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PCR-RFLP DETECTION AND TYPISATION OF HUMAN PAPILLOMAVIRUSES (HPVs)

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Abstract

Human papillomaviruses (HPV) are the members of the family *Papovaviridae*. Viral particles are spherical, with icosahedral symetry, 55 nm in diameter, containing double strand molecule of DNA (mol. weight $8x10^3$). HPV cause benign and malignant lesions of epthelial and mucous tissue. On the basis of epidemiological and experimental data it is known that HPV is the main etiological causative agent of cervix cancer which can be transmited sexual contact. This virus has very unstable genome which mutates very often. At present, there are more than 70 different HPV types divided into three following groups:
HPVs of low oncogenic risk (types 6 and 7);
HPVs of intermediate oncogenic risk (types 31, 35 and 45);
HPVs of high oncogenic risk (types 16, 18 and 33).

It is very important in therapeutical and preventive sense early detection of HPV and corresponding tzpisation. Now it is possible to achieve this by combination of two molecular methods such as PCR (*Polymerase Chain Reaction*) and RFLP (*Restriction Fragment Length Polymorphism*) which are adapted for analysing of L1-region HPV-DNA (*1, 2, 3*).

We analysed total 123 specimens by HPV-DNA-PCR (L1-ORF) method (41 cervix specimens, 41 urine-supernatant and 41 urine-pellet specimens). Samples detected as positive (4, 5, 28, 33, 38, 39 and 40) in all PCR analysis are typed by RFLP.

Out of 16 HPV-DNA-PCR positive specimens analysed by RFLP detected HPV types were: 6, 2a, 16, 33 and 10a.

The percentage of successful typisation of HPVs by RFLP was 40%. For five specimens after RFLP analysis, electrophoretic patterns were unclear. These PCR products and other which showed undefined restriction fragments must be controled and confirmed by sequencing method.

Key words: *HPVs, PCR, RFLP*

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Introduction

Till today it has been identified over 70 types of Human papillomaviruses (HPVs) according to their ability to induce formation of lesions in mucous tissue and derm. 25 types infect anogenital tract of men and women. This type differentiation is also done according to the type of clinical lesions. So, anogenital HPVs a divided into the three groups:

HPVs of low oncogenic risk (types **6** and **7**);

HPVs of intermediate oncogenic risk (types **31, 35** and **45**) usually cause genital warts and rarely CIN (*cervical intraepithelial neoplasia*) or cancer;

HPVs of high oncogenic risk (types **16, 18** and **33**) often cause CIN lesions and invasive cervix cancer (*4, 5, 6, 7, 8*).

The papillomaviruses are currently classified as a genus within the *Papovaviridae* family. Viruses are non-enveloped, small, icosahedral, with double-stranded DNA genome and infect mainly mammals. There are genus *Papillomavirus* and *Polyomavirus* within *Papovaviridae* family, differing in size and characteristics of genome, genus-specific antigens and biological properties.

Papillosis and papillomatosis are benign tumours (skin, mucous membranes, epithelial tissue) caused by HPV infection. Different types of HPV cause morphologically different papillosis among animal hosts. Some of the HPV types can be the major etiologic factor for malignancy develop. HPV infection alone is not sufficient for malignant transformation. The mechanisms of transformation are not clear at all but it is known that HPV-DNA integrates into the host genome (integrated form of HPV-16 DNA was often found in malignant tumors of human genital tract). HPV-DNA has 7.904 nucleotides. Period from 1985. till 1996. is specific because of acumulation of datas supporting the hypothesis that HPV infection is one of the major factor of pathogenesis some of the human epithelial benign and malignant neoplasia (*9*).

Very important cytologic parameters such as abnormal mitosis and nucleus atypia show to precursor states before forming of cancer. Specific lesions named as cervical intraepithelial neoplasia- CIN are caused by HPV and divided into 4 grades (*10*).

