ORIGINAL ARTICLE

# Genotyping of High Risk Human Papillomavirus (HPV) Among Cervical Precancer and Cancer Patients

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**ABSTRACT**

**Introduction**: Human papillomavirus (HPV) is a DNA virus which has tropism for epithelial cells, is the major etiological factor for development of cervical precancerous and cancerous lesions. Nearly 100 different types of HPV have been characterized and thereare a large number of other types. HPV infection is one of the most common causes of sexually transmitted disease in both men and women worldwide. It is associated with a variety of clinical conditions that range from innocuous lesions to cancer. Genital HPV types are divided into high and low-risk types, according to the oncogenic potential. Molecular and epidemiologic studies have solidified the association between high risk HPV types (especially HPV-16 and HPV-18) and cervical squamous cell carcinoma. HPV infection is often transient and self-limiting but infection may persists and progress to high grade lesions and cancer. In addition to persistent high-risk HPV infection, other viral factors such as high viral loads, HPV variants, infections with multiple high-risk HPV types and genetic predisposition contribute to the development of cervical cancer. The aim of the present study was to detect HPV DNA and identify high risk HPV genotype among women having cervical intraepithelial neoplasia and carcinoma and to evaluate potential efficacy of prophylactic HPV vaccine. **Methods:** Cervical swab from histopathologically diagnosed CIN (n=51) and carcinoma (n=39) patients were taken and high risk HPV DNA was detected by HC II assay. Polymerase Chain Reaction was used to identify high risk HPV genotype. **Result**: HPV DNA was detected in 41 (45.56%) patients by HC II assay. HPV type 16 was detected in 27 (81.82%) followed by type 18 in 3 (9.09%) and type 45 in 2 (6.06%) cases of cervical carcinoma. Among precancerous cases, only type 16 was detected. **Conclusion**: Knowledge based on HPV prevalence and genotype could be used to predict the efficacy of cost effective prophylactic vaccine, introduction of newer generation vaccine and management of cervical carcinoma.

**Keywords:** Genotyping, Human papillomavirus, Polymerase chain reaction

## INTRODUCTION

Human Papillomavirus infection is one of the most common sexually transmitted diseases worldwide. Up to 70% of sexually active women globally may become infected with HPV during their lifetime.[1] Infection with oncogenic HPV types is the most significant risk factor for developing cervical precancers and cancer.[2] HPV is responsible for development of cervical cancer & virtually every cervical cancer (99.7%) is HPV positive.[3] Some studies demonstrated that more than 90% of cervical cancers causes by HPV DNA, while some studies have demonstrated HPV in all cases of cervical cancer.[4] The risk of cervical neoplasia is greatest among women who develop persistent high risk HPV infections.[5]

HPVs are small circular double-stranded DNA viruses that belong to the *Papovaviridae* family. There are over 100 different HPV types, among which 40 are known to infect the genital tract.[6] HPV genotypes can be classified into high-risk and low-risk groups according to the propensity for malignant progression of the associated lesions.[6] Among these, 15 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) are classified as high risk HPV types; 3 HPV types (26, 53, 66) are classified as probable high risk[7] and 12 HPV types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81) are classified as low risk types.[8] Low-risk HPV types, such as 6 and 11 induce benign lesions with minimum risk of progression to malignancy. By contrast, high-risk HPVs (HR-HPV) have higher oncogenic potential. In North India, the percentage prevalence of high risk HPV in case of squamous cell carcinoma in

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decreasing order are- HPV 16 (64.8%), 18 (14.7%), 45 (6.4%), 33 (6.4%), 35 (5%), 58 (3.8%), 59 (2.1%), 56 (1.9%), 31 (1.7%) and 51 (1.4%) and in case of adenocarcinoma percentage prevalence are- HPV 16 (51.7%), 18 (34.5%), 31 (6.9%), 62 (5.9%), 33 (3.5%), 42 (3.5%) and 45 (3.5%) (Bhatla*et al*., 2008). Globally, HPV type 16 and 18 contribute to over 70% of all cervical cancers, while HPV types 31, 33, 35, 45, 52 and 58 are responsible for approximately 20% cases.[9]

Certain cervical intraepithelial neoplasias (CINs) with persistent HPV infection progress to invasive cervical cancer though a fraction of them regress during the course of time – 60% in case of CIN I, 40% of CIN II and 33% in CIN III.[9] Thirty percent of CIN I persist, 10% progress to CIN III, and 1% progress to invasive cancers. Approximately 40% of all CINs persist and only about 1% of CIN I, 5% of CIN II and 15% of CIN III advance to invasive cancers.[10]

In a study from Bangladesh, the prevalence of high risk HPV infection was detected in 60% of different grades of CIN and 4.1% of control women.[11] Age specific incidence rates of cervical cancer in Bangladesh are highest compared to Southern Asia and the World (WHO/ICO Information Center on HPV Summary Report 2010).[12]

