Detection of E-6 Antigen (Human Papilloma Virus-16) and H11B2C2 Antigen (Habp) in Cervical Cancer Tissues

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Abstract

Background: Cervical cancer is one of the most common types of women's reproductive cancers. A major risk factor for cervical cancer is exposure to a group of common sexually transmitted viruses called Human papillomavirus (HPV). The over expression of HPV 16 i.e., E6 antigen in the tumors of suspected cervical cancer tissues. however E-6 antigen has hyaluronan binding protein site.

Methods: The procedure carried out by using 15 benign and 50 different grades of cervical cancer tissue samples to study the expression of HPV 16 i.e., E6 antigen and H11 antigen(HABP) by biochemical method.

Results: The HPV 16 i.e., 18KD is expressing along with hyaluronic acid binding proteins 57KD and 92KD. This shows that E-6 antigen itself reacting with hyaluronic acid binding sites.

Conclusion: The H11 antigen (HABP) reacted with anti E-6 protein indicating hyaluronic acid binding protein 57KD and 92 KD is reacting with anti E6 antibody. These results showed a partial homology between E6 and H11 antigen (HABP). Thus the homology between the two proteins in tumor cells is an important parameter and a clinical diagnostic marker for progressive human tumors.

Key Words: Human papilloma virus (HPV); H11 antigen (HABP); Cervical cancer tissue; E-6 antigen

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Introduction

Human Papilloma Virus (HPV) infection is a major cause of cervical cancer, the second most common cancer in women worldwide. Papilloma viruses are small nonenveloped viruses with 55nm-diameter icosahedral capsids that contain double-stranded DNA genomes of approximately 8,000 bp. They are widely distributed throughout the animal kingdom and specifically infect squamous epithelial and cause the generation of warts. Harald Zur Hausen's laboratory was the first to demonstrate that genital warts contain human papillomavirus genomes [1, 2].

One of the most common virus groups in the world today affecting the skin and mucosal areas of the body is the human papilloma virus. It infects the epithelial cells of skin and mucosa. The epithelial surfaces include all areas covered by skin and/or mucosa areas of the mouth, throat, tongue, tonsils, vagina, penis, and anus. Transmission of the virus occurs when these areas come into contact with a virus allowing it to transfer between epithelial cells.

The invasive sampling method of screening is one of the reasons why women do not participate. The efficiency of cervical cancer screening could be increased if less invasive tests were available. Today, there is extensive scientific evidence that infection with high-risk human papillomavirus (HPV) is associated with the development of cervical cancer. An international survey of more than 1000 cervical cancers showed that HPV DNA was present in 93% of all tumours. [4]. Further investigation of the HPV negative carcinomas showed that, with improved methodology, 99.7% of all cervical tumors contained HPV DNA [5] Recently, it has been suggested that self-sampled vaginal material can be used for HPV detection. Several investigations—on a limited number of women have shown a good correlation between self sampled vaginal material and a cervical sample taken by a professional [6, 7].

The most dangerous aspect of the human papillomavirus is its potential to cause cancer. A highly studied topic is HPV's ability to cause cervical cancer. Two genes, Rb and p53 regulate normal cell

division. Rb segregates the transcription factors necessary for progression through the cell cycle. This means that the Rb prevents the cell from dividing until it has isolated enough proteins for cell division. The important protein, which Rb segregates, is E2F. This makes Rb a tumor suppressor gene/protein. It does not allow the cell cycle to continue until it has accumulated enough proteins, especially the E2F protein. High-risk HPVs encode two oncoproteins, E6 and E7, which subvert crucial cellular regulatory mechanisms that reactivate and maintain DNA synthesis in the host cell. E6 binds to the wild-type p53 protein and promotes its ubiquitin-dependent degradation [8]. E6 accelerates proteosomal degradation of the p53 tumor suppressor protein, whereas, E7 inactivates the retinoblastoma protein, interfering with the action of both p16 INK4a [6] and the cyclin-dependent kinase inhibitor p21 Cip1 [7, 9].When a cell is infected with HPV, the E7 gene binds to Rb so that the Rb releases E2F and the other proteins. This is a signal for the cell cycle to progress. As long as the E7 stays attached to Rb, the cell cycle will continue to happen, thus causing a cycle of uncontrolled cell reproduction, which is one of the definitions of a malignant cell.E7 binds to the retinoblastoma protein Rb and disturbs the complex between Rb and the E2F transcription factor family, which controls the expression of genes involved in cell-cycle progression [10]. Therefore, the development of anticancer therapies that tergent HPV E6 and E7 might be specific and effective treatment for cervical cancer. In fact, RNA interference (RNAi) of E6 and E7 inhibits growth of HPV-positive cancer cells [11, 12, 13]. Both E6 and E7 high-risk HPV oncoproteins independently induce genomic instability in normal human cells [14, 15]. Only a small portion of precursor lesions infected with HPV, however, develops into invasive carcinomas [5]. Therefore, additional genetic and micro environmental factors subsequent to HPV infection are thought to play an important role in the initiation and progression of cervical neoplasia [5, 15, 16]. The two most harmful human papillomaviruses are HPV 16 and HPV 18. Both of these are genital viruses, which are spread through sexual contact. These types of the human papillomavirus have E6 and E7 proteins with very strong binding capabilities. This allows HPV 16 and HPV 18 to reproduce quickly and in great numbers, leading to uncontrolled reproduction of viral cells, and eventually cancer. It is well established that HPV 16 and HPV 18 are causative factors in cervical cancer.

