Prevalence of HPV Infection in Head and Neck Cancer Patients in Egypt: National Cancer Institute Experience.

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Research Article

Keywords: HPV, HNSCC, HPV Direct Flow CHIP System, HPV66, PCR, genotyping

Posted Date: July 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-49031/v1>

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Abstract

Background: The new classification of Head and Neck Squamous Cell Carcinoma (HNSCC) according to their association with human papillomavirus (HPV) infection into HPV positive tumors with good prognosis and HPV negative tumors with poor prognosis has changed the treatment strategy of HNSCCs dramatically worldwide. However, data concerning HPV infection in HNSCCs patients is still limited and there are no studies regarding HPV genotypes in HNSCC among Egyptians. Therefore, the aim of the current study is to investigate the frequency of HPV and to detect the most prevalent HPV genotypes in the patients with oral cavity and oropharyngeal carcinoma from Egypt

Methods: This cross sectional study was conducted from January 2016 to November 2017. Ninety-two oral brushing samples (76 oral cavity and 16 oropharynx) were collected by cytobrushing in CYTO-fast solution. HPV-DNA was subjected to two methods which are the: (1) HPV Direct Flow CHIP system for automatic detection and genotyping (2) conventional PCR for detection of HPV using consensus primers (MYO9/MYO11). SPSS software was used for statistical analysis.

Results: Out of the 92 cases assessed, HPV DNA was present in three samples only (3/92; 3.3%) and the HPV66 genotype was the only genotype detected by HPV Direct Flow CHIP System.

Conclusion: The Results of this study may (1) indicate that the HPV-associated HNSCC among Egyptian patients is very low and (2) highlight the presence of HPV66 genotype in these malignancies. Other studies from other countries in general and from different governorates in Egypt are still needed to determine the exact frequency of HPV infections in head and neck cancer.

Introduction

Head and Neck Squamous Cell Carcinomas (HNSCCs), which include cancers of the oral cavity, oropharynx, and larynx, have an estimated annual burden of 330,000 deaths and more than 650,000 incident cases, making it the sixth most common cancer worldwide [1]. In Egypt studies have shown that HNSCCs account for about 17–20% of all cancers [2]. Tobacco smoking, alcohol consumption and infection by Human Papillomavirus (HPV) are considered the major risk factors for the development of these diseases [3].

HPV is a small, non-enveloped, double stranded, circular DNA virus with a genome of approximately 8000 base pairs that infects the skin and mucous membranes [4]. To date, more than 220 HPV genotypes have been identified. They are divided into two main groups according to their association with cancer: the low risk (LR) HPV types (6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84 and 89 (CP6108)) and the high risk (HR) HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 53, 56, 59, 66, 68, 70, 73, and 82) [5]. Recently, HNSCCs are classified according to their association with HPV into HPV positive (+) and HPV negative (-) where HPV + HNSCCs patients have an improved outcome to the current treatment options [6].

In the last few decades, a significant increase of HPV infection in younger males with higher Socioeconomic status was observed in the United states [7]. However, the situation in Egypt is still unclear where limited data is available with only few studies in this issue (8,9). This highlights the need to assess this topic and to investigate the association between HPV infection and HNSCC in Egyptian patients.

The majority of published studies investigating the prevalence of HPV among HNSCCs patients used many different methods for HPV testing in fresh frozen, and/or formalin fixed paraffin-embedded tumor tissue samples including the polymerase chain reaction (PCR), in situ hybridization (ISH) or immunohistochemistry (IHC) using P16 expression.(10,11). Taken in consideration that each method has different sensitivity and/or specificity.

Among the HPV detection methods is the HPV Direct Flow CHIP (e-BRID System™) (Hospitex Diagnostics, Florence, Italy), which is an automated instrument that is able to identify 18 high-risk and 18 low-risk human papillomavirus (HPV) genotypes rapidly and sensitively. It is based on direct PCR from crude-cell extracts, automatic flow-through hybridization, and colorimetric detection. This test has been used mainly for HR-HPV testing in cervical specimens. However, it has not been routinely implemented in testing for HR-HPV in the head and neck SCC cases.(12,13). Another obvious advantage using the e-BRID System™ is that it has a very high sensitivity for HPV detection and it can be performed in a very short time compared to other systems and thus reducing the total processing time from hours to minutes.