Early diagnosis of HPV is of great terapeutic and prophylactic importance before histological and clinical signs. Extremly sensitive and specific HPV-DNA PCR in combination with RFLP is adapted for this purpose analysing L1 ORF (*open reading frame)* of HPV genome using MY11/MY09 and GP5/GP6 primers specifically amplifying L1 region (position 6772-7170, **figure 1**). L1 HPV-DNA region is useful for detection of HPV types **11, 16** i **18,** however E5 HPV-DNA region

is often analysed by specific detection primers for determination of HPV type 6. Molecular method such as PCR and RFLP helping us to research mentioned sequences which are specific for the most important types of HPV (*11*).

In 1989. the first identification of HPV-DNA was done by PCR technology (*1*) and this technology found application in epidemiology research of genital HPV infections. PCR technology in combination with next two methods were used for HPV-DNA research:

1. ELISA- (*Enzyme-Linked Immunosorbent Assay*)
2. RFLP- (*Restriction Fragment Length Polymorphism)*

PCR-RFLP analysis of L1-ORF HPV in different clinical specimens is fast and sensitive method for detection and typisation of HPV-DNA and for determining of oncogenic potential, as well. For typisation of HPV by ELISA high specific biotin labelled non-radioactive hybridization probes are used for types 6, 11, 16, 18 and 33. RFLP analysis understands usiness of restriction endonucleases for amplificated HPV-DNA digestion (*12,13*).

On the basis of available references there are no similar research for HPV in BiH region that has been done by PCR-RFLP for parallel collected specimens of the cervix and urine.

Material and methods

Cervical and urine specimens were selected and taken in the period of 1997-1999. Cervical specimens (in 2 ml PBS) and urine (20 ml) were treated after delivery.

Treatment of specimens (extraction and incubations DNA-HPV)

Cervical specimen was transferred into the 1.5 ml tube and after centrifugation at 13.000 rpm, 3 min. pellet was resuspend in 200 µl of cetus buffer, treated with Proteinase K (12 µl Proteinase K and 200 µl of specimen was incubated 1 hr at 55°C), incubated 10 min. at 96°C, transferred on ice for 2 min. and left on -20°C.

Urine specimen was at first centifugated 10 min. at 3.500 rpm. Then the 500 µl of supernatant was treated with 30µl of Proteinase K Digestion was done at 55°C for 1 hr. After 10 min. incubation at 95°C specimen was transferred on ice shortly and left on -20°C.

Two round amplification detection of HPV using specific primers, agarose gel electrophoresis with PCR markers. PERKIN ELMER thermocycler (GENE AMP PCR SYSTEM 2400).

Positive control for PCR were CaSki cells (*Carcinoma Skin Cells*), 600 copies of HPV-DNA (dilutions 10^{-4}, 10^{-5} and 10^{-6}). As a negative control we used Rnase free destiled water.

RFLP (Restriction Fragment Length Polymorphism) procedure

For HPV typisation by detection method named restriction fragment length polymorphism of DNA (RFLP) were used PCR positive cervix and urine specimens after 1st round amplification. Positive PCR products were digested by these restriction endonuclease: *Rsa* I, *Hae* III and *Pst* I. Those enzymes cut phosphodiesteric bonds of nucliotide DNA strand at the specific sites. *Hae* III endonuclease isolated from *Haemophilus aegyptius* cuts the DNA at GG↓CC. *Pst* I endonuclease is isolated from bacteria *Providencia stuartii*. Restriction sites of this enzyme is CTGCA↓G. *Rsa* I endonuclease isolated from bacteria *Rhodobacter sphaeroides* recognises palindromic restriction site GT↓AC (*14*).

RFLP markers

Plasmid pBR 322 DNA digested by *Hae* III endonuclease gave the fragments of this size: 587, 540, 504, 458, 434, 267, 234, 213, 192, 184, 124, 123, 104, 89, 80, 64, 57, 51, 21, 18, 11 and 8. 1 kb leader DNA had lineary differing fragments in 1018 bp (from 12.216-1.018 bp) used for RFLP typisation analysis and also this system contained vector's DNA fragments of size: 506, 396, 344, 298, 220, 201, 154, 134 and 75 bp.

