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Human Papilloma Virus Genotypes 16 and 18 among the South-Eastern Regional Bangladeshi Women

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Abstract

Introduction: Cervical cancer ranks as the fourth most common cancer worldwide in terms of both incidence and mortality among women, accounting for 6.6% of all female cancer cases and 7.5% of cancer-related deaths in women. The primary cause of most cervical neoplasms, as well as other anogenital and oropharyngeal cancers, is infection by the human papillomavirus (HPV).

Objective: This study aimed to determine the prevalence and distribution of HPV genotypes 16 and 18 among women in southeastern Bangladesh.

Methods: This cross-sectional descriptive study was conducted at the Department of Laboratory Medicine in Epic Health Care Limited, Chattrogram, Bangladesh, from December 2023 to May 2024. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0. Descriptive statistical analyses were performed, and the results were presented in tables. Chi-square tests were applied to assess the relationship between age groups and the prevalence of specific HPV genotypes, with a significance level set at $p<0.05$.

Results: A total of 284 study participants were enrolled, and cervical scrape samples were collected from all participants. However, 13 samples were unsuitable for PCR HPV detection, leaving 271 samples for HPV testing and genotyping for HPV 16 and 18. The most common age group among the participants was 31-40 years (121 women, 44.64%), with a mean age of 38.57 ± 8.93 years. HPV was detected in 10 cases, representing 3.69% of the study population. Genotype 16 was found in 3 women (1.10%) and genotype 18 in 1 woman (0.36%). Three cases of genotype 16 were detected in the 41-50 age group, while one case of genotype 18 was found in the 51-60 age group. No statistically significant association was observed between age groups and the prevalence of HPV genotypes 16 and 18 ($P>0.05$).

Conclusion: This study offers valuable insights into the prevalence of HPV genotypes 16 and 18 among women in southeastern Bangladesh. Although these genotypes are recognized globally for their high risk of cervical cancer, the study found a relatively low prevalence (3.69%) among the women in this region. The study suggests enhancing public health initiatives, such as HPV screening and vaccination programs, to reduce the risk of cervical cancer in Bangladeshi women.

Keywords: Human, Papilloma, Virus, Genotypes, South-eastern, Bangladeshi, Women

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Introduction

Cervical cancer is the fourth most prevalent cancer globally in terms of both incidence and mortality among women, representing 6.6% of all female cancer cases and 7.5% of cancer-related deaths in women. The main cause of most cervical neoplasms, as well as other anogenital and oropharyngeal cancers, is infection with human papillomavirus (HPV) [2], which is responsible for approximately 5% of cancers globally [3]. Over 200 subtypes of HPV have been identified and classified as either high-risk (HR) or low-risk (LR) based on their link to malignancies, particularly cervical cancer. The International Agency for Research on Cancer (IARC) has designated HPV-16, HPV-18, and several other types as HR-HPV, which are responsible for 95% of cervical cancer cases [5,6]. On the other hand, LR-HPV types, such as HPV-6 and HPV-11, are associated with genital warts and mild changes in cervical cells. HPV infection rates vary across regions, countries, and subpopulations [7], with a global prevalence estimated at 11.7%, but specific country rates ranging from 1.6% to 41.9% [8]. Developing regions have a higher HPV prevalence (14.3%) compared to developed areas (10.3%). Additionally, HPV type distribution differs geographically [9, 10], with certain types being more prevalent in the Asia-Pacific region [11]. Numerous risk factors contribute to acquiring and persisting HPV, with age being a key predictor, though regional age-related patterns differ [12, 13]. Other factors include sexual behaviors, socioeconomic conditions, male circumcision, condom use, oral contraceptives, smoking [14-16], public bathhouse usage [17], and lower education levels [18]. High parity is also recognized as a risk factor for HR-HPV infection [19]. While some research has been done on HPV in South Asia [20], there is limited data on HPV prevalence in specific Bangladeshi populations, such as female sex workers [21] and cancer patients in tertiary hospitals [22]. Moreover, no studies have explored HPV prevalence and genotype distribution across different regions of Bangladesh. This paper seeks to assess the prevalence and distribution of HPV genotypes 16 and 18 among women from the southeastern region of Bangladesh.