In the current study, we sought to assess the prevalence of HPV among Egyptian patients with head and neck carcinomas, as well as to identify the most common HPV genotypes in Egypt using the HPV Direct Flow CHIP System. The results will be compared to those obtained by testing the same oral brushing samples using the conventional PCR.

Materials And Methods

Sample Collection

A total of 92 tissue samples (76 oral cavity and 16 oropharynx) were collected from patients with clinically obvious lesions during their first consultation at the Surgical Pathology Unit, National Cancer Institute, Cairo University in the period from January 2016 to November 2017 and before any treatment, (Whether radiotherapy or chemotherapy was received). The samples were taken by rubbing the lesional mucosa of the patient with an Orcellex Brush ® (Rovers Medical Devices B.V., Oss, The Netherlands) and rotating the brush 5–10 times. The brush was then swirled vigorously in 17 mL of a CYTO-Fast™ Collection Vial for preservation. Cases with insufficient number of cells and those with missed clinical data were excluded from the study. The clinicopathological features of the tested patients (age, sex, tumor grade and stage, lymph node metastasis, a history of smoking, and the marital status) were collected from patients' files.

DNA Extraction

Although the HPV Direct Flow CHIP System works properly with direct PCR from crude-cell extracts without DNA extraction, we started with a purified DNA in order to avoid any contamination and to ensure that we have enough DNA for the direct PCR by the e-BRID System™. In addition, purified DNA was also used for HPV-DNA amplification by the conventional PCR technique. The cytobrush samples were collected in CYTO-Fast™ Collection Vials and centrifuged at 2000 rpm for 10 minutes at room temperature. The samples were then washed with PBS (Phosphate Buffer Saline), centrifuged again at 2000 rpm for 10 minutes at room temperature and the supernatant was then removed and the pellet was treated using 100 µL of proteinase K. The DNA was finally extracted by using (QIAamp® DNA Mini Kit), according to manufacturer's instructions and

the extracted HPV and human genomic DNA were immediately stored at – 20° C until used for PCR amplification.

The quantity and quality of the extracted DNA were determined in terms of concentration and purity using the UV spectroscopy (NanoDrop8000, Thermo scientific, USA).

HPV Testing

Two molecular HPV assays were used in parallel for the detection and genotyping of HPV from the extracted genomic DNA.

HPV detection and genotyping by HPV Direct Flow CHIP

Six µl of the purified DNA of each sample was mixed with specific formulation of the PCR mix and Taq DNA polymerase in the HPV Direct flow CHIP kit (Master Diagnóstica, Granada, Spain). This allows PCR to be completed within 60 min only in which amplification of a 268 bp fragment of the human beta-globin gene (internal control) and a 150 bp fragment of the HPV L1 region (GP5+/GP6+) occurs. The PCR products were denatured at 95 °C for 5 min (in a thermocycler) just before hybridization and then were chilled on ice for at least 2 min. The reverse dot blot hybridization and read-out of the results were then performed automatically in the e-BRID System™ (Hospitex Diagnostics, Florence, Italy), which takes 90 minutes only to complete the run of 15 samples.

A positive sample is defined when a signal in the spot “B” (hybridization control), spot “C” (PCR control), spot “U” (HPV Universal probe) and in the corresponding HPV genotype probes appears.

HPV detection by Conventional polymerase chain reaction (PCR)

The purified DNA was subjected to conventional PCR amplification that amplifies 450 bp of the HPV-L1 region using consensus primers MY09 (5_ CGTCCMARRGGAWACTGATC- 3) and MY11 (5_- GCMCAGGGWCATAAYAATGG- 3). A known HPV positive control and a negative control (water) were included in each run. The amplification products were visualized by means of electrophoresis analysis on 1% agarose gels containing ethidium bromide (0.5 mg/ml).

Statistical analysis

The data were analyzed using the **SPSS** (Statistical Package for the Social Science V.21). The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. The association between categorical variables was tested using the Chi-square test while Fischer exact test was used when the expected cell count was less than 5. Continuous variables were presented as mean ± SD (standard deviation) and the two groups were compared with Student *t* test.

