µl of extracted nucleic acid and incubated at 70°C for 5 min in a heating block. Then chilled on ice for 2 min The total volume in tube was 21 µl. After that Step 2 mix was prepaired (10 x buffer II, 75 mM MgCl2, dNTPs, M-MuLV RT, RNase-free water) and added in 14 µl to each tube. Reaction mix is incubated for 10 min at room temperature and for 1h at 37°C in a water bath. After incubation samples are pulsed spin at 13 000 rpm for 15 s and incubated at 95°C for 5 min in a thermocycler or in a heating block. Chilled tubes on ice for 2 min and the total volume in tube after Step 2 was 35 µl.

Ist round PCR

- We used *20 µl of First round mix* (10 x buffer II, 75 mM MgCl2, dNTPs, Taq polymerase 5 U/ µl, NCR2 and OKA3 primers 20 pmol/µl, RNase free water) and *5 µl of template* (cDNA from RT reaction). The total volume of reaction mix was 25 µl which is the reaction volume for the PCR amplification. After brief spin in microcentrifuge (5 s) the samples are ready for PCR.
- *Positive control:* HCV positive plasma sample.
- *PCR cycle: 25* PCR cycles (denaturation at 94°C for 25 s, primer annealing at 50°C for 35 s and primer extension at 68°C for 2.5 min), finishing of polymerization reaction is performed at 68°C for 9.5 min and at the end, cooling at 4°C. All reactions are performed in PCR thermocycler machine (Perkin Elmer GeneAmp PCR system 2400).
- *20 µl of Second round mix* (10 x buffer II, 75 mM MgCl2, dNTPs, Taq polymerase 5 U/µl, NCR4 and OKA1 primers 20 pmol/µl, RNase free water) and *2 µl of the first round PCR product.* The total volume of reaction mix is 22 µl.

- *PCR cycle:* is the same as in the first round amplification.

VI Visualisation of PCR products

Gel electrophoresis

10 μl aliquots of amplification products from the last PCR round are analyzed by 3% NuSieve agarose gel electrophoresis. Gel contained 0.5 μg/ml ethidium bromide and electrophoresis was runing at 45 V 30-60 min in 1 x TAE solution. After electroforesis gels were photografized under UV illumination (transilluminator) by CDD camera with terminal printer. Marker of length that was used is a 50, 150, 300, 500, 750 and 1000 bp ladder.

Results

- The polymerase chain reaction (PCR) assay is an extremely sensitive technique for the detection of specific DNA and RNA fragments. Numerous laboratories perform (RT) PCR using in-house laboratory methods and reagents.
- In our laboratory we perform the procedures for qualitative PCR assays, in consideration of Addenbrooke's Hospital Cambridge - Public Health and Clinical Microbiology.
- For this PCR testing individual plasma samples were analysed. Virus deoxyribonucleic acids were extracted by QIAamp nucleic acids extraction method or by guanidine isothiocyanate (GTC)/silica gel extraction of nucleic acids. The HCV ribonucleic acid was extracted by High Pure Viral RNA Kit (Roche) also from plasma samples. We detected integrated form of HIV and HBV-viral DNA but in the case of HCV it was RNA from free viral particles present in the blood of patient. So, for HCV detection an additional reverse transcription step before PCR amplifications was performed. Using specific complementary primers to the virus sequences we were able to detect a low amount of virus at early phase of viral infection by amplification reaction. Amplified virus-specific sequences by specific complementary primers were detected by agarose-gel electrophoresis. Primers used for HIV identification were specific for gag region (figure 1.) resulting in *412 bp* PCR product after second round of amplification. One round amplification of specific HBV DNA region HBS gene sequence (figure 2.) was identified by *259 bp* PCR product. Primers for HCV detection were complementary to *5' UTR* region (figure 3.) resulting in *235 bp* PCR product after second round amplification. The predicted length of specific PCR products was determined by comparing fragment length with known markers of length (50, 150, 300, 500, 750 and 1000 bp). After electrophoresis gels were photographing under UV illumination (transilluminator) by camera.

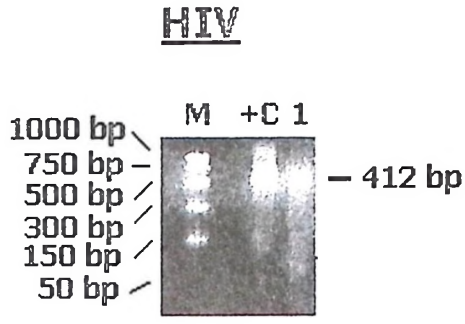

Figure 1. Amplification of specific HIV DNA region- *gag* sequence - by specific PCR primers. Length of amplified fragment is 412 bp. (M - marker of length, +C - positive control, 1- HIV positive specimen).

