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RESEARCH PAPER

Distribution of Type Specific Human Papilloma Virus DNA Among Women of Cervical Cancer in a Tertiary Level Hospital

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Abstract

Background: Infection with high-risk human papilloma virus (HPV) has been recognised as a causal factor for development of cervical pre-cancerous and cancerous lesions. So far more than 150 types of HPV are identified. Their distribution varies from country to country, also from region to region. Background knowledge about the distribution of HPV genotypes in invasive cervical cancer is crucial to guide the introduction of prophylactic vaccines.

Objective: The study was aimed to assess the distribution of type specific human papilloma Virus DNA among cervical cancer patients.

Methods: The study was a cross sectional, observational and single centred one. It was carried out in the department of gynaecological oncology, of Bangabandhu Sheikh Mujib Medical University (BSMMU). Purposive sampling was done according to the availability of patients.

Results: HPV type 16 was detected in 35 cases (76.1%) followed by type 18 in 4 (8.7%) cases of cervical carcinoma. HPV 39, 56 and 68 were also detected, each was in 1 case (2.2 %). It was to be also found that 1 co-infection (2.2%) with HPV 33+35. HPV 16 was detected in 79.4 % of squamous cell carcinoma and in 70.0% of adenocarcinomas. HPV 18 was detected in 5.9 % of squamous cell carcinoma and 20.0% of adenocarcinomas. HPV 39 was detected in one case of small cell carcinoma (100.0%). One case of HPV 68 (2.9%) and one co-infection with HPV 33+35 (2.9 %) were found in squamous cell carcinoma. One case of HPV 56 (10.0%) was detected in adenocarcinoma of cervix. HPV DNA was not found in 3 cases of squamous cell carcinoma of cervix.

Conclusion: The distribution of HPV infection among Bangladesh women is similar to other regions of Asia. However, type specific patterns are different. The study findings will guide the formulation of HPV vaccination policies in Bangladesh, impact of vaccination programmes, to predict the efficacy of cost effective prophylactic vaccine, introduction of newer generation vaccine and finally prevention of cervical carcinoma in the country.

Keywords: Cervical cancer, Human papilloma virus, HPV DNA, Nanovalent vaccine

Introduction

Cervical cancer remains a major public health problem worldwide particularly in less developed countries. Globally, it is the fourth most common cancer among women with an estimated 569847 new cases and 311365 deaths in 2018.[1] Around 85% of these new cases and 80% of deaths occur in less developed countries.[2] In Bangladesh, it is the 2nd most common cancer among females with an estimated 11956 new cases and 6582 deaths in 2018.[1]

HPV is one of the most commonly acquired sexually transmitted infection which is identified in 99.7% cases of invasive cervical cancer.[3,4] Infection with oncogenic HPV types is the most significant risk factor for developing cervical precancers and cancer. HPV is a small non enveloped DNA virus of about 55 nm in diameter containing viral genome of circular double stranded DNA infecting mucosal and cutaneous keratinocytes.[5] So far, more than 150 sub types of HPV are known and are differentiated on the basis of their genetic sequences.[6] Upto 70% of sexually active

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women globally may become infected with human papilloma virus (HPV) during their lifetime.[7]

Mucosal infecting HPV are subdivided into low or high risk on the basis of their oncogenic potential. Low risk HPV types lead to the development of benign neoplasms such as warts and condyloma acuminatum whereas persistent infection of approximately 15 high risk HPV genotype causes almost all cases of cervical cancer (99.7%) and its immediate precursor lesions. Fifteen anogenital types are often termed as oncogenic.