Increased development of cervical cancer has been associated with other risk factors, which are responsible for interaction between host and virus which also increases the susceptibility of HPV infection. These factors are early age of sexual contact, multiple sexual partners, increased parity, poor sexual hygiene, prolonged use of oral contraceptives, smoking and altered immune status of the patients.[13, 14, 15] Other sexually transmitted diseases such as *Chlamydia trachomatis*, Herpes simplex virus type II and Human immunodeficiency virus as well as some poorly known dietary factors are likely intervening factors.[16]

Identification of individual HPV types is essential to investigate the epidemiology and clinical characteristics of particular types. Detection of high-risk HPV types in genital specimens has been approved in several countries for women with a cytological diagnosis of atypical squamous cells of undetermined significance (ASCUS) and also for primary cervical cancer screening in women aged 30 years and above as an adjunct to cytology.[17] In the management of women with ASCUS, HPV characterization helps improve the understanding of prevalence, individual risk stratification, persistence, reinfection, co-infection and development of effective vaccines.[18] Detection of type-specific HPV DNA in abnormal smears may be used as a more specific predictor of high-grade cervical intraepithelial neoplasia[19] and identification of high-risk HPV genotypes may permit selection of patients who are at increased risk for disease.[20]

Cervical cancer usually develops slowly from precursor lesions. Therefore, regular screening should be performed in order to provide a significant impact on its morbidity and mortality. Screening every year or every three years play an important role in cervical cancer prevention programs.[21]The most powerful application of HPV genotyping is in primary screening.[22] Genotyping should be done to understand the prevalence of HPV of the respective country because there is regional variation in HPV genotypes among the female population.[23] Persistence of oncogenic HPV types is important risk factor and predictor of CIN's progression and cancer.[24] An effective genotyping test for HPV will contribute to identify woman who are at risk of developing cervical cancers, to monitor genotype specific HPV infection, towards the development of type specific vaccines and to determine the appropriate clinical management strategy. Presently, two vaccines are available: one is a quadrivalent vaccine named Gardasil (Merck & Co. USA) against type 6,11,16,18. Another bivalent vaccine prepared against type 16 and 18 named Cervarix (GlaxoSmithKline, UK) is being used in Bangladesh. Knowledge of prevalent genotypes in pre-cancer and cancer patients will contribute to the development of effective type specific vaccines.

First generation HPV16/18 vaccines have the potential to provide 75–80% protection against invasive cancer in India. HPV 45, 33, 35 and 58 which account for an additional 20% cases of cervical cancer should be considered for inclusion in second-generation HPV vaccines.[25] Theoretically, an HPV vaccine with 100% efficacy in preventing HPV 16 and 18 infections could potentially reduce the cervical cancer burden by more than 60%, assuming 100% coverage.[26]

The two methodologies most widely used for HPV DNA detection are PCR and Hybrid capture II system.[27] HPV DNA can be detected by hybridization techniques in approximately 89.0% of cervical cancers and approximately 33.8% of low-grade lesions.[28] It is designed to provide quantitative estimate loads, which may correlate with grade and natural history of cervical pathology.[29] It detects the DNA of the virus from cervical samples even years before this virus causes genital disease or cervical cancers that may spread to other organs of the reproductive system. The hybrid capture assay has proved to be a reliable, accurate and reproducible HPV test method for routine clinical practice.[30]

Polymerase Chain Reaction is particularly useful for detection of HPV types with known DNA sequences from small amount of tissue specimens.[31] Among cervical carcinoma HPV DNA can be detected in 70% cases by type specific PCR.[32] This is a highly sensitive method[33], easy to interpret and can characterize multiple virus types in case of multiple infection[34] or at least one virus type in cases of multiple infections.[35]

In a study from Bangladesh, the most prevalent high-risk HPV types among women of high risk behavior were types 16 (33.1%), 18 (14%), 58 (10.9%), 45 (4.8%), 31 (4.1%) and 33 (3.1%).[36] Information on HPV type specific prevalence in women with or without cervical lesions is not available for Bangladesh and there are no systemic population based studies to estimate HPV prevalence.

Therefore, the present study has aimed to detect high risk genotypes of HPV among cervical pre-cancer and cancer patients in Bangladesh by using HC II assay and type specific PCR for E6/E7 gene amplification. The type specific PCR detects target HPV-DNA from different carcinogenic HPV types (high risk genotypes: 16, 18, 31, 33 and 45).

### Objectives

- To detect the presence of high risk HPV genotypes and quantitative high risk HPV DNA among cervical pre-cancer and cancer patients.

