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Original Article

Seroprevalence 36 Months after a Single-dose Bivalent Human Papillomavirus Vaccination among Nine to Fifteen-year-old Girls in Dhaka, Bangladesh: A Cross-sectional Study

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Abstract

Background and objectives: Immunization against human papillomavirus (HPV), particularly with a single-dose vaccine, offers a cost-effective strategy for cervical cancer prevention. This study aimed to evaluate the seroprevalence following a single-dose bivalent HPV vaccine among adolescent girls in Bangladesh and to examine its association with sociodemographic characteristics.

Methods: A cross-sectional study was conducted among 648 adolescent girls (aged nine to fifteen years) in Dhaka, Bangladesh, who received a single dose of the bivalent HPV vaccine in November 2019. Participants were recruited from ten local schools. At 36 months post-vaccination, blood samples were analyzed for HPV16/18 L1-specific immunoglobulin G using enzyme-linked immunosorbent assay. Sociodemographic data were collected and analyzed using logistic regression.

Results: Most participants were aged nine to thirteen years (82.4%), with a mean age of 11.89 ± 1.59 years. The overall seroprevalence was 72.8% for HPV16 and 82.4% for HPV18. Seropositivity for HPV16 was significantly lower among participants aged 14–15 years [adjusted odds ratio (aOR) = 0.61; 95% confidence interval (CI): 0.39–0.95; $p = 0.020$] and those in grades nine to ten (aOR = 0.50; 95% CI: 0.28–0.89; $p = 0.004$). For HPV18, significantly reduced odds of seropositivity were observed among participants from households with monthly incomes up to Taka 10,000 (aOR for Taka 10,001–20,000 = 0.41; 95% CI: 0.26–0.67; $p < 0.001$; aOR for Taka 20,001–50,000 = 0.21; 95% CI: 0.11–0.40; $p < 0.001$).

Conclusions: A single-dose bivalent HPV vaccine induces sustained immunity in Bangladeshi adolescent girls, with lower HPV16 seropositivity among older girls and those in higher grades, and higher HPV18 seropositivity is linked to lower household income.

Introduction

Cervical cancer (CC) remains a major public health challenge globally, particularly in low- and lower-middle-income countries (LLMICs), where nearly 90% of CC-related deaths occur.[1] In Bangladesh, CC is the second most common cancer among women, with approximately 9,640 new cases and more than 5,826 deaths annually.[2] The burden is especially high among women in low-resource urban and rural settings, highlighting the urgent need for effective prevention strategies.[3] Persistent infection with high-risk human papillomavirus (HPV), especially types 16 and 18, accounts for 70–80% of all CC cases worldwide.[4,5] Preventive vaccination against HPV has shown remarkable success in reducing HPV infection and subsequent cervical precancerous lesions.[6] The bivalent HPV vaccine (targeting types 16 and 18) induces ro-

Keywords: Human papillomavirus; HPV; HPV vaccine; Single-dose; Cervical cancer; Seroprevalence; Bivalent vaccine; Bangladesh.

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Table 1. Distribution of study participants according to selected schools

Name of the school	Initially vaccinated	Recruited at follow up for seroprevalence
Begum Rahima Adarsha School	45	36
Uttarkhan Collegiate School	176	130
Monija Rahaman Girls School	122	91
Diabari Primary School	51	39
Bailjhuri School	60	42
Shotodol School	105	72
Rowshan Ara Uchya Bidyaloy	170	120
Shere Bangla Girls School	130	88
Nagar Matri Sadan Nagar Health Center	41	30
Kamrangirchar 31 Bed Hospital	70	20
Total	970	648

bust immune responses and has demonstrated effectiveness in both high-income and resource-constrained settings.[7,8] In countries with similar socioeconomic and healthcare contexts, such as India and Tanzania, bivalent vaccines have shown high seroconversion rates and protective efficacy following immunization of adolescent girls.[9]

Recognizing the critical role of HPV vaccination in CC prevention, the World Health Organization (WHO) launched a global strategy in November 2020 to eliminate CC as a public health problem. One key target is to ensure that 90% of girls are fully vaccinated with the HPV vaccine by the age of 15 by the year 2030.[10] Although both bivalent and quadrivalent vaccines have been recommended by WHO since 2009, their adoption into national immunization programs has been limited in many LLMICs, including Bangladesh. Challenges such as the high cost of vaccines, the need for multiple doses, and logistical constraints have hindered widespread implementation.[11]