HPV (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 and 12 HPV types (6,11,40,42,43,44,54,61,70,72,81) are classified as low risk types.[8] 16 and 18 account for about 70% of global cervical cancer cases with HPV-16 causing about 55-60% and HPV-18 about 10-15%.[9]

The estimated global HPV prevalence is reported to be 11.7%. While Country specific prevalence ranges were from 1.6% to 41.9% which is higher (14.3%) in developing regions than developed region (10.3%).[10] Prevalence of HPV infection among the general populations varies from (7-14) % in India, Bangladesh, Nepal and Srilanka. Hospital based statistics indicate that cervical cancer constitutes 20-35% of female cancers in different parts of Bangladesh and India.[11-13] In a study of Bangladesh, the prevalence of high risk HPV infection was detected in 60% of different grades of CIN and 4.1% of control women. Age specific incidence rates of cervical cancer in Bangladesh are highest compared to Southern Asia and the World (WHO /ICO Information Centre on HPV Summary Report 2010). The geographical variations may be due to prevalence of different sub types of HPV and host related factors. Moreover, demographic, cultural, socioeconomic variables, multiparity, long term contraceptive use, young age at first coitus, multiple sexual partners, low socioeconomic status, low education level, poor genital hygiene, cigarette smoking, genital tract infections, altered immune status of the patient etc are probable co-factors that increase the risk of cervical cancer in women with HPV infection.[14-16]

HPV can be detected by two methods. Hybrid capture method – it can identify whether a patient is infected with high risk HPV and Real Time PCR-by this method specific type of HPV can be detected.

Vaccination is a part of primary prevention to prevent cervical cancer. Vaccines are available that can protect against certain HPV infections. HPV vaccines that have been developed are based on recombinant expression and self-assembly of the major capsid protein-1 into virus like particle (VPL s) that resemble the outer capsid of whole virus. The HPV VPLs contain no DNA and are not life attenuated virus. Three vaccines are approved by the FDA to prevent HPV infection. It was in 2006 that HPV vaccines were licensed in the USA for use in females 9-26 years of age with the aim of preventing cervical cancers, precancerous lesions and genital warts. Currently vaccines available in global market are bivalent Cervarix (GlaxoSmithKline, UK), Quadrivalent Gardasil (Merck & Co USA) and Nano valent Gardasil 9. All three vaccines prevent infections with HPV types 16 and 18, two high risk HPV type that cause about 70 % of cervical cancers and an even higher percentage of some of the other HPV associated cancers. Gardasil in addition also prevents infection with HPV 6 & 11 which cause 90% of genital warts. Gardasil 9 prevents infection with same four HPV types plus five additional high risk types (31, 33, 45, 52, 58).[17,18]

HPV vaccines has been introduced in 2016 for the first time in Bangladesh by the Ministry of Health with support from Global Alliance for Vaccines and Immunizations (GAVI).[19] In Bangladesh, bivalent Cervarix and Quadrivalent Gardasil are available which were mainly developed to use for two main cancer causing HPV type 16 and 18. Knowledge of prevalent genotypes in cancer patients in Bangladesh will contribute to the introduction of effective type specific vaccines. The exact mechanism of HPV associated carcinogenesis of rare types of HPV is known little due to lack of insufficient epidemiological evidence. The biological properties of rare HR-HPV types have only been investigated in a few studies which included mostly lesions that were CIN and a few cases of invasive cervical cancer.

First generation HPV 16/18 vaccines have the potential to provide 75-80% protection against invasive cancer in India. HPV 45, 33, 35 and 58 which accounts for an

additional 20% cases of cervical cancer should be considered for inclusion in second generation HPV vaccines.[20] It is important to realise that no vaccine provides complete protection against all cancer causing types HPV, so routine cervical cancer screening is still necessary.

Materials and Methods

The study was a cross sectional, observational and single centered study. It was carried out in the department of gynaecological oncology, BSMMU, Dhaka during January-June 2019. Purposive sampling method was used according to the availability of patients. For safeguarding confidentiality and protecting anonymity each of the patient was given an ID number which was followed during examination and each and every step of the procedures. Signed informed consent was taken from the patient. Data collection sheet was enclosed for which a short interview was required. A total of 46 women were included in the study having age 21-65 years, all were histopathologically diagnosed case of carcinoma of cervix. Swab was collected from endocervix and also from ectocervix in case of growth or ulcer. Fourteen high risk genotype of HPV (type 16,18,31,33,35, 39,45,51,52,56,58,59,66 and 68) were detected by Real Time Polymerase Chain Reaction (PCR) using AmpliSens HPV High Carcinogenic Risk (HPV HCR) genotype-titre-FRT PCR kit, Russia after sample processing and DNA extraction. Then data were collected and analysed using SPSS software for windows.