## MATERIALS AND METHODS

### Study Population

After taking informed written consent, the patients were interviewed for detailed history which was recorded in a pre- designed data collection sheet. With all relevant history 150 patients, who were VIA positive and had clinically suspected sign symptoms for cervical precancerous and cancerous lesions were preliminarily selected and referred for colposcopy to BSMMU hospital. VIA test positive patients were advised to do colposcopy after 14 days of the test. After examining the cervix by Cuscos speculum, 2 cervical samples were collected by gynecologist from each patient using the Cervex-Brush (Digene, Germany) and processed by using a liquid-based cytology medium (Digene, Germany). Samples of primarily selected 150 cases were stored at -20$^0$C at the Department of Virology, BSMMU. After that, colposcopy was performed. Out of the 150 preliminarily selected patients, 120 patients were diagnosed with cervical lesions by colposcopy and colposcopy guided cervical tissue was obtained for biopsy from these 120 patients. When all biopsy reports were available, 90 cervical pre-cancer and cancer cases were segregated. Then Hybrid Capture II assay and type specific PCR were carried out with the cervical swab samples of these 90 histological positive cases. The experimental protocol was approved by the Ethical Committee of the Bangabandhu Sheikh Mujib Medical University and written informed consent was obtained from all the subjects.

### Determination of HPV DNA by Hybrid Capture II Assay

The test was done by Hybrid Capture II (HC II) High-risk HPV DNA test kit (Digene Corporation, Gaithersburg, MD 20878, USA; catalog no- 5197-1330) according to the manufacturer's instructions.

### Genotyping by Polymerase Chain Reaction

#### *DNA extraction*

DNA was extracted by a commercially available kit (Sacace DNA- sorb-A DNA extraction kit, Sacace Biotechnologies Srl, Italy, REF- k-1-1/A) according to the manufacturer's instructions. DNA concentration was measured in ng/µl by spectrophotometer (Nanodrop 2000 UV-Vis spectrophotometer) measured at the ratio of absorbance at 260 and 280 nm.

#### *PCR protocol*

**PCR primers:** The selection of primers was based on the published work by Maki[37] for HPV type 16, Sotlar[38] for type 18, Shikova[39] for type 31 and 33 and Guo[40] for type 45.

**Summary of characteristics of the primers**

| Primers | Sequence (5′-3′) | Size of PCR product (amplicon) in bp |
|---|---|---|
| Type 16 F | AGCTCAGAGGAGGAGGATGA | 203 |
| Type 16 R | GGTTTCTGAGAACAGATGGG | |
| Type 18 F | CACTTCACTGCAAGACATAGA | 322 |
| Type 18 R | GTTGTGAAATCGTCGTTTTTCA | |
| Type 31 F | ATGGTGATGTACACAACACC | 514 |
| Type 31 R | GTAGTTGCAGGACAACTGAC | |
| Type 33 F | ATGATAGATGATGTAACGCC | 455 |
| Type 33 R | GCACACTCCATGCGTATCAG | |
| Type 45 F | GGACAGTACCGAGGGCAGTGTAA | 71 |
| Type 45 R | TCCCTACGTCTGCGAAGTCTTTC | |

Note: F: Forward, R: Reverse

### Statistical Analysis

Statistical analysis was done with SPSS (Statistical Packages for Social Science) software version 19.The statistical significance was evaluated by Chi-square test.A p value of <0.05 was considered as statistically significant.

## RESULTS

Of the 90 patients enrolled in the study 41 (45.56%) cases were detected by HC II assay and 33 (36.67%) cases by type specific PCR. Out of 29 CIN I cases 2 (6.90%) cases, 18 CIN II cases 2 (11.11%) cases and 4 CIN III cases 2 (50%) cases were detected by HC II assay. By type specific PCR, 1 (3.45%) CIN I cases, 1 (5.56%) CIN II cases and 1 (25%) CIN III cases were detected. Among 39 carcinoma cases, 35 (89.74%) cases detected by HC II assay and 30 (76.92%) cases were detected by type specific PCR(Table 1). Out of 90 cases in this study, maximum 34 (37.8%) belonged to the 31-40 years age group, followed by 22 (24.4%) women > 50 years. Only 1 (1.1%) case was < 20 years of age. According to the age at first sexual exposure among HPV positive 53 women with cervical lesions, 26 (49.06%) had history of exposure before 18 years of age, while 16 (40.54%) women among 37 cases had history of sexual exposure after the age of 18 years (Figures 1-5)

It was observed that HPV positive cases increased with the number of parity. Out of the 28 cases who had 4 or more than 4 children, 20 (71.43%) had HPV infection, among 21 cases who had 3 children,11 (52.38%) had infection with HPV, 5 (19.23%) HPV infected patient had 2 children. Out of 2 nulliparous women both had HPV infection. A statistically significant relation was observed between HPV positivity and parity (p value= 0.001). History of contraceptive use revealed that 23 (56.10%) women took oral contraceptive pills (OCP), 6 (40%) women used barrier method, 4 (40%) women used intrauterine device, and 8 (33.33%) women could not gave the history of any contraceptive methods or they never used any contraception in their lifetime.