Human papilloma viruses (HPV) are the necessary cause of Invasive Cervical Cancer (ICC). Of the many different types identified so far, only a few of them account for the great majority of cases worldwide, with geographical differences in their distribution. Data on the local distribution are now of interest in view of the soon-to-come introduction of HPV type-specific prophylactic vaccines (Human papillomavirus typing of invasive cervical cancers in Italy,[17] Therapeutic vaccines, however, are expected to have an impact on cervical cancer or its precursor lesions, by taking advantage of the fact that the regulatory proteins (E6 and E7) of HPV are expressed constantly in HPV-associated cervical cancer cells[18]. Recent studies have shown that HPV- 16 confers an increased risk of high-grade CIN and cervical cancer [19]. HPV infections and HPV-associated malignancies by targeting non-structural early viral antigens of HPV such as E6 and E7 [20]. Based on the central role of carcinogenic human papillomavirus (HPV) types in the development of cervical cancer [4, 6,21] and its immediate precursor lesions [9], HPV testing has now been approved in the worldwide as an adjunct to cytology for triage at all ages and for general screening in women aged 30 years and above [5]. One possible method to expand HPV-based cervical cancer screening to underserved populations is self-collection of cervicovaginal specimens. Cervical cancer screening by papanicolaou (Pap) smear has shown its use in reducing both incidence and mortality. Nowadays, cervical tumors are mostly diagnosed in women who were not or not properly screened.

Cervical tissue is commonly infected with human papilloma virus. In the present study, 15 benign and 50 different samples of cancer cervix were screened for E6 protein using a polyclonal antibody E-6. Also screened for $H_{11}B_2C_2$ antigen (HABP) using mAb $H_{11}B_2C_2$

Materials and Methods

HPV16, E-6 (Goat polyclonal IgG, Santa Cruz Biotechnology). Regular laboratory chemicals and reagents of analytical grade were purchased either from E-Merck or Sigma, USA or Calbiochem, USA or Ranbaxy, India. Media and glasswares for mammalian cell culture were purchased from Gibco, BRL, Germany, Nunclon, Germany and Millipore, Germany.

Production of monoclonal antibody [mAb $H_{11}B_2C_2$]:

The antibody was originally produced by the fusion of a myeloma variant NS1 with spleenic lymphocytes from SJL/J mice, immunized with semi-

purified hyaluronic acid binding protein [22]. Hybridomas producing IVd4 antibody were selected, whose interaction with antigen was competed out by hyaluronic acid and hyaluronan oligomers [22]. Subsequent hybridomal clonal selections were performed by heat shock treatment, growing them in bovine serum and finally subcloned in filtered human serum of different blood groups received from the hospitals. Furthermore the hybridoma was selected in HAT and HT media in DMEM. One of the clones $H_{11}B_2C_2$ was selected. The antibody production in human serum of any blood groups did not affect $H_{11}B_2C_2$ antibody in recognizing the human antigen expressed in tissues derived from malignant tumors. The clone $H_{11}B_2C_2$ was grown in DMEM containing 10% (v/v) human serum. After 14 days the media was collected. The media collected was taken and an equal volume of cold saturated ammonium sulphate solution was added with constant stirring at 4^0C overnight and centrifuged at 12000rpm for 30min. The pellet was dissolved in PBS and dialyzed. After dialysis the antibody solution was lyophilized and antibody was dissolved in PBS whenever required.