Results

Regarding demographic variable of the study population, it was observed that the mean age of the study population was 48±10.8 years, with range from 21 to 65 years. Moreover, more than half (52.2%) of the study subjects belonged to age 50-60 years. Age of marriage was found 17±3.6 years with range from 11 to 24 years. Majority of patients (73.91%) were housewives. 31 (67.39%) had completed primary education. More than a half (60.87%) of patients came from 10000-20000 taka monthly income family. The mean age at first child birth was 19.1±3.7 years with range from 13 to 29 years.

Table I: Distribution of the study population by demographic variable (n = 46)

Age at marriage in years	No	Percentage
<= 18 years	30	65.21%
> 18 years	16	35%
Mean±SD	17±3.6	
Range(min, max)	11-24	
Educational status		
Illiterate	6	13.4%
Primary	31	67.4%
SSC	7	15.22%
HSC & above	2	4.34%
Monthly income (taka)		
10,000-20,000	28	60.87%
21,000-30,000	15	32.6%
> 30,000	3	6.52%
Occupational status		
House wife	34	73.91
Government worker	11	23.91
Service holder	1	2.17
Age at 1st delivery		
< 18	28	60.87
> 18	18	39.13
Mean±SD	19.1±3.7	
Range (min, max)	13-29	

More than half of patients (56.5%) took OCP followed by injectable contraceptive (30%) and other type of contraceptives (6.13 %).

Cervical carcinoma is associated with high parity. It was observed that, 33.72% patients had parity >= 5 followed by 11.24 % had para 2-4.

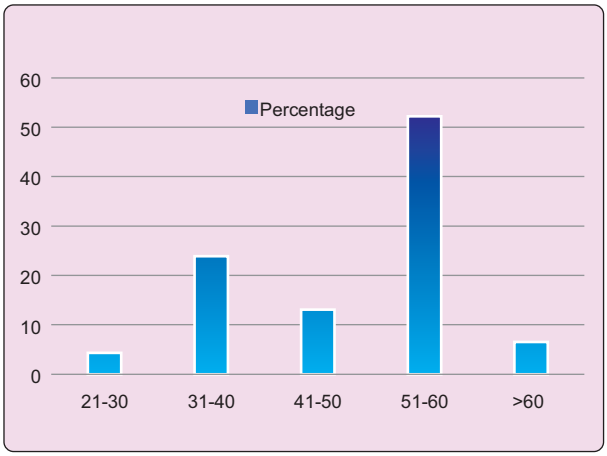

Figure 1: Age distribution of study population

Among 46 study population, Real time PCR could detect 93.47 % cases of HPV DNA in cervical cancer.

Table II: Distribution of study population by HPV DNA test and histopathological findings

DNA test (PCR)	No of patient	Percentage	
Negative	3	6.52%	
Positive	43	93.47%	
Histopathological Findings	no of population	percentage	*p* value
Invasive Squamous cell carcinoma	34	73.91	
Adenocarcinoma	10	21.74	
Adeno squamous	1	2.174	<0.001*
Neuroendocrine/ small cell carcinoma	1	2.174	
Others	0	0	

Regarding histopathological findings, it was observed that majority patients were found invasive squamous cell carcinoma 73.9 % (n=34) followed by adenocarcinoma 21.74% (n=10). The other two histopathological findings were adenosquamous 2.17 % (n=1) and Neuroendocrine or small cell carcinoma 2.17 %. (n=1).