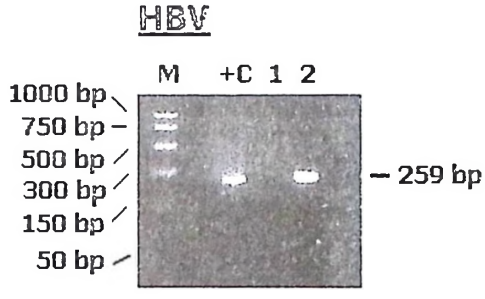

Figure 2. Amplification of specific HBV DNA region - *HBS gene* sequence - by specific PCR primers. Length of amplified fragment is 259 bp. (M - marker of length, +C - positive control, 1,2 - specimen). Specimen 1 did not contain virus specific sequence but specimen 2 is HBV positive.

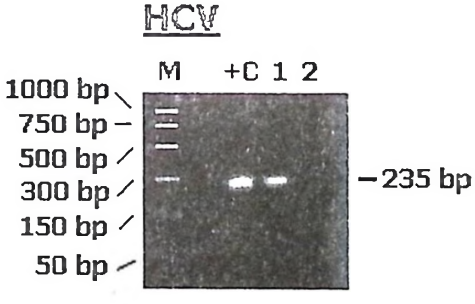

Figure 3. Amplification of specific HCV DNA region- *5' UTR* sequence - by specific PCR primers. Length of amplified fragment is 235 bp. (M - marker of length, +C - positive control, 1,2 - specimen). Specimen 2 did not contain virus specific sequence but specimen 1 is HCV positive

Year	Medical Institution	Number of HIV tested persons		
		Total	Positive	Negative
from July 1998	Clinic of Infectious Diseases	5	0	5
	Clinic of Neurosurgery	1	0	1
	Personal request	12	2	10
Total		**18**	**2**	**16**
Percentage		100%	11.1%	88.9%
1999	Clinic of Neurosurgery	3	0	3
	Department of Oral Surgery	2	0	2
	Clinic of Haematology	1	0	1
	Clinic of Infectious Diseases	3	0	3
	Institute of Nephrology	3	0	3
	Personal request	110	5	105
Total		**122**	**5**	**117**
Percentage		100%	4.1%	95.9%
2000	Clinic of Cardiac diseases and Rheumatism	1	0	1
	Clinic of Infectious Diseases	2	1	1
	Institute of Nephrology	1	0	1
	Clinic of Haematology	1	0	1
	Clinic of Endocrinology	1	0	1
	Clinic of Pulmonary Diseases	1	0	1
	Clinic of Psychiatry	1	0	1
	Personal request	36	0	36
Total		44	**1**	**7**
Percentage		100%	2.3%	97.7%
T o t a l (1998-99-2000)		**184**	**8**	**176**
Percentage (1998-99-2000)		100%	4.4%	95.6%

HBV, HCV

Year	Medical Institution	Number of tested persons								
		HBV			HCV			HBV+HCV		
		Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative
1999	Clinic of Infectious Diseases	0	0	0	3	1	2	0	0	0
	Institute of Nephrology	1	0	1	43	1	42	0	0	0
	Institute of Vascular Diseases	0	0	0	2	0	2	0	0	0
	Clinic of Otorinolaringology	0	0	0	1	0	1	0	0	0
	Post transfusion	1	0	1	1	0	1	0	0	0
	Pediatric Clinic	0	0	0	1	0	1	1	0	1
	Clinic of Gastroenterohaepathology	6	1	5	45	2	43	4	0	4
	Medical staff	0	0	0	7	0	7	1	0	1
	External medical institution	0	0	0	10	0	10	2	0	2
Total		8	1	7	113	4	109	8	0	8
Percentage		100%	12.5%	87.5%	100%	3.7%	96.3	100%	0%	100%

Year	Medical Institution	Number of tested persons								
		HBV			HCV			HBV+HCV		
		Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative
2000	Clinic of Infectious Diseases	0	0	0	1	0	1	0	0	0
	Institute of Nephrology	0	0	0	1	0	1	0	0	0
	Institute of Vascular Diseases	1	0	1	0	0	0	0	0	0
	Clinic of Cardiac deseases and Rheumatism	1	0	1	1	0	1	0	0	0
	Pediatric Clinic	2	1	1	0	0	0	0	0	0
	Clinic of Pacdiatric Surgery	0	0	0	1	0	1	0	0	0
	Clinic of Gastroenterohaepathology	31	6	25	21	3	18	1	0	1
	Clinic of Urology	1	0	1	0	0	0	0	0	0
	External medical institution	10	3	7	10	2	8	3	0	3
Total		46	10	36	35	5	30	4	0	4
Percentage		100%	27.8%	72.2%	100%	16.7%	83.3%	100%	0%	100%
Total (1999-2000)		54	11	43	148	9	139	12	0	12
Percentage (1999-2000)		100%	20.4%	79.6%	100%	6.1%	93.92%	100%	0%	100%