Extraction of protein from benign and malignant human cervical tissues

Fresh tissues from benign and malignant cervical cancer samples were collected from the hospitals in cold PBS and stored at–20^0C. Before extraction the samples were resuspended in lytic buffer and then homogenized (1:4w/v) in lysis buffer in a glass-Teflon homogenizer. The lysate was centrifuged at 10000rpm for 45min and an aliquot of the supernatant was assayed for protein concentration using Bradford method.

Detection of E-6 antigen (HPV-16) in benign and malignant human cervical tumor tissue using a polyclonal antibody E-6

After extraction, equal amount of protein (250µg) from each of the tissue sample extract was taken and electrophoresed on a 12.5% SDS-PAGE [23] at 25mA constant current and electro transferred to a PVDF (Immobilon-p, Millipore) membrane at 200mA for 1hr at room temperature. The membrane was washed extensively with Tween-TBS buffer. The membrane was incubated with E-6 polyclonal antibody (1:200 dilutions) incubate overnight at 4^0C. Next day the membrane was washed with Tween-TBS. The membrane was then incubated with secondary antibody (goat anti rabbit IgG biotin conjugated 1:1500 dilution) for 1hr at room temperature. The membrane was washed with Tween-TBS. The membrane was treated with HPO-9 (Strepta avidin peroxidase, Sigma 1:3000 dilution) for 1hr at room temperature. After extensive washing with Tween-TBS, the immuno-reactive proteins were visualized with ECL western blotting detection reagents (Pierce).

Immunoprecipitation analysis of E-6 antigen from benign and malignant cervical tissues and cross-reaction with $H_{11}B_2C_2$ antigen [HABP]

Two hundred fifty milligram of total protein was taken from different benign and malignant cervical tissue extracts. Dilute the extract if required with lysis buffer. Add 25ml of protein A-CL agarose, 5ml of protein G-CL agarose and incubated at 4^0C with intermittent mixing. Spin the tube for 10min in a cooling centrifuge and transfer the supernatant to a fresh tube. To the supernatant add 25ml [5mg] of E-6 polyclonal antibody and incubate the tube at 4^0C for overnight with intermittent mixing. After overnight incubation the antigen-antibody complex is pulled down using 20ml of protein A-CL agarose and 10ml of protein G-CL agarose for 1hr at 4^0C on a mixer. Spin the lysate for 20mins in a cooling centrifuge at 10000rpm and discard the supernatant. Wash the pellet by resuspending in 1ml of TTBS and placing on a rotary mixer at 4^0C for 5mins. Spin in a cooling centrifuge for 5mins and repeat the wash steps twice or more. After final wash resuspend the pellet in 40ml of 1x sample buffer and elute the antigen by heating the tube to 1000C for 5min. in a water bath. Run samples under reducing conditions on a 12.5% SDS-PAGE at 25mA constant current. Same samples were run simultaneously in two different lanes and separated in the gel and were transferred onto a PVDF membrane in a western blotting unit at 200mA constant current for 1hr. After transblotting the membrane was incubated with mAb $H_{11}B_2C_2$ monoclonal antibody 1:100 dilution overnight at 4^0C. Next day the membrane was washed with Tween-TBS. The membrane was then incubated with secondary antibody (goat anti rabbit IgG biotin conjugated) 1:1500 dilution for 1hr at room temperature. The membrane was washed with Tween-TBS. The membrane was then treated with HPO-9 (Strepta avidin peroxidase, Sigma) 1:3000 dilution, for 1hr at room temperature. After extensive washing with Tween-TBS, the immunoreactive proteins were visualized with ECL western blotting detection reagents (Pierce).

Immunoprecipitation analysis of $H_{11}B_2C_2$ antigen [HABP] from benign and malignant cervical tissues and cross-reaction with E-6polyclonal antibody.