Methods

This cross-sectional descriptive study was carried out at the Department of Laboratory Medicine, Epic Health Care Limited, in Chattrogram, Bangladesh, from December 2023 to May 2024. The risks and benefits of the study were explained to participants in their local language, and informed consent was obtained. A consecutive sampling method was used, and 284 married women of varying ages, referred to Epic Health Care Limited for HPV genotyping of types 16 and 18, were included in the study. Cervical scrape samples were obtained following the standard procedure, using a cytobrush to collect cells from the ectocervix and transformation zone, and the specimens were stored in tubes containing phosphate-buffered saline (pH 8.6). Then, the specimen tubes were vortexed, cytobrushes were discarded, and tubes were centrifuged to pellet the cells, which were suspended in 1 mL of phosphate-buffered saline. Aliquots of each fresh specimen were made and stored for a short duration at −20°C until further processing. The experiment was carried out with Gene Proof Human Papillomavirus (HPV) screening PCR kit. This kit used real-time PCR with TaqMan probe based fluorescnce detection to amplify and quantify specific target sequence. In this kit Envelop protein genes (E1 and E2) of the HPV was the target sequence. HPV High Risk (HR) variants as well as differentiation of type 16, 18 and 45 could be achieved using this kit. Human GAPDH gene was used as internal control (IC). Applied Biosystems 7500 real time PCR system was used to detect HPV in this experiment. Successfully sampling and PCR was confirmed by the amplification of IC which was labeled with fluorophore JOE. Positive HPV was identified if fluorescence in the FAM channel was seen. Further differentiation of positive HPV was done if fluorescence was seen in either channel Cy5 which means HPV16 or TEXRED which means HPV18. This machine is unable to detect HPV 45, so only HPV16 and HPV18 were evaluated in this study. The gathered data were systematically organized and analyzed using version 23.0 of the Statistical Package for Social Sciences (SPSS) software. Descriptive statistical analyses were conducted, and the results were presented in tables. Chi-square tests were employed to assess the relationship between the women's age groups and the prevalence of specific HPV genotypes, with a significance level set at $p<0.05$.

Results

A series of 284 study subjects were enrolled in this study and a total of 284 samples of cervical scrapes were collected from the study subjects. Of them 13 samples were not applicable for PCR HPV detection and genotyping and finally (284-13) = (N=271) samples were tested for the detection of HPV and its genotyping 16 and 18. Out of 271 study subjects, the most frequent 121(44.64%) women were between 31-40 years, followed 74(27.29%), 20-30 years, 51(18.81%), 51-60 years, 20(7.38%), 61-70 years, 4(1.47%), and 1 (0.4%) in (71-80) years. The mean age of the study women was 38.57 ±8.93) years with a median age of 37 years. The mode age of the study women was 30 years and the age range of the women extended from 29.64-47.50 years (Table-1). Out of the 271study subjects, overall HPV was detected in 10 cases, accounting for 3.69% of the total population and HPV genotype-16 was detected in 3(1.1%) women followed HPV genotype-18 in only 1(0.36%) case. The most frequent 4(1.47%) HPV detection was found within the age group 41-50 years followed by 3(1.11%) 31-40 years and similarly 3(1.10%) 20-30 years which was not statistically significant (p=0.718),(Table-3). Among, the overall HPV prevalent

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cases, 3(1.10%) cases were detected within the age group (41-50) years and only 1(0.36%) case was identified as HPV genotype-18 within the same age group (51-50) years. The association between the prevalence of genotype 16 and 18 and the age groups of the study subjects was nor observed statistically significant (P>0.05).

Table 1: Age distribution of the study women (N=271)

Age Category(years)	Frequency	
	N	%
20-30	51	18.81
31-40	121	44.64
41-50	74	27.29
51-60	20	7.38
61-70	4	1.47
71-80	1	0.36
Total	271	100
Mean Age(years)	38.57±8.93	
Median	37	
Mode	30	
Range	29.64-47.50	

Age group was determined by one decade (10 years)

Table-1 presents the age distribution of the study women, categorized into specific age groups. The most frequent 121(44.64%) women were between 31-40 years, followed 74(27.29%), 20-30 years, 51(18.81%), 51-60 years, 20(7.38%), 61-70 years, 4(1.47%), and 1 (0.4%) in (71-80) years. The mean age of the women was 38.57 ±8.93) years with a median age of 37 years. The mode age of the patients was 30 years. The age range of the women extended from 29.64-47.50 years.