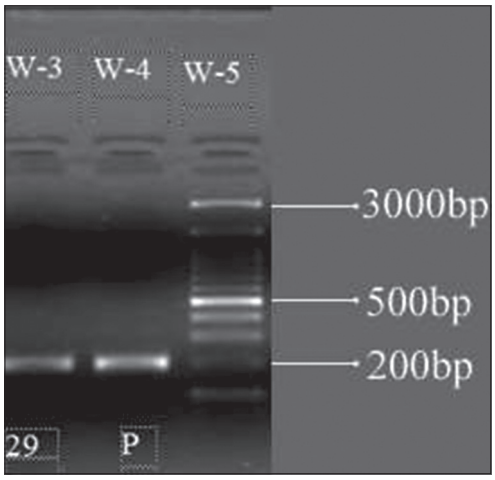


**Figure 1:** Shows HPV type 16 in agarose gel. In well-5, 100 bp, DNA ladder is used as molecular weight marker. In well-4, band, represents positive control. Band in well-3 indicate, patient sample. Molecular weight of type 16 is 203 bp

**Table 1: Distribution of HPV positive cases among women with cervical lesions**

| Histo-pathological diagnosis | No. of patient | HPV positive cases (HC II assay)* | HPV positive cases (PCR)¤ |
|---|---|---|---|
| CIN I | 29 | 2 (6.90%) | 1 (3.45%) |
| CIN II | 18 | 2 (11.11%) | 1 (5.56%) |
| CIN III | 4 | 2 (50%) | 1 (25%) |
| Carcinoma | 39 | 35 (89.74%) | 30 (76.92%) |
| Total | 90 | 41 (45.56%) | 33 (36.67%) |

Note: a) *HC II assay detects 13 HR HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), b) ¤PCR detects 5 HR HPV (types 16, 18, 31, 33 and 45)

**Table 2: Relation between cervical lesions and HPV infection detected by HC II assay**

| Histological diagnosis | No. of total cases | HPV positive cases | HPV negative cases |
|---|---|---|---|
| CIN's | 51 | 6 (11.76%) | 45 (88.24%) |
| Carcinoma* | 39 | 35 (89.74%)¤ | 4 (10.26%) |
| Total | 90 | 41 (45.56%) | 49 (54.44%) |

Note: a) CIN's includes CIN I, CIN II and CIN III. b) *All the carcinoma cases were tagged under broad term carcinoma. These included 37 squamous cell carcinoma and only two adenocarcinoma cases. c) ¤33 squamous cell carcinoma and 2 adenocarcinoma cases

Table 2 shows the relation between histopathological findings and HPV DNA test in study population. Among 51 CIN's positive cases, 6 (11.76%) cases were HPV DNA

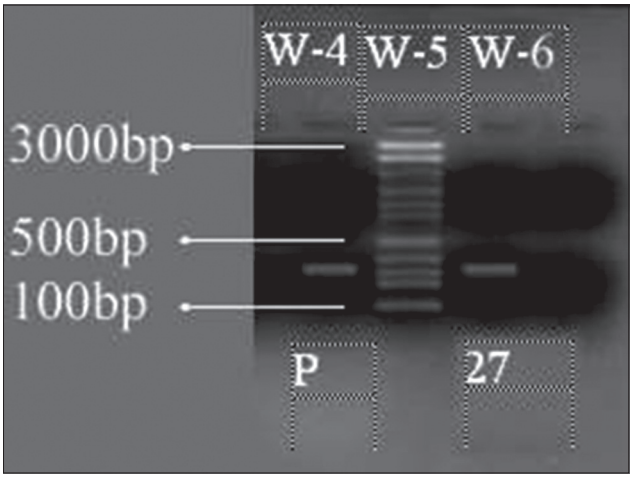


**Figure 2:** Shows HPV type 18 in agarose gel. In well-5 100 bp DNA ladder is used as molecular weight marker. In well-4, band represents positive control. Band in well-6 indicate patient sample. Molecular weight of type 18 is 322 bp

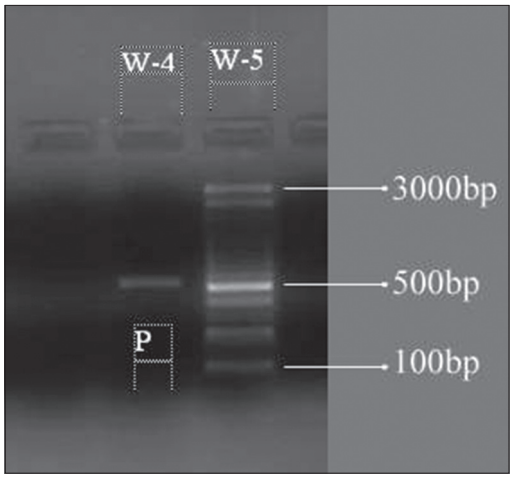


**Figure 3:** Shows HPV type 31 in agarose gel. In well-5 100 bp DNA ladder is used as molecular weight marker. Well-4 band represents positive control. Molecular weight of type 31 is 514 bp