RFLP- digestion by restriction endonuclease

1st round PCR amplification products that were HPV-DNA positive after 2nd round PCR amplification and detection were typed by RFLP. 30-40 µl of PCR I product were added to 22 µl mix of endonucleases *Rsa* I, *Hae* III and *Pst* I (1:1:2). So, to 18 µl of 1xL buffer (*Rsa* I buffer) was pipetted 1 µl of each, *Rsa* I and *Hae* III, and 2 µl of *Pst* I were added. Samples were 1 hr incubated at 37°C, heated 5 min. at 95°C in a dry heating block and than put on ice for 2 min.

Separation of digested HPV-DNA by electrophoresis

Separation of digested HPV-DNA PCR products was done by 3% agarose gel electrophoresis in 1x TBE buffer in the absence of ethidium bromide (EtBr). 20 µl of each RFLP product was added to 10 µl of sample buffer (*Orange G*) and 10 µl of 1xTE buffer. The first and the last sample well of agarose gel was reserved for markers (35 µl).

Electrophoresis was performed in Midi gel apparatus (LKB Pharmacia) in 1xTBE buffer for 2.5-3 hrs at 100 V (voltage). Gel was stained in 1xTBE 45 min. which contained EtBr (10 mg/ml) 100 µl na 100 ml 1xTBEbuffer. Gel was analysed by UV transilluminator and photographied by polaroid camera. Determination of HPV-types was done on the basis of well known values of HPV-DNA digestion fragments for determined HPV types found in restriction maps. Standard HPV-DNA restriction fragments or as a control group for comparation of our experimental datas and restriction maps as well are showed in the table 2. Precise computer determination of HPV-DNA fragments after restriction endonuclease digestion (*Hae* III, *Pst* I, *Rsa* I) was done according known lengths of RFLP markers (pBr 322, *Hae* III DNA and 1 kb DNA *leader*).

Results

PCR-HPV analysis

It was analysed 41 cervical and 82 urine specimens in parallel (41 supernatant and 41 pellet samples) by HPV-PCR. At each round of amplification the positive result had sample no. 40. Samples 28, 33, 38 and 39 were HPV-DNA negative in urine supernatant. Sample no. 4 was negative in urine supernatant (first amplification round) and pellet (first amplification round), sample no. 5 was negative in urine pellet (first amplification round).

Samples no. 4 (cervix, urine-pellet), 5 (cervix, urine-supernatant), 28 (cervix, urine-pellet), 33 (cervix, urine-pellet), 38 (cervix, urine-pellet), 39 (cervix, urine-pellet) and 40 (cervix, urine-supernatant and pellet) were HPV-DNA-PCR positive and proceeded to RFLP analysis for typisation, respectively, determination of their oncogenic potential.

RFLP analysis

Typisation of HPV-DNA positive specimens (cervix, urine) was done according the polymorphism of HPV-DNA restriction fragments

after digestion by restriction endonucleases *Hae* III, *Pst* I and *Rsa* I. Fragment separation was done in 3% agarose gel..

Order of analysed fragments is shown in figure 1 (A and B) and represents electrophoregram of HPV-DNA restriction fragments from positive PCR samples of cervix and urine:

Paths: 1, 2 - sample no. 4 (cervix, urine-pellet)
3, 4 - sample no. 5 (cervix, urine- supernatant)
5, 6 - sample no. 28 (cervix, urine-pellet)
7, 8 - sample no. 33 (cervix, urine-pellet)
9, 10 - sample no. 38 (cervix, urine-pellet)
11 - control (dH_2O- Rnase free water)
12, 13 - sample no. 39 (cervix, urine-pellet)
14 - sample no. 40 (cervix)
15, 16 - sample no. 40 (urine- supernatant and pellet)

Label M in each figure of electrophoregram means RFLP markers.

According the known size of RFLP marker fragments the size of HPV-DNA restriction fragments separated in agarose gel was calculated. Table 1 (A, C) shows the size of of those fragments labeled as Mol.Wt. for markers and and (QUERIES) for samples in figure 1 (A, B).

Compairing the known and experimental size of HPV-DNA fragments for individual types of HPVs we determined the presence of the next HPV types in analysed specimens: HPV-6, HPV-16, HPV-2a, HPV-33 and HPV-10a. For the rest of the samples with unclear HPV type it is necessary to perform DNA sequencing (table 1. P).