Discussion

Epidemiologically, the main resource of HIV, HBV and HCV infection are infected persons with no clinical symptoms of disease. The number of HIV infection in the world reaches tens million. The diagnosis of HIV, HBV and HCV infection can be made by qualitatively detecting of viral nucleic acid using gene amplification techniques (e.g., PCR, RT-PCR respectively).

- Screening for viral infection most often relies on the detection of antibody to specific proteins in the serum of infected persons. In some situations, antibody testing for i.e. HIV is not useful, such as in patients with early or "latent " HIV infection, when virus may be present without the production of detectable amounts of antibody, or neonates who have HIV antibodies from their infected mothers. HIV antigen assays are not useful in these situations because of relatively insensitive antigen test. The PCR assay is extremely sensitive and may detect even a few molecules of a specific DNA or RNA sequence.
- Using PCR as a validated qualitative methodology routine we checked 184 persons on **HIV** infection from July 1998. up to the end of 2000. *8 (4.4%)* of them were HIV-RNA positive. One of positive persons was a patient from Clinic of Infectious Diseases, other 7 HIV-RNA positive (6 injection drug users and 1 from promiscuity-risk group) were tested on their personal request. In 1999 number of infected persons with HIV was relatively high compairing it with results in the year before and after. During 1999 and 2000 we performed 54 tests for identification HBV infected individuals and found *11 (20.4%) HBV* positive persons. 7 of them were patients from Clinic of Gastroenterohaepathology, 1 from Paediatric Clinic and 3 from external medical institution. The HCV tests for the same period included 148 tested persons. *9 (6.1%)* of them were *HCV*-RNA positive. It was 1 patient from Clinic of Infectious Diseases, 1 patient from Institute of Nephrology, 5 patients from Clinic of Gastroenterohaepathology and other 2 patients were from external medical institution. None of PCR-tested persons had HBV/HCV or other dual positivism.
- Quantitative assays for measuring the concentration (titer) of viral nucleic acid have been developed and are available from commercial laboratories, including a quantitative RT-PCR (Amplicor HCV MonitorTM, Roche Molecular Systems, Branchburg, New Jersey) and a branched DNA (deoxyribonucleic acid) signal amplification assay (QuantiplexTM HCV RNA Assay -bDNA-, Chiron Corp., Emeryville, California). A quantitative assay for virus infection would be important for the evaluation of new drugs and vaccines or for monitoring disease progression. Because of yet mention reasons our main aim in the near future will be

employment of quantitative assay as additional test to qualitative PCR method.

SUMMARY

- For early diagnostics of HIV, HBV and HCV it is inevitable to have fast laboratory method with high sensitivity and specificity to avoid and reduce the risk of viral transmission. For this purpose serological tests and culture methods are replaced with PCR amplification techniques for detection of virus in clinical specimens especially during the window period before appearing of the clinical symptoms of diseases. Serological tests and culture methods can be useful in a postsymptomatic terapeutic treatment of patients.
- HIV, HBV and HCV have been the viruses most intensively subjected to PCR analysis. It has been necessary to have an overview of incidence of infectious diseases caused by these viruses for a transition-state country such as BiH. In consideration of number of our population there is relatively high percentage of *HIV* (the most affected risk group have been the intravenous drug users- 6 of 8 infected individuals- tested on their personal request), *HCV* (the most frequently cases were identified in patients from Clinic of Gastroenterohaepathology with acute or chronic hepatitis- 5 of 9 infected persons) and especially *HBV* positivism (5 of 7 infected persons have been also the patients from Clinic of Gastroenterohaepathology). But, in consideration of the extent national migrations in last few years and a large arrival and exchange of foreign citizens and soldiers in our country, the number of infected individuals by those viruses is not alarming.
- It would be necessary to realize better transmission control preventing infections by these viruses in BiH for the public health.
- For monitoring disease progression or for evaluation of new medicaments and vaccines of early antiviral therapy it would be important to employ a quantitative assay with the present diagnostics method. A quantitative assay as additional test to qualitative PCR method would be our next goal.

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