**Table 3: Relationship between viral load of HC II positive samples and different cervical lesions**

| Histological diagnosis | Viral load (RLU/COV) | | | | P value |
|---|---|---|---|---|---|
| | Low (1 to <10) | Moderate (10 to <100) | High (100 to <1000) | Very high (≥ 1000) | |
| CIN's (n=6) | 3 (50%) | 1 (16.67%) | 2 (33.33%) | 0 | 0.04 |
| Carcinoma (n=35) | 3 (8.57%) | 11 (31.43%) | 10 (28.57%) | 11 (31.43%) | |
| Total (n=41) | 6 (14.63%) | 12 (29.27%) | 12 (29.27%) | 11 (26.83%) | |

Note: Chi square test was done to measure the level of significance Chi square value-8.314, degree of freedom- 3

positive by HC II assay. Among 39 histologically diagnosed carcinoma cases, 35 (89.74%) cases were HPV DNA positive. Of these 35 HPV DNA positive carcinoma cases, 33 (94.28%) were squamous cell carcinoma and only 2 (5.7%) were adenocarcinoma.

Association of viral load with different types of histologically proven cervical lesions is shown in Table 3. Among very high viral load group, 11 (31.43%) cases were from carcinoma group. Among high viral load group, 10 (28.57%) cases had carcinoma, 2 (33.33%) case had cervical intraepithelial lesions. In case of moderate viral load, 11 (31.43%) cases had carcinoma, and only 1 (16.67%) case had CIN lesions. Among low viral load group, 3 (8.57%) cases had carcinoma, 3 (50%) case had CIN lesions. There was significant relationship between different groups of cervical lesions and viral load (p value=0.04).

Table-4 Shows HPV infection in different cervical lesions by type specific PCR. Among 90 study patients, a total of 33 (36.67%) cases were HPV positive when detected by type specific PCR. Of these, 3 (5.88%) case was from CIN lesion, while the majority 30 (76.92%) cases were from carcinoma group. Among these 30 HPV positive carcinoma cases, 28 (93.33%) squamous cell carcinoma cases and both (6.67%) cases of adenocarcinoma were detected by type specific PCR.

Table-5 summarizes different HPV genotypes detected among CIN's and cervical carcinoma patients by type specific PCR. Among 33 genotyped HPV cases, 27 (81.81%) were type 16, 3(9.09%) were type 18 and 2 (6.06%) cases were type 45, while only one (3.03%) case was co-infection with type 18/45.Out of 33 genotyped cases, 30 (90.9%) were from carcinoma group and 3 (3.03%) case each was genotyped from CIN I, CIN II and CIN III cervical lesions. The presence of oncogenic types increased with the severity of CIN and carcinoma. In cervical carcinoma a total of 30 (90.9%) type specific genotypes were identified, of these the most prevalent 24 (80%) cases were identified as type 16. Type 18 and 45 were identified only in carcinoma cases. By using specific primers, type 18 was identified in 3 (10%), and type 45 in 2 (6.67%) cases respectively. Co-infection with type 18/45 was observed in only one (3.03%) carcinoma patient. In case of squamous cell carcinoma type most cases were type 16, type 18 and 45 also identified and in 2 cases of adenocarcinoma, one co-infection with type 18/45 and one type 16 was identified. Among CIN, only type 16 was identified in all three groups.

**Table 4: Detection of HPV infection by type specific PCR**

| Histological diagnosis | No. of total patients | HPV positive cases | HPV negative cases |
|---|---|---|---|
| CIN's | 51 | 3 (5.88%) | 48 (94.12%) |
| Carcinoma* | 39 | 30 (76.92%)¤ | 9 (23.04%) |
| Total | 90 | 33 (36.67%) | 57 (63.33%) |

Note: a) CIN's indicates CIN I, CIN II and CIN III. b)* All the carcinoma cases were tagged under broad term 'carcinoma'. These included 37 squamous cell carcinoma and only two Adenocarcinoma cases. c) ¤28 squamous cell carcinoma and two adenocarcinoma case