Results

Study population

This study was conducted on ninety-two (92) patients who were diagnosed clinically and histopathologically as HNSCCs: seventy-six (82.6%) patients had oral cavity SCC and the other sixteen (17.4%) patients had oropharyngeal SCC. The mean age of the studied patients was 57.63 years with the highest frequency in patients ≥ 60 years old. Males represented 55.4% of the studied group and females represented 44.6%. Regarding Marital status and smoking, 98.9% of the studied cases were married and out of those 38% were smokers. Twenty-nine (31.5%) patients were positive for the lymph node metastasis and (68.5%) were negative. The clincopathologic data of the 92 patients according to HPV positivity are summarized in (Table 1).

Table 1
Clinicopathologic data of the patients according to HPV positivity:
(n = 92)

Clinicopathologic data	n(%)	Positive HPV (n = 3) n(%)
Sex		
Male	51 (55.4)	2 (66.7%)
Female	41 (44.6)	1 (33.3%)
Age		
< 50 y	15 (16.3)	0 (0%)
50–60 y	37 (40.2)	2 (66.7)
≥ 60 y	40 (43.5)	1 (33.3)
Tumor location		
Oral cavity	76 (82.6)	2 (66.7)
OP	16 (17.4)	1 (33.3)
Marital Status		
Married	91 (98.9)	3 (100.0)
Single	1 (1.1)	0 (0.0)
Smoking		
Smoker	35 (38.0)	1 (33.3)
Non–Smoker	57 (62.0)	2 (66.7)
Tumor grade		
Hyperplasia	1 (1.1)	0 (0.0)
Grade 1	9 (9.8)	0 (0.0)
Grade 2	71 (77.2)	3 (100)
Grade 3	11 (12.0)	0 (0.0)
Lymph node metastasis		
Positive	29 (31.5)	2 (66.7)
Negative	63 (68.5)	1 (33.3)

(OP) oropharyngeal
HPV Results

Using the e-BRID System™, a total of 92 patients were examined for the presence of HPV DNA as well as for genotyping. Only three specimens out of all the studied specimens were positive for HPV (3.3%) and all of them were HPV 66 genotype only (Fig. 1).

When the same specimens were validated by subjecting them to the conventional PCR to detect the HPV DNA, the same results were obtained where we found the same three positive cases (3.3%). This exactly 100% similarity of the results from the two techniques was reported in the detection of HPV, however, an exact conclusion cannot be reached since the number of positive cases is too small.

Regarding the anatomical location of the tumor in the positive cases; the oral cavity showed the highest prevalence of HPV DNA (66.7%) compared to the oropharynx (33.3%). All positive cases were married, two of them were males (one smoker and one non-smoker) and one female (non-smoker). The median age of the HPV positive cases was 57.67 (range 51–64 years).

Discussion

Numerous studies confirmed the involvement of HR-HPV types in the development and progression of HNSCCs, especially in the oropharynx. (5,7,14) However, the prevalence of HPV in the HNSCCs differs between various studies and populations. These differences may be attributed to geographical difference among them, the differences in risk factor-associated behavior between population under study from different countries, or the use of different technical detection methods. In this study, we were able to detect HPV positivity in only 3.3% of all studied HNSCCs patients who ranged in age from 51 to 64 years using both the HPV Direct Flow CHIP System and the PCR suggesting that the infection of HPV associated HNSCCs in Egypt is very low.

Comparable results have also been reported in a recent study by Alsbeih et al who investigated the association between HPV infection and HNSCCs in patients from Saudi Arabia. The authors proved that in Saudi Arabia, the proportion of HNSCC attributed to infection with HPV is very low as in Egypt since they also found only a 3.5% prevalence of HPV DNA among the studied cases (6/28 oropharyngeal and 4/257 Oral cavity cancers) [15]. Another study in Jordan by Qatouseh et al examined the prevalence of HPV DNA in 108 paraffin embedded tissue samples also showed a relatively low prevalence of HPV DNA (14.8%) [16].