Figure 1.A. *RFLP electrophoregram. 1, 2-sample no.4; 3, 4-sample no.5; 5, 6-sample no.28; 7, 8- sample no.33; 9, 10- sample no.38; 11-negative control; 12, 13- sample no.39; 14, 15, 16- sample no.40. M-RFLP markers.*

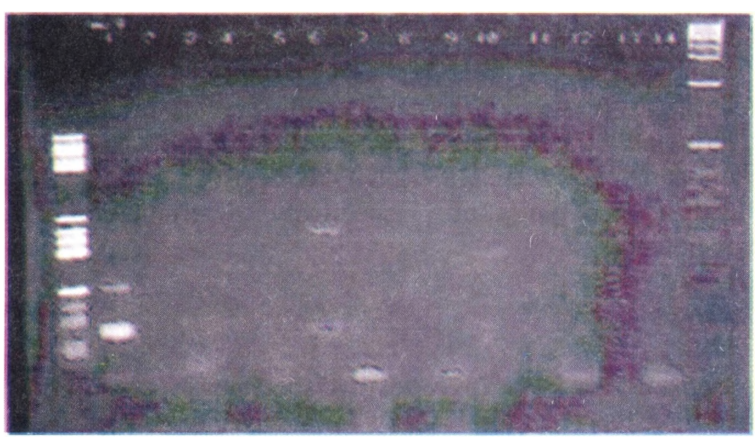


Figure 1.B. *Computer analysis of RFLP electrophoregram (figure 1A) (ISI 1000 Digital Imagining System). M- RFLP markers.*

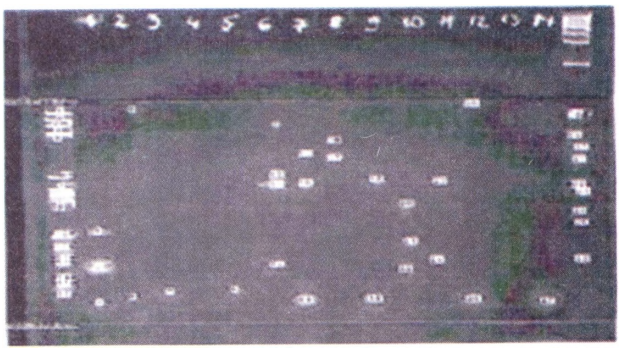


Figure 3. *RFLP electrophoregram sample no.40 (lines 15 and 16). M-RFLP markers.*

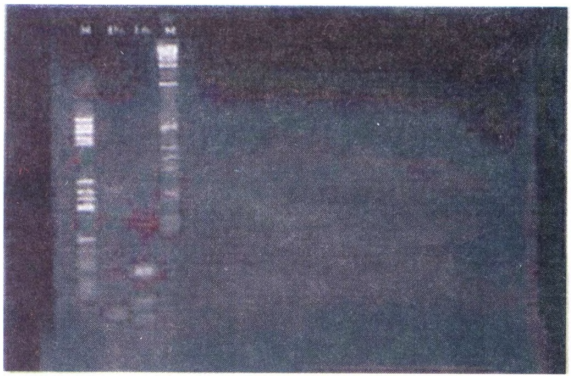


Figure 4. *Computer analysis of RFLP electrophoregram (figure 3) (ISI 1000 Digital Imaging System) M-RFLP markers.*