Recent studies have demonstrated that a single dose of the bivalent HPV vaccine can provide durable immunogenicity comparable to multi-dose regimens. A landmark study in Costa Rica found that over 90% of women remained seropositive for HPV16 and 18 even 11 years after a single dose, with stable antibody levels throughout the follow-up period.[12] Systematic reviews and trials from India, Kenya, and other regions further support the long-term protective efficacy of single-dose HPV vaccination.[13–15]

The cost of the HPV vaccine and the necessity of a multidose schedule are significant barriers to HPV vaccine introduction and sustainability in many LLMICs. The cost of vaccine procurement and delivery has been a major obstacle to the government's commitment to national HPV vaccine introduction.[16,17] Additionally, the global HPV vaccine shortage has become a barrier to the introduction and expansion of national HPV programs in some countries.[18] Therefore, implementing a single-dose schedule would aid the effective elimination of CC by reducing vaccine costs and simplifying program logistics, including supply, delivery, and accessibility in LLMICs. This regimen has been proven immunogenic and adequate for long-term protection against HPV-associated diseases.[19–21] For countries like Bangladesh, a single-dose regimen presents an opportunity to simplify vaccine delivery, reduce program costs, and overcome logistical barriers, making nationwide implementation more feasible and sustainable. These factors indicate the necessity of introducing a single-dose regimen to increase coverage of girls in the target age group to achieve the WHO global elimination target in Bangladesh. In November 2019, the National Center for Cervical and Breast Cancer Screening and Training (NCCBCST), Bangladesh Medical University (BMU), conducted an HPV vaccination program at NCCBCST and ten schools in Dhaka. Participants were engaged through advocacy meetings, provided with essential information, and their ages were verified via birth certificates. Teachers organized the events and supported the participants, while staff of the Expanded Program on Immunization (EPI) administered the vaccines free of charge, maintaining proper cold chain standards. Post-vaccination, the girls were observed for 30 minutes for adverse effects. A total of 970 girls received a single dose of the bivalent HPV vaccine. This study aimed to assess the seroprevalence of HPV types 16 and 18 antibodies 36 months after a single-dose bivalent HPV vaccination among girls aged nine to fifteen years in Dhaka. Additionally, the study examined the association between seropositivity and key sociodemographic factors such as age, school type, and parental education.

Materials and methods

Study design and setting

This was a cross-sectional, follow-up seroprevalence study conducted between January 1 and June 30, 2023, at the NCCBCST, BMU, Dhaka, Bangladesh. The objective was to assess the long-term seroprevalence of HPV types 16 and 18 antibodies in adolescent girls approximately three years after receiving a single dose of the bivalent HPV vaccine. In November 2019, an HPV vaccination campaign was implemented by NCCBCST in collaboration with the EPI, Bangladesh. The campaign targeted girls aged nine to fifteen years from ten purposively selected secondary schools across Dhaka (Table 1). These schools were purposively selected from government schools to provide wide geographic representation of Dhaka city. A single dose of the bivalent HPV vaccine (Cervarix®, GlaxoSmithKline) was administered, and health education sessions on HPV and CC prevention were conducted. A total of 970 girls were vaccinated during this initiative, serving as the cohort for the present follow-up study.

Study population and recruitment

The target population consisted of 970 adolescent girls aged nine

to fifteen years at the time of vaccination. For the follow-up, all vaccinated individuals were contacted through phone calls, community outreach, and written invitations facilitated by school authorities. Inclusion criteria for the follow-up study were: (1) receipt of a single dose of the bivalent HPV vaccine during the 2019 campaign; (2) current residence in Dhaka or surrounding areas; and (3) provision of written informed consent from a parent or guardian, along with verbal or written assent from the participant. Participants were excluded if they had a known history of HPV infection or cervical lesions; had received any prior HPV vaccination before the study; were immunocompromised or had chronic illnesses that could interfere with immune response (e.g., HIV/AIDS, cancer); had serious illness at the time of enrollment; declined to provide informed consent/assent; or were unwilling to provide a blood sample.