Two hundred fifty milligram of total protein was taken from different benign and malignant cervical

tissue extracts. Dilute the extract if required with lysis buffer. Add 25ml of protein A-CL agarose and 5ml of protein G-CL agarose and incubated at 4^0C with intermittent mixing. Spin the tube for 10mins in a cooling centrifuge and transfer the supernatant to a fresh tube. To the supernatant add 25ml (5mg) of $H_{11}B_2C_2$ monoclonal antibody and incubate the tube at 4^0C for overnight with intermittent mixing. After overnight incubation the antigen-antibody complex is pulled down using 20ml of protein A-CL agarose and 10ml of protein G-CL agarose for 1hr at 4^0C on a mixer. Spin the lysate for 20min in a cooling centrifuge at 10000rpm and discard the supernatant. Wash the pellet by resuspending in 1ml of TTBS and placing on a rotary mixer at 4^0C for 5min. Spin in a cooling centrifuge for 5mins and repeat the wash steps twice or more. After final wash resuspend the pellet in 40ml of 1x sample buffer and elute the antigen by heating the tube to 100^0C for 5min in a water bath. Run samples under reducing conditions on a 12.5% SDS-PAGE at 25mA constant current. Same samples were run simultaneously in two different lanes and separated in the gel and were transferred onto a PVDF membrane in a western blotting unit at 200mA constant current for 1hr. After transblotting the membrane was incubated with E-6 polyclonal antibody, 1:200 dilutions overnight at 4^0C. Next day the membrane was washed with Tween-TBS. The membrane was then incubated with secondary antibody (goat anti rabbit IgG biotin conjugated) 1:1500 dilution for 1hr at room temperature. The membrane was washed with Tween-TBS. The membrane was then treated with HPO-9 (Strepta avidin peroxidase, Sigma) 1:3000 dilution, for 1hr at room temperature. After extensive washing with Tween-TBS, the immunoreactive proteins were visualized with ECL western blotting detection reagents (Pierce).

Results

E-6 antigen expression by Western blot

The expression of E-6 antigen was analyzed by western blotting method by taking malignant cervical tumor and benign tumor tissues. Proteins were resolved in a 12.5% SDS-PAGE, then transblotted it on PVDF and treated with E6 polclonal antibody in all of the samples, the E6 protein was expressed. When compared with molecular weight markers it was found to be 18KD. Graphs showing the intensity of 18KD protein in each lane of the blots were done using Scion Image analysis software. These results are shown in Figure1.

$H_{11}B_2C_2$ antigen expression by Western blot

The expression of H11 antigen (HABP) was analyzed by western blotting method by taking malignant cervical tumor tissues Proteins were resolved in a 12.5% SDS-PAGE, then transblotted and treated with mAb $H_{11}B_2C_2$ in all of the samples, $H_{11}B_2C_2$ protein (HABP) was expressed. When compared with molecular weight markers they major bands were found to be 55-57KD and 30KD. Graphs showing the intensity of 57KD protein in each lane of the blots were done using Scion Image Analysis software. These results are presented in Figure2.

Immunoprecitated analysis of E-6 antigen and cross-reaction with H11antigen

The immunoprecitated E-6 protein from benign cervix crude sample, malignant cervix crude sample and malignant cervix sample purified on Q-Sepharose (5omM) fractions were separated on 12.5% SDS-PAGE under reducing conditions. They were transblotted on PVDF membrane and reacted with mAb $H_{11}B_2C_2$.From the markers, it was identified as a 18KD protein in each lane The blots were done using Scion Image Analysis. These results are given in Figure3.

Immunoprecitated analysis of H_{11}antigen and cross-reaction with polyclonal antibody E-6

The mAb $H_{11}B_2C_2$ immunoprecitated protein of the benign cervix crude sample, malignant cervix crude sample and malignant cervix sample purified on Q-Sepharose (5omM) fractions were separated on 12.5% SDS-PAGE under reducing conditions. They were transblotted on PVDF membrane and reacted with polyclonal antibody E-6. Probably E6 protein may have binding affinity for H11 antigen (HABP). The blots were done using Scion Image Analysis. These results are presented in Figure4.

Discussion

The presence of HPV in almost all of the cervical carcinomas is one of the most compelling evidence in the present study. In invasive squamous cell carcinomas the over expression of HPV 16 was observed using biochemical experiments. Low-risk HPV E6 and E7 proteins critically contribute to viral life cycle [24]. These results suggest that HPV 16 has a very high prevalence among women with invasive cervical cancer, therefore, the use of a prophylactic vaccine for HPV types 16 and 18 could prevent up to 75% of invasive cervical cancers. Current concept is the identification of human papillomavirus (HPV) as a cause of cervical cancer and their precursor lesion