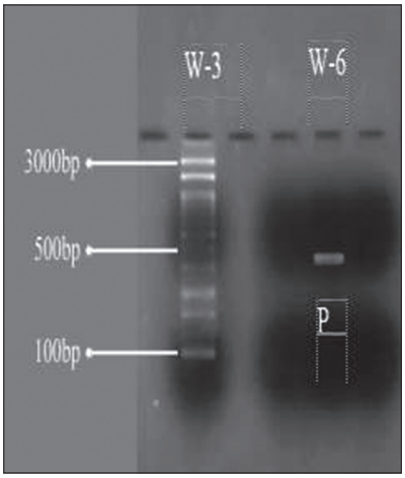


**Figure 4:** Shows HPV type 33 in agarose gel. In well-3, 100 bp DNA ladder is used as molecular weight marker. In well-6 band represents positive control. Molecular weight of type 33 is 455 bp

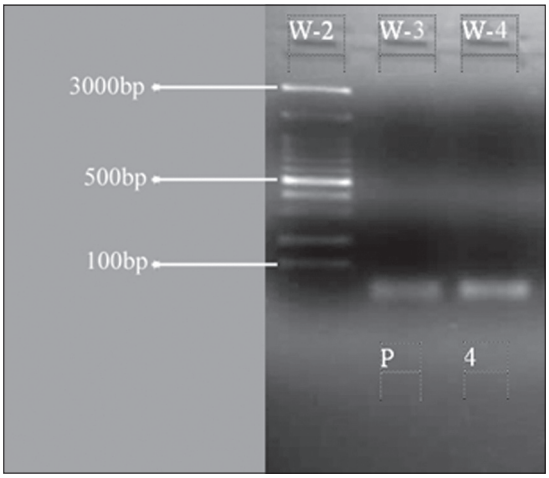


**Figure 5:** Shows HPV type 45 in agarose gel. In well-2, 100 bp DNA ladder is used as molecular weight marker. In well-3 band represents positive control and well-4, band indicate patient sample. Molecular weight of type 45 is 71 bp

**Table 5: Detection of HPV genotypes by type specific PCR**

| Histological diagnosis | PCR positive (%) for genotypes | | | | | |
|---|---|---|---|---|---|---|
| | Type 16 | Type 18 | Type 31 | Type 33 | Type 45 | Type 18/45 |
| CIN's (n=3) | 3 (100%) | 0 | 0 | 0 | 0 | 0 |
| Carcinoma (n=30) | 24 (80%) | 3 (10%) | 0 | 0 | 2 (6.67%) | 1 (3.33%) |
| Total (n=33) | 27 (81.82%) | 3 (9.09%) | 0 | 0 | 2 (6.06%) | 1 (3.03%) |

## DISCUSSION

Accurate HPV genotyping is essential for adequate classification of patients into low-risk or high-risk groups. A number of studies have demonstrated that cervical cancer is associated with high-risk human papilloma virus (HPV) genotypes.[3] Presence of multiple genotypes may reflect repeated exposure HPV infection.[5] Persistence of HPV infection has been identified as an important risk factor for cervical carcinoma and should be included in clinical testing.[41] HPV infections can only be classified as truly persistent if identical subtypes are detected in consecutive samples during follow-up studies. Persistent HPV infection is more common with the high-risk oncogenic HPV types and is an important determinant in the development of cervical cancers.[16] HPV viral load may also be a valuable predictor of disease. Thus, the present study was undertaken to detect HPV DNA and genotyping of some common high-risk HPV types among cervical precancer and cancer patients attending the BSMMU hospital.

HPV prevalence was related to some risk factors in the present study. Before 18 years of age, 26 (49.06%) HPV positive patients had history of first sexual exposure. In India, the association of infection of high-risk HPVs with the age of marriage below 18 years was found to increase the risk of cervical cancer by 22 fold.[42] Another study from India showed that the risk of HPV infection was higher in married women below 20 years of age (odds ratio, 1.80).[43] Women who began to have sexual experience before the age of 16 were found to have a two-fold higher risk of high-grade CIN and cervical cancer (OR=2.17, 95% CI=1.3-3.7) than women who had exposure for the first time after the age of 16.[44] This increased risk may be due to the fact that younger age may have exposure to a persistent HPV infection for a longer time than women having exposure at later age.[45] The present study also demonstrated similar associations.

Significant association with parity and HPV infection (p value=0.001) was observed in the present study. A previous study observed that the risk of HPV infection was higher in women with parity ≥4 (odds ratio, 1.04).[43] In the large IARC study, women with seven or more full-term pregnancies had a four-fold increase in the risk of developing squamous-cell carcinoma as compared with nulliparous women (odds ratio = 3.82, 95% CI: 2.66-5.48). Events related to the second and third trimesters of pregnancy or to delivery may be relevant with this factor. The concentrations of oestrogens and progesterone in blood are known to increase progressively during pregnancy to reach the highest levels in the last weeks of pregnancy. These hormonal changes are probably responsible for the alterations in the junction between the squamous and columnar epithelium (transformation zone) occurring during pregnancy, thus facilitating the direct exposure to HPV.[46]