Blood sample collection and storage

Venous blood (5 mL) was collected from each participant by trained medical technologists and nurses using standard aseptic techniques. All samples were transported to the laboratory at the Department of Virology, BMU, while maintaining the cold chain. Blood samples were centrifuged at 3,000 rpm for 10 m to separate the serum. The separated serum was aliquoted into sterile cryovials and stored at –80°C until laboratory analysis. All procedures adhered to national biosafety standards and quality assurance protocols.

Laboratory analysis: detection of HPV-specific antibodies

To assess the presence of type-specific immunoglobulin G (IgG) antibodies against HPV types 16 and 18, a two-step laboratory testing approach was used. First, serum samples were tested using a commercial enzyme-linked immunosorbent assay kit (CUSABIO Technology LLC, USA; Catalog No. CSB-EQ027477HU for HPV16 L1 IgG and CSB-EQ027476HU for HPV18 L1 IgG) designed to detect antibodies against the HPV L1 capsid protein. The enzyme-linked immunosorbent assay plates were pre-coated with virus-like particles (VLPs) specific to HPV-16 and HPV-18. A 100 µL aliquot of each participant's serum was added to the wells and incubated according to the manufacturer's instructions. Following incubation, wells were washed and incubated with anti-human IgG conjugated with horseradish peroxidase. A chromogenic substrate was then added, resulting in color development proportional to the amount of bound antibody. Optical density was measured at 450 nm using a calibrated microplate reader. Based on the kit's validated cutoff, an optical density value greater than 2.1 was considered seropositive for antibodies to HPV-16 or HPV-18. All assays were conducted in duplicate and included appropriate positive and negative controls to ensure assay reliability and reproducibility. In a subset of serum samples, pseudovirion-based neutralization assays were also performed to assess the presence of functional neutralizing antibodies against the HPV L1 protein. These assays further confirmed the immunogenicity of the vaccine and the presence of biologically relevant antibodies. All laboratory analyses were conducted at the Department of Virology, BMU, maintaining rigorous quality control measures throughout the testing process.

Data collection and variables

Sociodemographic and vaccination history data were collected through a structured questionnaire administered during the follow-up visit. Variables recorded included age group, participant's education level, mother's education, mother's occupation (homemaker or employed), and household monthly income categorized in Bangladeshi Taka (BDT) as less than 10,000, between 10,000 and 20,000, and more than 20,000.

Statistical analysis

Sample size justification

This study aimed to follow up the entire cohort of 970 adolescent girls who received a single dose of the bivalent HPV vaccine in 2019. However, due to logistical and practical constraints, including participant availability, informed consent, and eligibility, a total of 648 girls were ultimately enrolled in the follow-up seroprevalence assessment, representing approximately 67% of the original vaccinated cohort. Based on an expected seroprevalence of 90%, a 5% margin of error, and a 95% confidence level, the minimum required sample size was calculated to be 122 participants. Therefore, the achieved sample size of 648 far exceeded this requirement, yielding a narrower margin of error (±1.33%) and ensuring high statistical power to estimate overall seroprevalence accurately. This also provided greater robustness against potential non-response or missing data, thereby enhancing the validity and generalizability of the study findings.

Data analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0. Descriptive statistics, including frequencies and percentages, were used for categorical variables, and means with standard deviations were calculated for continuous variables. Bivariate associations between HPV antibody seropositivity (for HPV 16 and HPV 18) and participant characteristics were evaluated using chi-square (χ^2) tests. To identify factors associated with seropositivity, two separate binary logistic regression models were developed, one for HPV 16 and another for HPV 18. In these models, the dependent variable was serostatus (positive or negative), and independent variables included participants' age group, education level, mother's education, mother's occupation, and monthly family income. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were reported, and statistical significance was defined as a *p*-value less than 0.05.

Ethical considerations

Ethical clearance was obtained from the Institutional Review Board of BMU Ethics and Scientific Review Committee (Ref: No BSMMU/2020/686, dated 16/01/2020). This study was performed following the principles of the Declaration of Helsinki (as revised in 2024). All participants and their parents/guardians provided written informed consent and assent.

Results

A total of 970 adolescent girls received a single dose of the bivalent HPV vaccine during a campaign in November 2019. All vaccinated girls were invited to participate in a follow-up seroprevalence study 36 months later. Of these, 648 girls were available, met the eligibility criteria, and provided informed consent, forming the final study sample (Fig. 1).