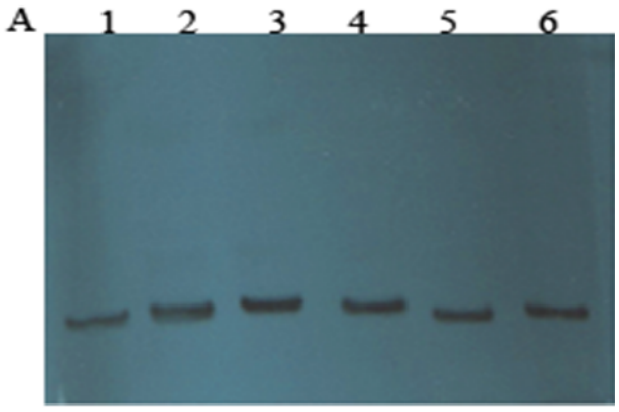

Figure1. Western blooting pattern of E6 protein (18KD) treated with E6 polyclonal antibody.
Lane: 1,2 and 3 Ca. cervix
Lane: 4,5 and 6 benign cervix.

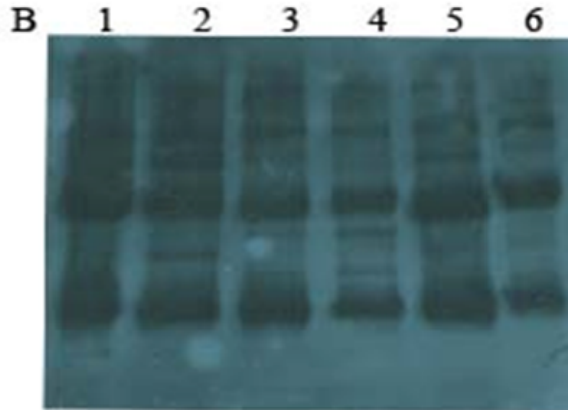

Figure2. Western blooting pattern of H_{11} antigen (HABP) protein from Ca.cervix treated mAb $H_{11}B_2C_2$ showing two proteins of mol wt 55-57KD and 30KD

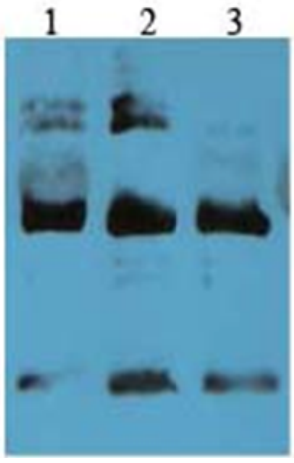

Figure3. Western blooting pattern of immunoprecipitated E6 Protein treated with mAb $H_{11}B_2C_2$ as developed by ECL.
Lane: 1, Benign cervix, Lane: 2, 3, Ca. Cervix

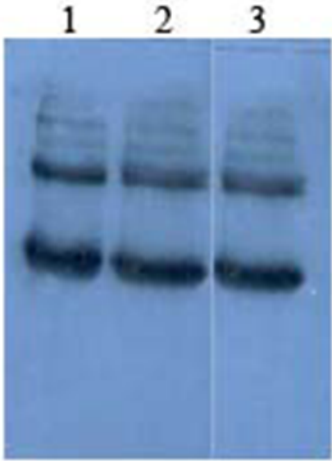

Figure4. Western blooting pattern of immunoprecipitated mAb H11

indicates that HPV vaccines can potentially be used to prevent or treat cervical cancer and other HPV-associated malignancies.

It was observed in the experiments that there is a very high concordance between H11 antigen (HABP) and cervical E- 6 antigen. It is not surprising that in the present study, it was found that in the tumors of suspected cervical cancer tissues the E6 antigen was expressed. Earlier results showed that H11 antigen (HABP) has hyaluronan binding protein sites and it is a common denomitor for all human tumors during tumor progression, however, whether E-6 antigen has hyaluronan binding protein site or not is still inconclusive. It was decided to do cross reaction experiments with mAb $H_{11}B_2C_2$ with E6 protein.

E-6 immunoprecitated proteins from cancer cervix tissue were reacted with mAb $H_{11}B_2C_2$. These results show that HPV 16 expressing 18KD protein along with hyaluronic acid binding proteins 57KD and 92KD. This shows that E-6 antigen itself reacting with hyaluronic acid binding sites. The HA-HA receptor and HPV 16 interaction both in intracellular and in cell surface activates hyaluronan mediated signaling for the malignant tumor cells during early stage of malignancy. The cumulative data suggests over-expression of $H_{11}B_2C_2$ antigen (HABP) in tumor cells is

an important parameter and a clinical diagnostic marker for progressive human tumors.