Long term oral contraceptive pill users are at risk of cervical cancers.[46] In this study, 23 (56.10%) HPV positive patients had history of taking oral contraceptive pills, while 6 (40%) HPV positive patients used barrier method for contraception. In terms of risk factor of hormonal contraception use, a study for International Agency for Research on Cancer, Multicentric Cervical Cancer Study Group showed that long-term use of oral contraceptives could be a cofactor as it increases risk of cervical carcinoma by up to four-fold in women who are positive for cervical HPV DNA. The odds ratio for use of oral contraceptives was 2.82 (95% CI=1.46- 5.42) for 5-9 years, and 4.03 (95% CI=2.09-8.02) for use for 10 years or longer.[14] In addition, a study from Thailand also observed that the risk of cervical cancer increased with parity and use of oral contraceptives, but not with injectable progesteron. The study also found that factors that may predispose to persistent oncogenic HPV 16 or 18 infections may include estrogens or progestins in the presence of estrogens.[47] Hormonal-related mechanisms may influence the progression from pre-malignant to malignant cervical lesions by promoting integration of HPV-DNA into the host genome, which results in deregulation of E6 and E7 expression (IARC, 1995).

In the present study, 41 (45.56%) cases out of 90 cases were positive by HC II assay. Most importantly, out of 39 carcinoma cases, 35 (89.74%) cases were positive by this detection method. Other studies have indicated that HPV DNA positivity increases with severity of CIN and carcinoma (p trend=0.004).[40] Using this method, high-risk HPV DNA was detected in 96.7% of cervical cancer patients from Bangladesh.[48] DNA of the high risk HPV types was found in over 90% of cervical cancers and in their precursor lesions.[49, 50]

Comparatively high viral load was detected in carcinoma cases than in other categories of histological diagnosis in the present study. Among 35 carcinoma cases, 11 (31.43%) cases had very high viral load. In HPV positive CIN's patients, 3 (50%) cases were from low viral load group indicating that probably when histological stage progresses, the viral load also increases. This was evident from the result of the present study where among 35 HC II positive carcinoma cases 3 (8.57%) had viral load <10 RLU index, 11 (31.43%) had viral load < 100 RLU index and 21 (60%) had viral load ≥ 100 RLU index. In this study, 60% of carcinoma showed a viral load of ≥ 100 RLU index. In a study, Cremoux[51] reported that 71% of cervical carcinoma had viral load ≥100 RLU index. Viral load of HR-HPV may vary between different individuals of same histological types. The replication of HPV can only occur in the presence of episomal form of the

genome, thus increasing the load rather than the integrated form. Co-infection with other types of HPV may decrease the viral load.[52]

There is very few data available regarding the analysis of viral load in HC II assay. In a similar study, Tozetti[53] stratified the load into 4 groups, but in a retrospective study Xu[54] categorized them into three groups. Moderate to high viral loads of HR- HPV were found to be significantly related to the histological grades of dysplasia (p=0.029).[54] Similar observations were also expressed by study conducted by Cremoux. In the present study, significant relation was observed when histological findings were analyzed with viral loads (Chi sq= 8.314, df= 3, p= 0.04). After comparative analysis of viral load and histological findings, it was observed that when histological severity increased the viral load also increased.

HPV genotype distribution among different categories of cervical histology by TS-PCR amplification of E6/E7 gene in this study detected 33 (36.67%) HPV positive cases. Importantly, out of 39 carcinoma cases, 30 (76.92%) cases were detected by this method. Increased prevalence of HR-HPV is related to the severity of cervical histopathology. So, the HR-HPV genotypes are associated with a risk of progression to cervical carcinoma.[55]

Genotyping may be a useful tool for classification of HPV positive women according to oncogenic potential and relative risk of progression to carcinoma, as well as for evaluating the efficiency and epidemiological impact of vaccination programs.[56] In this study, some common high-risk HPV genotypes were identified in CINs and cervical carcinoma. Among 33 genotyped HPV cases, 30 (90.9%) carcinoma cases were genotyped as type 16, 18 and 45. Types 16, 18, 45, 31, 33, 52, 58, and 35 accounted for 95 percent of the squamous-cell carcinoma positive for HPV infection. These findings have important implications for the prevention of cervical cancer. It implies that an effective vaccine against the five most common HPV types may prevent about 90 percent of cervical cancers worldwide. However, regional variation in the distribution of certain HPV types should be taken into account in the creation of vaccines tailored for different geographic regions.[21]