Sociodemographic characteristics of the participants

The majority of participants (82.4%) were aged between nine and thirteen years, with a mean age of 11.89 ± 1.59 years. Most were enrolled in grades three to eight, with 39.2% in grades three to five and 49.7% in grades six to eight. Regarding maternal education, 63.6% of mothers had education up to the primary level, while only 6.2% had completed graduate-level or higher education. The vast majority

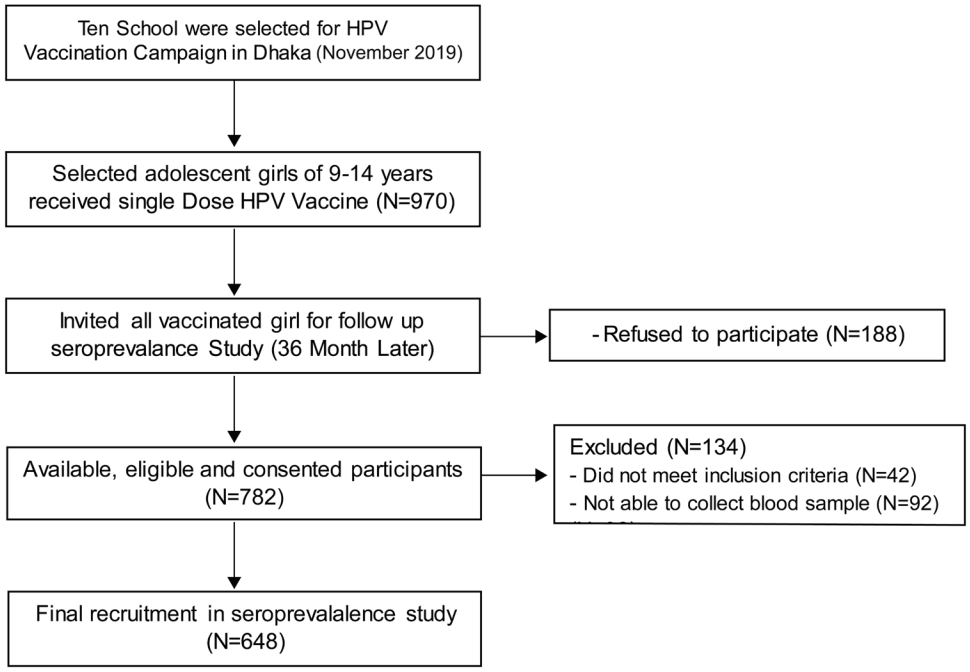

Fig. 1. Participant flow diagram in the study. HPV, human papillomavirus.

of mothers were housewives (97.1%), and the predominant paternal occupation was business (44.3%), followed by private jobs (23.5%) and government jobs (12.8%). Over half of the participants (52.3%) came from households with a monthly income of up to BDT 10,000, indicating a largely low-income population (Table 2).

Seroprevalence of HPV antibodies

At 36 months post-vaccination, the overall seroprevalence was 72.8% for HPV type 16 antibodies and 82.4% for HPV type 18 antibodies (Fig. 2).

HPV 16 antibody seropositivity

HPV 16 antibody seropositivity was significantly higher among participants aged nine to thirteen years (75.1%) compared to those aged 14–15 years (62.3%) ($p < 0.01$). Similarly, girls in lower grades (three to five) showed a higher prevalence (75.6%) than those in grades nine to ten (61.1%), with a marginally significant difference ($p = 0.05$). Although there was a trend toward increasing seroprevalence with higher maternal education, ranging from 57.9% among girls whose mothers had no formal education to 83.3% among those whose mothers had attained Higher Secondary School level education, this association did not reach statistical significance ($p = 0.08$). No significant associations were observed between HPV 16 antibody seropositivity and mother's occupation, father's occupation, or household income (Table 3).

HPV 18 antibody seropositivity

The seroprevalence of HPV 18 antibodies did not differ significantly by age group (82.8% in nine to thirteen years vs. 80.7% in 14–15 years; $p = 0.59$) or participant education level ($p = 0.35$). A higher prevalence was observed among daughters of mothers with HSC-level education (92.6%), although this association was not statistically significant ($p = 0.25$). In contrast, a significant association was found with household income: girls from households earning up to BDT 10,000 showed the highest seroprevalence (88.8%), while those from households earning BDT 20,001–50,000 showed the lowest (67.9%) ($p < 0.01$) (Table 4).