In contrast, a slightly higher prevalence (28%) of HPV DNA in oropharyngeal Squamous Cell Carcinoma (OPSCC) has been reported in a group of Egyptian patients assessed by Tealab et al, who investigated the presence of HPV in 99 paraffin embedded tissue samples.[8] This relatively high prevalence compared to our results may be due to the difference in methods and type of samples used, where they detected HPV by P16 IHC as a first line screening test followed by ISH for confirmation which is highly recommended by pathologists of the American Society of Clinical Oncology (ASCO).[17]. Although the low prevalence of HPV in our study might be due to the brush sampling that we used for the collection of the samples from the patients, However, previous studies provided evidence that brush sampling is considered a suitable, safe and non-invasive sampling method for the detection of HPV. It is also useful in obtaining information with few side effects from patients quickly. Broglie et al, also confirmed this as they demonstrated that the liquid-based brush cytology specimens obtained from oropharyngeal lesions accurately identified patients with OPSCC with positive HR-HPV status [18]. However, till now there is currently no preferred method for HPV detection from cytological samples and the choice of any method is dependent on the pathologist and the laboratory [19]. Although there

are numerous methods used for the detection of HPV we chose the HPV Direct Flow CHIP System (for the first time) in samples from HNSCC because of its sensitivity and ability to automatically detect 36 HPV genotypes either as single or multiple infection.

Our results may be contrast with other previously published data in developed countries. For example, in the United States, about 60–70% of their OPSCC (oropharyngeal squamous cell carcinoma) cases were HPV-related. [20]. Since HPV is a sexually transmitted virus and several Studies have shown higher numbers of lifetime oral sex partners (> 5) and a higher number of lifetime vaginal sex partners (> 25) have been associated with increased risk of HPV positive head and neck malignant tumors. Although these results might be normal and acceptable in the developed countries. However, in Egypt due to religious and ethical issues, these habits are strongly rejected by most Egyptians especially the elder as well as in the people who are living in the rural area. This might provide an explanation to the very low prevalence of HPV in the HNSCCs.

In relation to the anatomical location, the oral cavity appeared to have a greater HPV positivity (66.7%) compared to the oropharynx (33.3%). Our findings are similar to those reported by Vietía et al.[22].

At the end to our knowledge, there is no previous study in Egypt reported the prevalence of HPV genotypes in HNSCCs till now. The high incidence of high-risk HPV genotypes, especially genotypes16 and 18, have been well established and reported in several studies at all the anatomical location. (22,23). However, the role of HPVs as etiological factor for HNSCCs is still under debate. Interestingly, HPV66 genotype has been reported in all positive samples. HPV66 is an α -papillomavirus that infect mucosal membranes and belong to the high risk HPV group.[25]. The presence of HPV66 genotype in the Egyptian patients, as an interesting finding of this study, might be related to the geographical region. Moreover, the specific HPV genotypes detected in OPSCC which seem to be influenced by the geographic location recommend further studies in order to (1) evaluate the reasons for these genotypic differences in HR-HPV types in OPSCC. (2) provide an explanation for the exact reason of the low incidence of HPV infection in head and neck tumors in Egypt.

To our knowledge, the current study is the first study to both detect and genotype HPV in HNSCCs in Egyptian patients. Furthermore, it is the first study to report the association between HPV66 and different head and neck sub sites. Another important point that strengthen our study is the use of two HPV detection methods; the e-BRID System™ and the conventional PCR. The later used MY09 & MY11 set of primers which from previous studies were considered the best primers to amplify the HPV DNA compared to other HPV primers.[26].

On the other hand, one of the limitations of the current study is the low number of the included specimens that did not allow us to perform adequate analysis. Therefore, a follow-up study containing larger number of samples is highly recommended to assess and confirm the possible changes in trends of HNSCC among Egyptians in different regions of the country, especially urban areas in which the population have a higher socioeconomic status. Another limitation to our study is that we did not genotype the studied samples by PCR since we only used it as a confirmation for the presence of the virus.

In conclusion, the results of the current study confirm that (1) the HPV Direct Flow Chip System is suitable for routine analysis and allows the detection of HPV DNA from oral brush samples of head and neck cancer with a comparable sensitivity and specificity to conventional PCR. (2) The presence of HPV-DNA (HPV66) in only three cases in our study suggests that the proportion of HNSCC attributable to infection by HPV seems to be very low

in Egypt. Hence, further studies covering diverse geographical and socioeconomic groups including different governorates are needed to delineate the profile of HPV infectivity and genotypes in HNSCCs in Egyptian population.