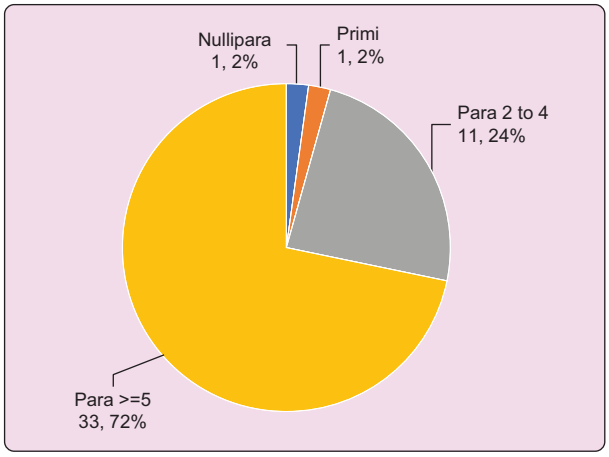

Figure 2: Distribution of parity of the study population

Among study population HPV 16 was detected in 76.1% (n=35) case followed by HPV 18 in 8.7% (n=4) cases. HPV 16 and 18 together contributed 84% of cervical cancer. HPV 39, 56, 68 each were present in 2.2 % cases individually. There was a co-infection with HPV 33+35 in 2.2 % case.

Among 34 cases of invasive squamous cell carcinoma, HPV 16 was detected in 27 cases (79.4%), HPV 18 was detected in 2 cases (5.9%). HPV 68 was detected in 1 case (2.9%), HPV 33+35 was detected in 1 case (2.9%) and in rest 3 cases of squamous cell carcinoma HPV DNA was not detected.

Among 10 cases of adenocarcinoma, HPV 16 was detected in 7 cases (70.0%), HPV 18 was detected in 2 cases (20.0%) and HPV 56 was detected in 1 case (10.0%). HPV 16 was detected in one case of adenosquamous carcinoma (100.0%) Again HPV 39 was detected in one case of Neuroendocrine/small cell carcinoma of cervix (100.0%).

Considering HPV is associated with 99.7% case of cervical cancer, the Sensitivity, Specificity, Accuracy, PPV and NPV of Type Specific HPV DNA detection by Real Time PCR was 93.7%, 100.0%, 93.8%, 100.0% and 4.6% respectively.

Table III: Distribution of type specific HPV DNA in relation to histopathological findings of carcinoma cervix

Histopathology	HPV 16		HPV 18		HPV 39		HPV 56		HPV 68		HPV 33+35		un detected		*p* value
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	> 0.05*
Invasive Sq cell carcinoma	27	79.4	2	5.9	0	0	0	0	1	2.9	1	2.9	3	8.8	
Invasive Adenocacrinoma	7	70	2	20	0	0	1	10	0	0	0	0	0	0	
Adeno squamous carcinoma	1	100	0	0	0	0	0	0	0	0	0	0	0	0	
Neuro endo crine (small cell type)	0	0	0	0	1	100	0	0	0	0	0	0	0	0	

**p* value was calculated by chi square test

Table IV: Validity test of type specific HPV DNA by real time PCR

Validity Test	Percentage
Sensitivity	93.8
Specificity	100
Accuracy	93.8
PPV	100
NPV	4.6

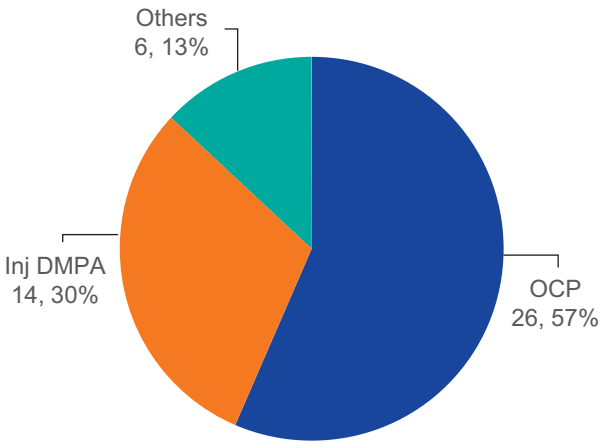

Figure 3: Proportion of study population receiving contraception

More than half of patients (56.5%) took OCP followed by injectable contraceptive (30%) and other type of contraceptives (6.13%).