Factors associated with HPV 16 antibody seropositivity

In multivariate analysis, participants aged 14–15 years had significantly lower odds of being seropositive for HPV 16 compared to those aged nine to thirteen years (aOR = 0.61; 95% CI: 0.39–0.95; $p = 0.02$). Similarly, participants in grades nine to ten had significantly reduced odds of HPV 16 seropositivity compared to those in grades three to five(aOR = 0.50; 95% CI: 0.28–0.89; $p = 0.01$). Additionally, a significant association was observed between paternal occupation in business and higher odds of HPV 16 seropositivity (aOR = 1.93; 95% CI: 1.10–3.39; $p = 0.02$). Other demographic variables, including parental education and household income, were not significantly associated (Table 5).

Factors associated with HPV 18 antibody seropositivity

In contrast, multivariate analysis for HPV 18 seropositivity identified household income as the only significant predictor. Girls from families earning BDT 10,001–20,000 and BDT 20,001–50,000 had significantly lower odds of seropositivity compared to those with income ≤ BDT 10,000 (aOR = 0.41; 95% CI: 0.26–0.67; $p < 0.001$ and aOR = 0.21; 95% CI: 0.11–0.40; $p < 0.001$, respectively). No significant associations were found with age, education, or parental occupation (Table 6).

Discussion

The study demonstrated seroconversion after a single dose of HPV 16 and 18 vaccinations among young girls in Bangladesh. The majority of the study population were between the ages of nine and thirteen. Among the participants, 72.8% had HPV 16 antibodies and 82.4% had HPV 18 antibodies persisting for 36 months after

Table 2. Distribution of sociodemographic characteristics of the participants (N = 648)

Characteristics	Frequency	Percentage
Participants' age (years)		
9–13	534	82.4
14–15	114	17.6
Mean ± SD	11.89±1.59	
Participants' educational qualification		
Grades 3–5	254	39.2
Grades 6–8	322	49.7
Grades 9–10	72	11.1
Mothers' educational qualification		
No schooling	19	2.9
Up to primary	412	63.6
SSC	123	19.0
HSC	54	8.3
Graduate and above	40	6.2
Mothers' occupation		
Housewife	629	97.1
Service holder	19	2.9
Fathers' educational qualification		
No schooling	27	4.2
Up to primary	381	58.8
SSC	107	16.5
HSC	59	9.1
Graduate and above	74	11.4
Fathers' occupation		
Farming	71	11.0
Government job	83	12.8
Private job	152	23.5
Business	287	44.3
Living abroad	41	6.3
Driver	14	2.2
Monthly income		
Up to Taka 10,000	339	52.3
Taka 10,001–20,000	219	33.8
Taka 20,001–50,000	78	12.0
Taka 50,001 and above	12	1.9

HSC, Higher Secondary Certificate; SD, standard deviation; SSC, Secondary School Certificate.

a single-dose vaccination. This indicates that at least 70–80% of unmarried girls in Bangladesh remained immune for 36 months following a single-dose vaccination. Serological monitoring may be a significant additional method to observe the impact of HPV vaccination. Instead of collecting HPV DNA samples from the vagina or cervix, serum collection is easier, particularly for younger age groups who are less sexually active within the sociocultural context of Bangladesh. Kramer et al. also mentioned population-based sero-epidemiological studies to regularly monitor the impact of mass vaccination against HPV.[22]

Several trials have already reported high efficacy of a single-dose HPV vaccine against persistent HPV16/18 infection, among which