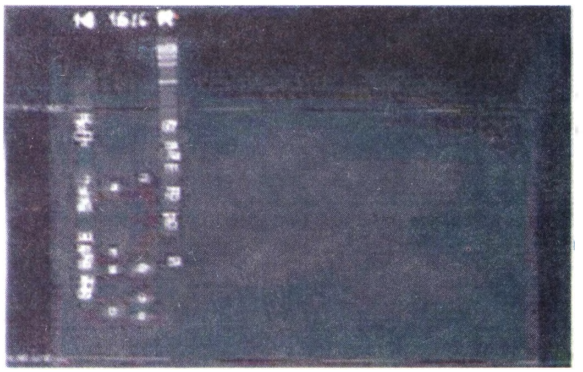
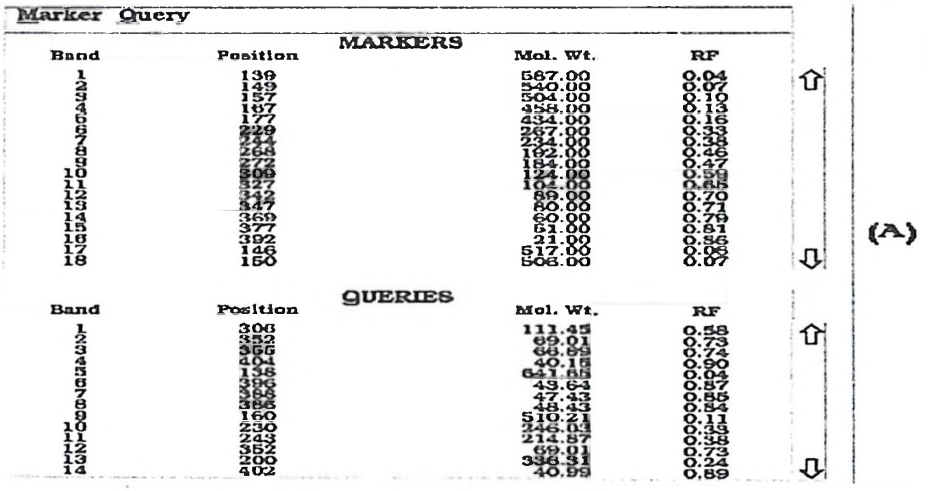


Table 1-A. *Restriction fragment size (Mol. Wt.) of RFLP markers (MARKERS) and analysed samples (QUERIES).*



Marker Query

MARKERS

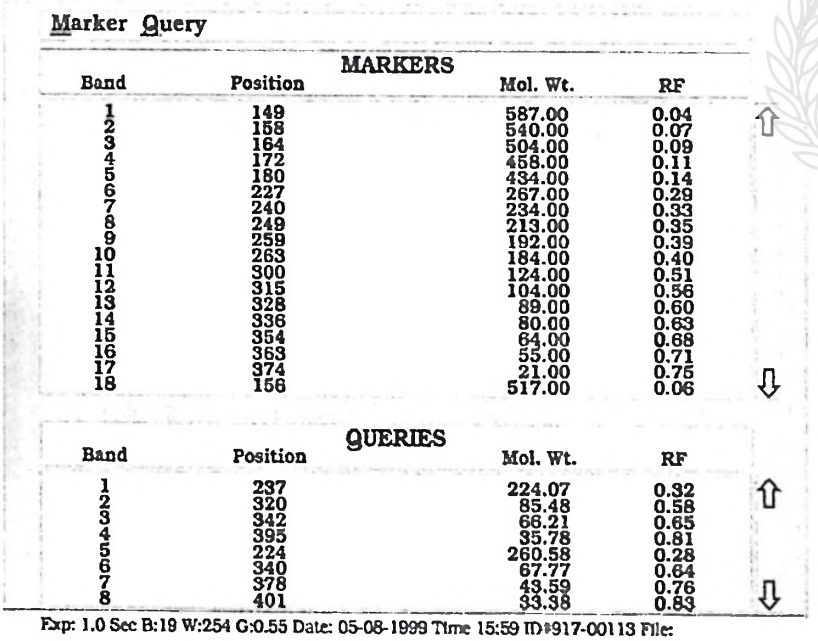
Band	Position	Mol. Wt.	RF
1	139	567.00	0.04
2	149	540.00	0.07
3	157	504.00	0.10
4	167	458.00	0.13
5	177	434.00	0.16
6	229	267.00	0.33
7	244	234.00	0.38
8	268	192.00	0.46
9	272	184.00	0.47
10	306	124.00	0.59
11	327	104.00	0.65
12	342	89.00	0.70
13	347	80.00	0.71
14	369	60.00	0.79
15	377	51.00	0.81
16	392	21.00	0.86
17	146	517.00	0.08
18	150	506.00	0.07

QUERIES

Band	Position	Mol. Wt.	RF
1	306	111.45	0.58
2	352	69.01	0.73
3	366	66.99	0.74
4	404	40.15	0.90
5	138	641.65	0.04
6	396	43.64	0.87
7	368	47.43	0.85
8	386	48.43	0.84
9	160	510.21	0.11
10	230	248.03	0.39
11	243	214.87	0.38
12	352	69.01	0.73
13	200	398.31	0.24
14	402	40.99	0.89