Discussion

This cross sectional study was carried out with the aim to observe the distribution of high risk human papilloma virus infection (HPV genotypes 16, 18, 31. 33, 35, 39, 45, 51, 52. 56, 58, 59, 66 & 68) among patients of cervical cancer in BSMMU. Cervical cancer was diagnosed by histopathological report.

In the present study, it was observed that peak age of cervical cancer was between the age ranges of 50-60 years which was similar to the study conducted by in Twin cites of Pakistan.[26] Where HPV induced cervical cancer rate was higher in the age group of 41-60 years.

In this study, 65.21%, patients had first sexual exposure before the age of 18 years. The association of infection of high risk HPVs with the age at marriage below 18 years was found to increase the risk of cervical cancer by 22 fold.[30]

In this study, women who began to have sexual experience before the age of 16 years were found to have a twofold higher risk of high grade CIN and cervical cancer (OR=2.17, 95% CI) than women who had exposure for the first time after the age of 16.[31] This increased risk might be due to the fact that younger age may have exposure to a persistent HPV infection for a larger time than women having exposure at later age. The present study demonstrated similar association.

Significant association with high parity and HPV infection was observed in the present study. In this study, it was observed that 33.7% patients had parity ≥5. Found a direct association between the number of full term pregnancies and squamous cell cancer risk: the odds ratio was 3.8 (95 % CI 2.7-5.5) compared with nulliparous woman and 2.3 (1.6 -3.2) compared with women who had one or two full term pregnancies.[32] There was no significant association between risks of adenocarcinoma or adenosquamous carcinoma and number of full –term pregnancies.

In this study, it was observed that majority (56.0%) patients took oral contraceptive pills (OCP) and 30.0% took injectable contraceptives (DMPA). It was to be found that OCP as a co-factor of development of cervical cancer.[33] The study showed 71% patients of cervical cancer, used OCP. Maximum (40%) used oral contraceptive pill for > 5 years whereas 31% for < 5 years.

56.10 % HPV positive patients had history of taking oral contraceptive pills. This findings is similar to the present study.[21]

In terms of risk factor of hormonal contraception use, a study for International Agency for Research on Cancer, Multicentre Cervical Cancer Study Group by showed that long term use of oral contraceptives could be a cofactor as it increases risk of cervical carcinoma by upto fourfold in women who are positive for HPV DNA.[33] 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.

In the present study, 43 cases (93.47%) out of 46 cases were positive for high risk HPV DNA by Real time PCR.

A study conducted in BSMMU, Dhaka among 90 cervical precancers and cancer patients, detection of high risk HPV by HC II assay and Real Time PCR, detection rate of high risk HPV by Real time PCR

was 76.92%. Furthermore, 98.8 % of samples of cervical cancer were HPV DNA positive by Real Time PCR.[34]

In the present study, HPV genotype were not detected in 3 cases of invasive squamous cell carcinoma. It may be due to low viral loads, tissue degradation and misdiagnosis of endometrial adenocarcinomas could account for less than 10% of HPV negative adenocarcinomas. Then there should be role of immunohistochemistry to differentiate endometrial or cervical adenocarcinomas. However due to lack of validated assay, we concluded that HPV negativity was largely attributable to technical faults in collecting or processing samples. The use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and PCR amplification) but also to assess the adequacy of sampling and storage of clinical material. If epithelial swab was taken incorrectly (the number of epithelial cells is insufficient i.e. 10^3–10^5 genome equivalents), the amplification signal of beta globin gene will be underestimated. DNA of high risk HPV types were found in over 90% of cervical cancers and precancers lesions.[35,36]

In the present study, the histopathological findings of the study population were observed that 73.91% population were found invasive squamous cell carcinoma followed by 21.74% were found adenocarcinoma.