Abbreviations

HNSCC

Head and neck squamous cell carcinoma.

HPV

Human papillomavirus.

PCR

Polymerase chain reaction.

ISH

In situ hybridization.

ICH

Immunohistochemistry.

HR-HPV

High risk Human papillomavirus.

IRB

Institutional Review Board.

PBS

Phosphate Buffer Saline.

SPSS

Statistical Package for the Social Science.

OPSCC

oropharyngeal Squamous Cell Carcinoma.

ASCO

American Society of Clinical Oncology.

Declarations

Ethics approval and consent to participate

The study was ethically approved by the Institutional Review Boards (IRB) of the National Cancer Institute, Cairo University. Organization No- IORG0003381 (IRB NO-IRB00004025).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by National Cancer Institute, Cairo University.

Authors' contributions

Doaa A. Salem carried out the practical work, data analysis, wrote and edited the manuscript. Sherif Bahaa, Mahmoud N. El Rouby and Yehia A. Osman participated in coordination and supervised the work. Sherif Bahaa supervised the collection of samples from patients. Abeer A. Bahnassy reviewed the manuscript critically for its scientific content. Abdel-Rahman N. Zekri participated in the study design and coordination and supervised all the work. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* [Internet]. 2018;68:394–424. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30207593>
2. Attar E, Dey S, Hablas A, Seifeldin IA, Ramadan M, Rozek LS, et al. Head and neck cancer in a developing country: A population-based perspective across 8 years. *Oral Oncol* [Internet]. Elsevier Ltd; 2010;46:591–6. Available from: <http://dx.doi.org/10.1016/j.oraloncology.2010.05.002>
3. Taberna M, Mena M, Pavón MA, Alemany L, Gillison ML, Mesía R. Human papillomavirus-related oropharyngeal cancer. *Ann Oncol*. 2017;28:2386–98.
4. IARC. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER IARC Monographs on the Evaluation of Carcinogenic Risks to Humans VOLUME 90 Human Papillomaviruses. *Int. Agency Res. Cancer*. 2007.
5. Tumban E. A Current Update on Human Papillomavirus-Associated Head and Neck Cancers. *Viruses*. 2019;11.
6. Božinović K, Sabol I, Dediol E, Milutin Gašperov N, Manojlović S, Vojtechova Z, et al. Genome-wide miRNA profiling reinforces the importance of miR-9 in human papillomavirus associated oral and oropharyngeal head and neck cancer. *Sci Rep*. 2019;9:1–13.
7. Boscolo-Rizzo P, Del Mistro A, Bussu F, Lupato V, Baboci L, Almadori G, et al. New insights into human papillomavirus-associated head and neck squamous cell carcinoma. *Acta Otorhinolaryngol Ital* [Internet]. 2013;33:77–87. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23853396> <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3665382>