(A)

Table 1-C. *Restriction fragment size (Mol. Wt) of RFLP markers (MARKERS) and analysed samples (QUERIES).*



Marker Query

MARKERS

Band	Position	Mol. Wt.	RF
1	149	587.00	0.04
2	158	540.00	0.07
3	164	504.00	0.09
4	172	458.00	0.11
5	180	434.00	0.14
6	227	267.00	0.29
7	240	234.00	0.33
8	249	213.00	0.35
9	259	192.00	0.39
10	263	184.00	0.40
11	300	124.00	0.51
12	315	104.00	0.56
13	328	89.00	0.60
14	336	80.00	0.63
15	354	64.00	0.68
16	363	55.00	0.71
17	374	21.00	0.75
18	156	517.00	0.06

QUERIES

Band	Position	Mol. Wt.	RF
1	237	224.07	0.32
2	320	85.48	0.58
3	342	66.21	0.65
4	395	35.78	0.81
5	224	260.58	0.28
6	340	67.77	0.64
7	378	43.59	0.76
8	401	33.38	0.83

Exp: 1.0 Sec B:19 W:254 G:0.55 Date: 05-08-1999 Time 15:59 ID:917-00113 File:

(C)

Table 1-P. *Size of experimental HPV-DNA restriction fragments after agarose gel electrophoresis was compaired with the size of known fragments for individual HPV types.*

Fragment length	HPV type	Length of known fragments for individual types
Path 1: 40 bp, 66 bp, 89 bp, 111 bp	6	37, 67, 89, 111
Path 2: 43 bp, 641 bp	?	-
Path 3: 47 bp	?	-
Path 4: had not intensive DNA bends	-	-
Path 5: 40 bp	?	-
Path 6: 69 bp, 214 bp, 246 bp, 510 bp	16	66, 68, 70, 218
Path 7: 40 bp, 217 bp, 336 bp	2a	40, 190, 225
Path 8: 315 bp, 405 bp	?	-
Path 9: 41 bp, 231 bp	2a	40, 190, 225
Path 10: 69 bp, 94 bp, 126 bp	33	38, 67, 90, 137
Path 11: control (RNase free dH_2O)	-	-
Path 12: 40 bp, 668 bp	?	-
Path 13: had not intensive DNA bends	-	-
Path 14: 39 bp	?	-
Path 15: 36 bp, 66 bp, 85 bp, 260 bp	10a	29, 67, 110, 229
Path 16: 33 bp, 43 bp, 67 bp, 260 bp	10a ?	29, 67, 110, 229

Discusion and conclusion

In these papers are shown the results of high sensitive and specific PCR analysis after the screening programme for the cervical and urineclinical specimens using specific detection primers for the L1-HPV-DNA region.

Till today, research of L1- ORF- HPV- DNA showed that the amplicons of this part of nucleotide sequence can be typed according to their restriction fragment size after digestion by endonucleases *Psa* I, *Pst* I and *Hae* III, and comparative study with standards in considered HPV-DNA restriction maps (*8*). For all HPV types with known DNA sequence the length of their restriction fragments after digestion by mentioned restriction enzymes are known and are used in HPV-DNA typisation in this work, as well. Results of our HPV-DNA-PCR-RFLP

research in comparation with the standard restriction map for HPV are shown in the table 2.

Table 2. *Results of HPV-DNA-PCR-RFLP analysis (A) in comparation with results of colleges from Cambridge (C) and standard lengths of HPV-DNA restriction fragments.*