Table 2: Overall prevalence of HPV genotypes among the study women (N=271)

Overall HPV distribution	Frequency	
	N	%
Human Papilloma Virus		
Detected	10	3.69
Not detected	261	96.3
Total	271	100
HPV genotypes distribution		
HPV Genotype-16		
Detected	3	1.1
Not detected	268	98.9
Total	271	100
HPV Genotype-18		
Detected	1	0.36
Not detected	270	99.63
Total	271	100

Table-2 summarizes the prevalence of human papilloma virus (HPV) and its specific genotypes among the study subjects. Out of the 271 women, HPV was detected in 10 cases, accounting for 3.69% of the total population, while 96.30% tested negative for HPV. HPV genotype-16 was detected in 3(1.10%) patients followed HPV genotype-18 in 1(0.36%) patients.

Table 3: Distribution of overall prevalence of HPV by age group of the study women (N=271)

Age category(years)	HUMAN PAPILLOMA VIRUS				P-value
	Not detected		Detected		
	N	%	N	%	
20-30	48	17.71	3	1.1	
31-40	118	43.54	3	1.1	
41-50	70	25.83	4	1.47	
51-60	20	7.38	0	0	0.718
61-70	4	1.47	0	0	
71-80	1	0.36	0	0	
Total	261	96.31	10	3.69	

Table-3 illustrates the distribution of human papilloma virus (HPV) prevalence among different age groups within the study population. Of the 271 patients, HPV was detected in 10(3.69%) cases, while the remaining 271(96.31%) patients tested negative. The most frequent 4(1.47%) HPV detection was found within the patients' age group 41-50 years followed by 3(1.10%) 31-40 years and similarly 3(1.10%) 20-30 years which was not statistically significant (p=0.718).

Table 4: Distribution of HPV genotype -16 by age group of the study women (N=271)

Age category	HPV_GENOTYPE16				Total	P-value
	Not detected		Detected			
	N	%	N	%	N(%)	
20-30	51	18.81	0	0	51(18.81)	
31-40	121	44.64	0	0	121(44.64)	
41-50	71	26.19	3	1.1	74(27.29)	
51-60	20	7.38	0	0	20(7.38)	0.201
61-70	4	1.47	0	0	4(1.47)	
71-80	1	0.36	0	0	1(0.36)	
Total	268	98.9	3	1.1	271(100)	

Table-4 details the age-specific distribution of HPV genotype-16 among the study women. HPV genotype-16 was detected in only 3(1.10%) women within the age group 41-50 years. .No cases of HPV genotype-16 were detected in the age group, 20-30 years(n=51), followed 31-40 years(n=121),

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51-60 years (n=20), 61-70 years (n=5) and 71-80 years (n=1). The overall P-value of 0.201 suggests no statistically significant association between age and the detection of HPV genotype-16 in this study population.

Table 5: Distribution of HPV genotype -18 by age group of the study women (N=271)

	Not detected		Detected		Total	P-value
	N	%	N	%	N(%)	
20-30	51	18.81	0	0.0	51(18.81)	0.633
31-40	121	44.64	0	0.0	121(44.64)	
41-50	73	26.93	1	0.36	74(27.28)	
51-60	20	7.38	0	0.0	20(738)	
61-70	4	1.47	0	0.0	4(1.47)	
71-80	1	0.36	0	0.0	1(0.36)	
Total	270	99.63	1	0.36	271(100)	

Table-5 presents the distribution of HPV genotype-18 among different age groups within the study population. Out of the 271 women, only 1(0.36%) case of HPV genotype-18 was detected within the age group 41-50 years. .No case of HPV genotype-18 was detected in the age group 20-30 years(n=51),31-40 years(n=121), 51-60 years (n=20), 61-70 years (n=5) and 71-80 years (n=1) . The overall P-value of 0.633 indicates no statistically significant association between age and the detection of HPV genotype-18 in this cohort.