90% cervical cancers were of squamous cell carcinoma and rest 10 % were adenocarcinoma.[30]

Here, we noticed, the incidence of adenocarcinoma is increasing day by day. This rise in adenocarcinoma could be a consequence of well-known limitations of cytology based screening of adenocarcinoma precursor lesions since they are frequently located in the endocervical canal, making them less accessible than squamous cell carcinoma precursor lesions for cytological detection.

Genotypic distribution of HPV among women of cervical cancer in present study showed 76.1 % was HPV 16, 8.7% was HPV 18, 2.2% was HPV 56, 2.2% was HPV 68 and 2.2% was co-infection with HPV 33+35. Here, HPV 16 and 18 together contributed 84.78% of cervical cancer.

A study was done at virology department, BSMMU, Dhaka, showed HPV type 16 was detected in 81.82% followed by HPV 18 in 9.09 % and HPV 45 in 6.06% cases of cervical cancer.[21]

Conducted a retrospective cross-sectional worldwide study among 38 countries of five continents.[27] Eight most common HPV types were 16, 18, 31, 33, 35, 45, 52 and 58 among invasive cervical cancer worldwide.[27] In Asia, most commonly found types were HPV 16 (60%), HPV18 (11%) and HPV 45 (6%). In present study, we did not find any HPV 45. According to WHO 2010, in India, HPV 16 in cervical cancer was (70-90%), and HPV 18 (3-26%), similar to present study.

In a study of 175 cervical cancer cases from Brazil found, 5 most frequent types were, HPV 16 (77.6%), HPV 18 (12.3%), HPV 31 (8.8%), HPV 33 (7.1%) and HPV 35 (5.9%). Here HPV 16 and 18 together contributes 90% of ICC. Distribution of HPV types were almost similar to present study.[34]

The distribution of HPV genotypes detected in cervical cancer varies depending on the histological type of the cancer. The most prevalent genotype detected in the present study was HPV 16 irrespective of clinical pathology. While HPV 16 is the most frequent genotype in squamous cell carcinoma, relative proportion of HPV 18 is much more in adenocarcinoma than in squamous cell carcinoma. Borna found in her study, in squamous cell carcinoma HPV 16 (80%), HPV 18 (10%) and HPV 45 in (6.67%) case.[21] In adenocarcinoma, co infection with HPV 18/45 (50%) and HPV 16 (50%) was present. Unfortunately, due to small sample size, the findings could not be confirmed in the present study. However, HPV 16 and 18 are the most prevalent HPV types in carcinoma cervix worldwide followed by HPV types 45, 31, 33, 52, 58 and 35.

Present study showed sensitivity and specificity of HPV DNA by Real Time PCR are 93.8 % and 100 % respectively. conducted a comparison study between HC II Assays and Real time PCR for detection of HPV infection and found sensitivity and specificity of Real Time PCR was 95.8% and 99.8% respectively, similar to this study. [37]

Among 46 cases, 42 cases were detected for single infection (91.3%) and only one case was detected for multiple infection (2.2%). The ratio between single infection and co- infection was 42.1.

Observed that multiple genotypes are less prevalent in carcinoma patients.[38] Some observed that 35% HPV positive patients with advanced cytological disorder and > 50% of HIV infected patients contained

multiple HPV genotypes. 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.

Conclusion

The study shows that along with most prevalent HPV type 16 and 18, other types like Type 33, 35, 39.56 and 68 also contributed in development of cervical cancer in Bangladeshi women. Vaccines available in Bangladesh are bivalent Cervarix (acts against type 16 and 18) and Quadrivalent Gardasil (acts against type 6, 11, 16 &18). To provide 100 % coverage against all strains of HPV subtypes causing cervical cancer, a new type of second generation polyvalent HPV vaccine covering wide range of oncogenic HPV, should be made available in Bangladesh.

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