Electrophoretic path	HPV-DNA restriction fragment length (bp) (A)	HPV type	Standard HPV-DNA restriction fragment length (*8*) (B)	Results of HPV-DNA PCR analysis in Cambridge (*11*) (C)
7/9	40, 217, 336/41, 231	2a		225, 190, 40
		3		220, 70
		5	464	377, 99
1	40, 66, 89, 111	6	122, 78, 73, 71, 67, 37	113, 68, 64, 34
		7		460
		8	288, 176	300, 184
15/16	33, 16, 85, 224/33, 43, 67, 260	10a		229, 110, 67, 29
		11	135, 124, 73, 71, 26, 10	140, 124, 75, 20
		14		303, 121, 40, 8
6	69, 214, 246, 510	16	216, 71, 70, 68, 25	218, 70, 68, 66
		17		330, 130
		18	134, 107, 85, 73, 38, 18	132, 110, 85, 70
		20		350, 130
		31	138, 118, 98, 73, 25	131, 124, 90, 80
10	69, 94, 126	33	140, 101, 96, 73, 39	137, 90, 67, 38
		49		200, 56, 10
		50		135, 110, 81, 67,10
		57	297, 153	303, 146, 10

According to reference datas (*8*) and compairing with standard restriction map, variability in fragment size is very often for HPV-DNA analysed by PCR-RFLP. We also confirmed that fact (table 2). It can be explained with the presence of partial digestion products which are made as a result of new restriction HPV-DNA sites that arise by point mutatuions in specific sites of nuclieotide HPV-DNA sequence.

Obtained results unidentical at all with standard HPV-DNA restriction maps could arise as a consequence of dual infection. In these cases regularly is detected larger number of restriction fragments than it is in standard restriction map. Among all reference datas there is only

one research that can be compaired with this work and was done in Cambridge (England) where were used the identical restriction enzymes and analysed identical clinical specimens (cervix, urine). The same amplification and detection primers (MY09/MY11 and GP5/GP6) were also used for L1-ORF HPV-DNA analysis.

Analysis that was done by research team from Cambridge was checked by sequencing so the HPV types were determined with great insurancy. They determined the presence of these HPV types (nucleotide sequence of HPV-DNA had more than 10% homology in consideration of nucleotide sequence of all known HPV: 2a, 3, 5, 6, 7, 8, 10a, 11, 14, 16, 17, 18, 20, 31, 33, 49, 50 and 57.

Our PCR-RFLP analysis showed the presence of these HPV types: 2a, 6, 10a, 16 and 33. For six samples we could not determine HPV type definitely but the large probability is that it was HPV type 10a in one of those samples (table 2.). In consideration of the fact that this work is the first for our territory and determines HPV types it would be interesting to investigate the sequence of HPV types 2a, 6, 10a, 16 and 33 and to consolidate the homology among their nucleotide sequence with HPV types from Camridge.

Investigation realized by several research centres showed the prevalence of these HPV-DNA types using PCR-RFLP typisation in clinical specimens of cervix: HPV-16 (48%) (*15*); HPV-16 (42%); HPV-18 (39%); HPV-6 (26%); HPV-11 (15%); HPV-45 (10%); HPV-52 (3%); HPV-31 (1.5%); HPV-68 (1.5%); HPV-33 (1.5%) (*16*); HPV-16 (10%); HPV-18 (10%); HPV-6 (2.2%); HPV-11 (2.2%) (*17*); HPV-16 (50%); HPV-18 (14%); HPV-45 (8%); HPV-31 (5%) (*18*). Predominant type in Indonesia is HPV-16, in Western Africa HPV-45, in Central and souh Africa HPV-39 and HPV-59 (*19, 20, 15*).

From large number of PCR positive samples those which were positive in cervix even in urine samples (pellet and supernatant) were further analysed for their oncogenic potential and typed by RFLP. Those were: sample no. 4 (cervix, urine-pellet), sample no. 5 (cervix, urine-supernatant), sample no. 28 (cervix, urine-pellet), sample no. 33 (cervix, urine-pellet), sample no. 38 (cervix, urine-pellet), sample no. 40 (cervix, urine- supernatant and pellet), sample no. 4 (cervix)- HPV- type 6, sample no. 28 (urine-pellet)- HPV- type 16, sample no. 33 (cervix)- HPV- type 2a, sample no. 38 (cervix)-HPV- type 2a, sample no. 38 (urine- pellet)_ HPV- type 33, sample no. 40 (urine- supernatant)- HPV- type 10a, sample no. 40 (urine-pellet)- HPV- type 10a.