Discussion

Persistent infection with high-risk human papillomavirus (HR-HPV) plays a key role in the development of cervical cancer, making HPV testing a valuable method for lowering both its incidence and mortality rates [23]. However, due to lacking awareness about HPV and its consequences among the large remote female population, creating a comprehensive database on HPV prevalence and genotype distribution remains challenging [24]. Consequently, there is a lack of comprehensive information on HPV prevalence and genotype distribution among Bangladeshi women. This current study aimed to determine the prevalence and distribution of HPV genotypes in women seeking medical examinations and treatment in a tertiary level health care center in Chattrogram, a southeastern city of Bangladesh. In this study, it was observed that out of the 271 women, HPV was detected in 10 cases, accounting for 3.69% of the total study population. Of them HPV genotype-16 was detected in 3(1.10%) women and HPV 18 was detected in only one woman. Another study found an overall HPV infection prevalence of 7.7%, with no significant variation between urban and rural women. The most prevalent high-risk genotypes identified were HPV16, HPV66, HPV18, HPV45, HPV31, and HPV53 [25]. These findings of that study are partially in the lineage of this study because only genotypes HPV-16 and 18 were observed among the study women. Moreover, they did not find any difference between urban and rural population in the findings of HPV genotypes but our study population was urban based and overall HPV prevalence of our study was 3.69% whereas their study observed 7.7% overall prevalence of HPV genotypes. Therefore, these findings suggest overall prevalence of HPV and HPV genotypes may differ from population to population and region to region of a country. This present study observed that HPV genotype-16 was detected in only 3(1.10%) women within the age group 41-50 years and .no case of HPV genotype-16 were detected in the age group, 20-30 years(n=51), followed 31-40 years(n=121), 51-60 years (n=20), 61-70 years (n=4) and 71-80 years (n=1). and there was no significant association was observed between age groups and the prevalence of HPV genotype-16 (P> 0.05). A meta-analysis reported that HPV genotype-16 was the most prevalent genotype, detected in a wide range of age groups [26].These findings of both the studies suggest that HPV-16 infections are common in any age group of the women but regional Bangladeshi women are more vulnerable to late 40 years. This study finally observed that out of the 271 patients, only 1(0.36%) case of HPV genotype-18 was detected within the age group 41-50 years. No case of HPV genotype-18 were detected in the age group 20-30 years(n=51),31-40 years(n=121), 51-60 years (n=20), 61-70 years (n=4) and 71-80 years (n=1). Another study reported a higher prevalence of HPV genotype-18, which was found to be one of the most common genotypes, particularly in younger women. The study showed that HPV-18 had a significant presence in the 20-30 and 31-40 age groups, which contrasts with our findings. Our findings indicate a particularly low prevalence in the studied population, possibly due to cultural and religious differences [27]. However, the absence of HPV-16 and HPV-18 in younger age groups, and their rare occurrence in middle-aged regional Bangladeshi women, suggests potential regional, cultural and religious variations in HPV infection patterns. This low prevalence could also reflect underreporting, limited screening, or cultural and healthcare access factors unique to this population. Future research with larger sample sizes and enhanced HPV awareness campaigns is essential to better understand the true burden of HPV and to improve prevention strategies, including vaccination and screening efforts in Bangladesh.

Limitations of The Study

The constraints of this study include a limited sample size and its concentration on a particular region, which may not accurately reflect the wider Bangladeshi population. The study specifically targeted HPV genotypes 16 and 18, thereby excluding other high-risk types from the analysis. The cross-sectional design of this study limits understanding of HPV persistence over time.

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Conclusion

This study provides an insightful understanding of the prevalence of human papilloma virus (HPV) genotypes 16 and 18 among south-eastern regional Bangladeshi women. Despite the globally recognized high-risk association of these genotypes with cervical cancer, our findings revealed a relatively low occurrence in the sampled population, with only 3 cases (1.10%) of HPV 16 and only 1 case (0.36%) of HPV 18 among the study women. Regional, geographical, religious and cultural factors of southeastern Bangladesh may influence this lower prevalence of HPV genotypes 16 and 18 in southeastern Bangladeshi women. Finally, this study suggests enhancing public health initiatives like HPV screening and vaccination programs to reduce the risk of cervical cancer in Bangladeshi regional women.

Recommendations

Future research should focus on increasing the sample size and including a wider age range to enhance the generalizability of findings. Investigating additional high-risk HPV genotypes beyond 16 and 18 is also important, and employing a longitudinal design would allow for better tracking of HPV persistence. Incorporating clinical outcomes, such as cervical lesions, along with behavioral and socioeconomic data, would offer a more complete understanding. Moreover, strengthening public health efforts, including HPV screening and vaccination programs, could play a key role in reducing cervical cancer risk in the population.

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