In this study, HPV type 16 was detected in 24 (80%) cases of cervical carcinoma. By using specific primers for 18 and 45, 3 (10%) and 2 (6.67%) cases were detected respectively. HPV 16 was observed to be the most prevalent (80%) high risk types in case of carcinoma, followed by type 18 (10%) and type 45 (6.67%). A similar study detected HPV type 16 as the most commonly (89%) present type in cervical cancer patients.[57] In India, HPV 16 in cervical cancer is 70-90% while HPV type 18 varied from 3 to 20% (WHO, 2010), which corresponds with this study. The five most frequent types from Brazil were HPV16 (77.6%), HPV 18 (12.3%), HPV 31 (8.8%), HPV 33 (7.1%) and HPV 35 (5.9%) in cervical carcinoma cases.[58]Most (75%) infections were caused by individual HPV types.[58] In case of squamous cell carcinoma, in most cases type 16 was identified and in 2 cases of adenocarcinoma, one coinfection with type 18/45 and one type 16 was identified. The distribution of HPV genotypes detected in cervical cancer varies depending on the histological type of the cancer. While HPV 16 is the most frequent genotype in SCC, HPV 18 is a predominant genotype in ADC. Some studies indicate that both HPV 16 and HPV 18 play a prominent role in the development of ADC of the cervix.[45]

The most prevalent genotype detected in the present study was HPV 16 irrespective of cervical pathology. This finding correlates with a study from Thailand by Lurchachaiwong.[55] HPV18 is mainly a risk factor for development of adenocarcinoma. The carcinoma samples for the present study mostly comprised squamous cell carcinoma with only two samples diagnosed as adenocarcinoma. Unfortunately, due to small sample size, this finding could not be confirmed in the present study. However, HPV16 and 18 are the most prevalent HPV types in cervical cancer worldwide, followed by types 45, 31, 33,52, 58 and 35.[24]

The present study detected co-infection with more than one type (18/45) in only 1 (3.33%) case of carcinoma patients. The prevalence of multiple HPV infections varies in relation to the method used to detect HPV DNA. Various studies have reported different prevalences, while 1.9% to 3.2% patients were reported to be infected with multiple HPVs in an individual visit.[59] In cervical carcinoma patients 8.1% were recorded to be infected with multiple types. Kleter[60] observed that multiple genotypes are less prevalent in carcinoma patients. However, some[61] observed that 35% HPV positive patients with advanced cytological disorder and >50% of HIV infected patients contained multiple HPV genotype. Single infection of HPV genotype have more significant effect on increasing risk of high-grade cervical lesions than multiple infection of HPV genotypes. Moreover, the multiple HPV infections are less frequent in high-grade than low-grade cervical neoplasia.[54]

There are various HPV types involved in cervical cancer depending on geographic distribution. In future, HPV testing will focus on primary screening and cervical cancer prevention. HPV genotype distribution will provide basic knowledge for HPV-based cervical cancer screening, cost-effective prophylactic HPV vaccine and assessment of vaccination against specific HPV infection in each geographic area.[56] A second generation HPV vaccine should focus on HPV 31, 33, 35, 45, 52 and 58 for prevention of

HPV infection in all regions of the world. In routine set-up or high-throughput, HPV typing is feasible.[60]

Since cervical cancer is the only cancer that is almost completely preventable through regular screening, further implementation of effectively organized screening programs and improvement of existing screening strategies and technologies will inevitably decrease the burden of this disease.[61] At present, a HPV genotyping assay has been adopted in developed regions as a main method for screening cervical lesions. A HPV vaccine is currently the first choice for the prevention of HPV infection and consequent reduction of the incidence of cervical cancers.[62]

Reliable identification of HPV genotypes may be relevant for patient management. In addition to study the effects of antiviral treatment or type specific vaccination, accurate HPV genotyping methods are essential. The knowledge of HPV genotyping of cervical cancer within a country or region is important for both primary screening and vaccination policy.

## CONCLUTION AND RECOMMENDATIONS

Very high viral load was observed in cancerous patient than precancerous patients. Type 16 (81.82%) was identified as the most prevalent HPV genotype, followed by type 18 (9.09%) and type 45 (6.06%). Type 31 and 33 could not be detected due to small sample size or due to the lesser prevalence. In all histological staging, type 16 was the most prevalent type. In the present study mixed infection with more than one type were also observed.

The data obtained from the present study provided some important background information that may be the basis of further elaborative and systemic studies. Higher education, better socioeconomic condition, awareness, detection and genotyping of HPV DNA can reduce the possibilities of progression of cervical carcinoma. Although various HPV types are involved in cervical cancer depending on geographical distribution, to achieve more reliable data and to detect these genotypes, testing should include all high risk HPV genotypes. Distribution of HPV genotype will provide basic knowledge for HPV based cervical cancer screening, cost effective HPV vaccines, follow up of efficacy of recently available vaccine, and introduction of newer and more effective vaccines.

## LIMITATIONS OF STUDY

- Since all high risk HPV types were not included in this study, less numbers of genotypes were identified.
- It was not possible to observe the genotypes with progression of lesions due to limited study period.
- The exact prevalence of HPV types in Bangladesh could not be identified as the present study was conducted on hospital based specimens due to time and budget constrains.

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