Determined HPV types ordered according the oncogenic potential:

HPV- types of low oncogenic potential (HPV 6);

HPV- types of intermediate oncogenic potential (HPV 33);

HPV- types of high oncogenic potential (HPV 16);

Currently, oncogenic potential of HPV types 2a, 10 and 10a is not clear at all. RFLP analysis showed the presence of HPV type 6 in sample no. 4 (cervix) which is the major etiologic agent of lesions and rarely give arise the progressive malignancy. HPV-33 was identified in sample no. 38 (urine-pellet). It belongs to a group of typical HPV representative that infect anogenital tract. Very often is identified in cervical malignant lesions and according its oncogenic potential is classified within a group of HPV- types with intermediate oncogenic potential. HPV-16 was identified in sample no. 28 (urine-pellet). This type is classified within the group of HPV- types with high oncogenic potential and is often detected in cervical malign lesions. For all these cases, we got a precise informations about HPV types that supplement diagnosis and help clinics to evaluate a risk of epithelial cervical cells malignancy development.

Experimental datas show that all samples with unclear results of RFLP analysis are checked by sequencing because that is the best way to determine and confirm mutations in HPV-DNA nucleotide sequence (*14*). Besides the practical importance of introducing the sequencing for the final results it is necessary to emphasis the sequencing as a prognostic factor of determining the integrated forms of HPV-DNA types with high oncogenic risk, especially for types 16 and 18 in consideration of episomal forms of HPV-DNA (*21,22*). Using these methods, PCR and sequencing, for differentation of integrated and episomal forms of HPV-DNA, needed conditions for HPV detection and its research will be realized in the purpose of effective preventive and therapy of patients.

Results of our research represent original contribution to knowledge of HPV in Bosnia and Herzegovina. It is sure that sequencing method and quantitative PCR would improve the quality of detection and research on the molecular level and increase the percentage of HPV typisation, what would have a great importance for definitive diagnostics and molecular-epidemiological research of HPVs in Bosnia and Herzegovina.

Apstrakt

Humani papilomavirusi (HPVs) pripadaju familiji papovaviridae. Virusne partikule su sferičnog oblika, ikosaedralne simetrije promjera 55 nm, a sadrže dvolančanu DNA (mol. mase $8x10^3$). Uzrokuju benigne i maligne leyije epitelijalnog i mukoynog tkiva. Na osnovu epidemioloških i eksperimentalnih podataka sasvim se pouzdano zna da je HPV glavni etiološki uzročnik kancera cerviksa a prenosi se seksualnim putem. Ovaj virus ima nestabilan genom koji vrlo često mutira, tako da je do danas utvrđeno preko 70 tipova ovog virusa koji su podijeljeni na tipove:

HPV tipovi niskog onkogenog rizika (tipovi 6 i 7);
HPV tipovi srednjeg onkogenog rizika (tipovi 31, 35 i 45);
HPV tipovi visokog onkogenog rizika (tipovi 16, 18 i 33).

Od izuzetnog značaja je u terapeutskom i preventivnom smislu rana detekcija HPV određivanja onkogenog potencijala (tipizacija). To se danas u svijetu postiže kombinacijom PCR (*Polymerase Chain Reaction*) i RFLP (*Restriction Fragment Length Polymorphism*) prilagođenim za analiziranje L1 regiona HPV-DNA (*1,2,3*).

Metodom HPV-DNA-PCR analizirano je ukupno 123 uzorka (41 uzorak cerviksa, 41 uzorak urina-supernatant i 41 uzorak urina-talog). Uzorci koji su bili pozitivni (broj 4, 5, 28, 33, 38, 39 i 40) bili su podvrgnuti procesu tipizacije pomoću RFLP metode (Polimorfizam restrikcionih fragmenata DNA).

Od analiziranih 16 HPV-DNA pozitivnih uzoraka sa RFLP metodom, utvrđeni su slijedeći HPV tipovi: 6, 2a, 16, 33 i 10a.

Procenat uspješne tipizacije HPV sa RFLP metodom je 40%. Za pet uzoraka, poslije RFLP analiza, elektroforeske trake su bile nejasne. Ovi PCR produkti i drugi koji pokazuju nedefinisane restrikcione fragmente moraju biti kontrolirani i potvrđeni metodom sekvenciranja.

Ključne riječi: *HPVs, PCR, RFLP*

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