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Conflict of Interest

The authors declare that they have no conflict of interest in this article.

Authors' Contribution

The biochemical experiments and writing the manuscript were done by all authors. The biochemical studies were done by SM.

References

1. De Villiers, E. M., L. Gissmann, H. zur Hausen. Molecular cloning of viral DNA from human genital warts. J. Virol. 1981; 40:932-5.

2. Gissmann, L., H. zur Hausen. Partial characterization of viral DNA from human genital warts (Condylomata acuminata). Int. J. Cancer, 1980; 25:605-9.

3. Zur Hausen, H. Papillomaviruses and cancer: from basic studies to clinical application. Nature, 2000; 2:342-50.

4. Bosch Manos N, Munoz M, Sherman A, Jansen J, Peto M, Schiffman H, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J. Natl. Cancer Inst. 1995; 87:796-802.

5. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol.1999; 189: 12-9.

6. Hillemanns P, Kimmig R, Huttemann U. Screening for cervical neoplasia by self-assessment for human papillomavirus DNA. Lancet 1999, 4; 354(9194):1970.

7. Wright TC, Denny L and Kuhn L,. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA, 2000; 283:81-6.

8. Scheffner M, Werness BA, Huibregtse J M, Levine AJ and Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell. 1990; 63: 1129-36.

9. Schiffman M., Herrero R,Hildesheim A, Sherman M. E, Bratti M., Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. JAMA. 2000; 283: 87-93.

10. Dyson N., Guida P, Münger K, Harlow E. Homologous sequences in adenovirus E1A and human papillomavirus E7 proteins mediate interaction with the same set of cellular proteins. J. Virol.1992; 66:6893-6902.

11. Jiang M, Milner J. Selective silencing of viral gene expression in HPV-positive human cervical carcinoma cells treated with siRNA, a primer of RNA interference. Oncogene, 2002; 21:6041-8.

12. Butz K, Ristriani T, Hengstermann A, Denk C, Scheffner M, Hoppe-Seyler F. siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. Oncogene. 2003; 22:5938-45.

13. Yoshinouchi M, Yamada T, Kizaki M, Fen J, Koseki T, Ikeda Y,et al. In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by E6 siRNA. Mol Ther.2003; 8: 762-8.

14. De Roda Husman AM, Walboomers JM, van den Brule AJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol.1995; 76: 1057-62.

15. Vassilakos P, Griffin S, Megevand E, Campana A. Cyto Rich liquid-based cervical cytologic test. Screening results in a routine cytopathology service. Acta Cytol. 1998; 42:198-202.

16. Draganov, P, Todorov S, Todorov I, Karchev T, Kalvatchev Z. Identification of HPV DNA in patients with juvenile-onset recurrent respiratory papillomatosis using SYBR Green real-time PCR. Int. J. Pediatr. Otorhinolaryngol. 2006; 70: 469-73.

17. Del Mistro A, Salamanca HF, Treyisan R, Bertorelle R, Parenti A, Bonoldi E, et al. Human papillomavirus typing of invasive cervical cancers in Italy. Infect Agent Cancer. 2006; 27:1-9.

18. Sin. J.I Human papillomavirus vaccines for the treatment of cervical cancer. Experts rev Vaccines .2006; 5: 783-92.

19. Gok M, Coupe VM, Berkhof J, Verheijen RH, Helmerhorst TJ, Hogewoning CJ, et al. HPV16 and increased risk of recurrence after treatmentforCIN. Gynecol Oncol. 2007; 104: 273-5.

20. Linn YY., Alphs. H., Hung CF, Roden RB, Wu TC. Vaccines against human papillomavirus. Front Biosci.2007; 12: 246-64.

21. Vassilakos P, Griffin S, Megevand E, Campana A. CytoRich liquid-based cervical cytologic test. Screening results in a routine cytopathology service. Acta Cytol. 1998; 42:198-202.

22. Banerjee S.D, Toole BP .The determinants of migrating with a pre-arranged job and of the initial duration of urban unemployment: an analysis based on Indian data on rural-to-urban migrants.J Dev Econ. 1991; 36:337-51.

23. Laemmli UK. Cleavage of structural proteins during the assembly of the Head of bacteriophageT4.Nature. 1970; 227:680-5.

24. Oh ST, Longworth MS, Laimins LA. Roles of the E6 and E7 proteins in the life cycle of low-risk human papillomavirus type 11. J. Virol. 2004; 78: 2620-6.