8. Tealab SH, Sedhom NFH, Hassouna A, Gouda I, Ismail H. Prevalence of human papilloma virus in oropharyngeal, tongue and lip squamous cell carcinoma: an experience from the Egyptian National Cancer Institute. *J Investig Med*. 2019;jim-2018-000968.
9. Mansour A, Ali M, Helmy H, Kassim S. Human papillomavirus-16 (HPV-16) infection association with CIAP-2 expression in head and neck cancer. *Med Oncol*. 2012;29:2459–65.
10. Kim KY, Lewis JS, Chen Z. Current status of clinical testing for human papillomavirus in oropharyngeal squamous cell carcinoma. *J Pathol Clin Res*. 2018;4:213–26.
11. Abreu ALP, Souza RP, Gimenes F, Consolaro MEL. A review of methods for detect human Papillomavirus infection. *Virol J*. 2012;9:1–9.
12. Herraéz-Hernández E, Preda O, Alonso S, Pardo RS, Olmo A. Detection and Genotyping of Human Papillomavirus DNA in Formalin-Fixed Paraffin-Embedded Specimens with the HPV Direct Flow CHIP System. *Open Virol J*. 2013;7:91–5.
13. Herraéz-Hernández E, Alvarez-Perez M, Navarro-Bustos G, Esquivias J, Alonso S, Aneiros-Fernandez J, et al. HPV Direct Flow CHIP: A new human papillomavirus genotyping method based on direct PCR from crude-cell extracts. *J Virol Methods* [Internet]. Elsevier B.V.; 2013;193:9–17. Available from: <http://dx.doi.org/10.1016/j.jviromet.2013.04.018>
14. Chaturvedi AK. Epidemiology and Clinical Aspects of HPV in Head and Neck Cancers. *Head Neck Pathol*. 2012;6:16–24.
15. Alsbeih G, Al-Harbi N, Judia S Bin, Al-Qahtani W, Khoja H, El-Sebaie M, et al. Prevalence of human papillomavirus (HPV) infection and the association with survival in saudi patients with head and neck squamous cell carcinoma. *Cancers (Basel)*. 2019;11.
16. Abu Qatouseh L, Sabri I, Alkhatib I, Atwa E, Arafat T. Detection of High-Risk Human Papillomavirus Genotypes 16 and 18 in Head and Neck Squamous Cell Carcinomas in Jordan. *Asian Pac J Cancer Prev*. 2017;18:1337–41.
17. Fakhry C, Lacchetti C, Rooper LM, Jordan RC, Rischin D, Sturgis EM, et al. Human papillomavirus testing in head and neck carcinomas: ASCO clinical practice guideline endorsement of the college of American pathologists guideline. *J Clin Oncol*. 2018;36:3152–61.
18. Broglie MA, Jochum W, Förbs D, Schönegg R, Stoeckli SJ. Brush cytology for the detection of high-risk HPV infection in oropharyngeal squamous cell carcinoma. *Cancer Cytopathol*. 2015;123:732–8.
19. Russell-goldman E, Krane JF. Molecular Diagnostics in Cytopathology. *Mol Diagnostics Cytopathol*. 2019;181–97.
20. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Paula Curado M, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol*. 2013;31:4550–9.
21. Young D, Xiao CC, Murphy B, Moore M, Fakhry C, Day TA. Increase in head and neck cancer in younger patients due to human papillomavirus (HPV). *Oral Oncol* [Internet]. Elsevier Ltd; 2015;51:727–30. Available from: <http://dx.doi.org/10.1016/j.oraloncology.2015.03.015>
22. Vietía D, Liuzzi J, Ávila M, De Guglielmo Z, Prado Y, Correnti M. Human papillomavirus detection in head and neck squamous cell carcinoma. *Ecancermedicallscience*. 2014;8:1–8.
23. Yete S, D'Souza W, Saranath D. High-Risk Human Papillomavirus in Oral Cancer: Clinical Implications. *Oncol*. 2018;94:133–41.

24. Li Y, Xu C. Human papillomavirus-related cancers. *Adv Exp Med Biol.* 2017;1018:23–34.

25. Kotsopoulos IC, Tampakoudis GP, Evaggelinos DG, Nikolaidou AI, Fytli PA, Kartsiounis VC, et al. Implication of human papillomavirus-66 in vulvar carcinoma: A case report. *J Med Case Rep.* 2011;5:2–6.

26. Qu W, Jiang G, Cruz Y, Chang CJ, Ho GYF, Klein RS, et al. PCR detection of human papillomavirus: Comparison between MY09/MY11 and GP5+/GP6+ primer systems. *J Clin Microbiol.* 1997;35:1304–10.

Figures

Figure 1

HPV results by the HPV Direct Flow Chip System. a- show the distribution of spots in the membrane where the spot “B” (hybridization control), spot “C” (PCR control), spot “U” (HPV Universal probe) and 36 HPV genotype probes in duplicates. b- A positive HPV 66 sample. a signal in the spot “B” (hybridization control), spot “C” (PCR control), spot “U” (HPV Universal probe) and in the corresponding HPV genotype probe

Figure 2

HPV results by conventional PCR on the agarose gel. Agarose gel 1% electrophoresis of PCR products for MY09/11 primers for HPV. M: 100 Molecular weight marker, lane 1: Negative Control, lane 2: Positive control, lanes 3-5 three negative samples, and lane 6: Positive sample (450 base pairs).