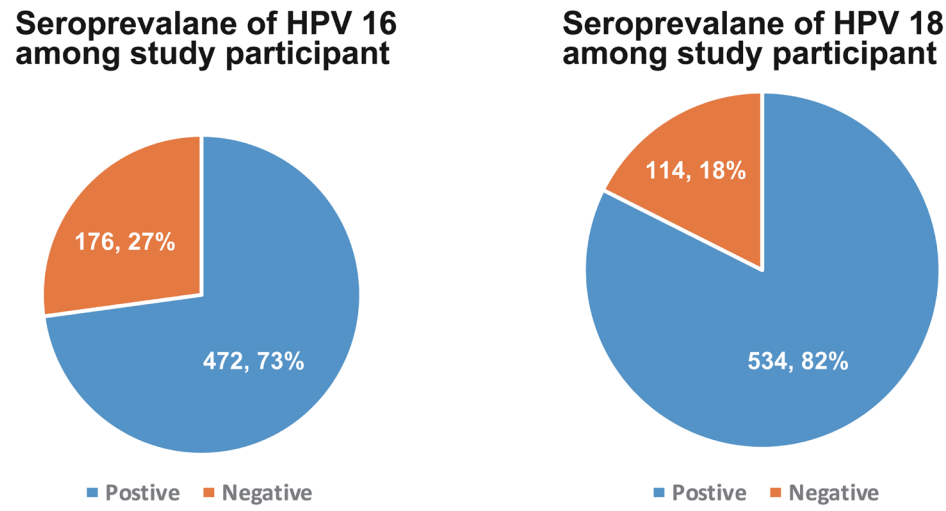

Fig. 2. Seroprevalence of HPV 16 and HPV 18 among study participants. HPV, human papillomavirus.

the Costa Rica Vaccine Trial (CVT) and PATRICIA trials are notable.[12,19] Data from the Finnish cohorts of these two international, randomized, phase 3 trials showed persistence of protective immunogenicity after the bivalent HPV vaccine for up to 12 years, with detectable antibody titers.[23] In the CVT, although the antibody levels against HPV16 and HPV18 remained lower than those produced by three and two doses, the levels persisted for seven years, indicating long-lasting protection.[24] Another study showed that over 90% of women remained HPV16 and 18 seropositive at both four and eleven years, with only 2.5% seronegative after one dose of the bivalent HPV vaccine.[13] The present study revealed lower seropositivity (72.8% for HPV 16 and 82.4% for HPV 18) than the CVT and other studies. However, in those studies, participants were tested for HPV infection prior to vaccination, allowing inclusion of only HPV 16 and 18 DNA-negative girls. In the present study, there was no opportunity to estimate pre-vaccination seropositivity status or presence of HPV 16/18 DNA in their cervix, nor was it possible to exclude participants who were sexually active. This may be one important reason for the lower seroprevalence observed. Nevertheless, this study provides a realistic picture of future seroprevalence after vaccination implementation among the target population of Bangladeshi girls. The durability of protection by the HPV vaccine is a critical concern, as immunized girls will be risk-free for many years. The present study evaluated immunogenicity for three years following a single-dose bivalent HPV vaccine. The overall rate of seroconversion was low but comparatively higher against HPV type 18 (534, 82.4%) than against HPV type 16 (472, 72.8%), persisting 36 months after single-dose vaccination. This indicates sustained protection against HPV 16 and 18 infections for at least 36 months among Bangladeshi girls. These findings are supported by long-term follow-up from the CVT, which found that over 98% of single-dose recipients remained seropositive for HPV16/18 up to 16 years post-vaccination, with only minor declines in antibody levels.[24,25] A 2024 systematic review by Jeong and Jang analyzing randomized controlled trials reported that while single-dose recipients generally exhibit lower antibody titers than those receiving three doses, protective efficacy against HPV infection remained substantial, ranging from 53.9% to 100.0%.[26] Surprisingly, there was no significant association between age groups and the presence of HPV 16 and HPV 18 antibodies. This suggests that the risk of acquiring these HPV types is not significantly influenced by age within this particular group.

However, the study revealed interesting associations with participants' education levels and their mothers' education and occupation. Development of HPV 16 antibodies was associated with participants' education up to grades five and six to eight, as well as mothers with no schooling or up to primary education. This implies that lower education levels of both participants and their mothers might be linked to an increased risk of developing HPV 16 antibodies. On the other hand, the development of HPV 18 antibodies was significantly associated with mothers' occupation as housewives, which may be explained by the fact that most mothers in the study population were housewives.

A study among Tanzanian girls aged nine to fourteen years showed that a single dose of the bivalent HPV vaccine induced a strong immune response up to 24 months and suggested this age group for vaccination.[9] The findings on durability of immunogenicity in the present study are important since a sustained immune response following a single-dose vaccination has not previously been reported among Bangladeshi girls. Further studies should be conducted with the seropositive population from this study to evaluate the additional sustainability of antibodies in this population.

Though HPV vaccines were initially approved as three-dose regimens, later on, to reduce costs, a two-dose schedule was introduced for adolescents (nine to fourteen years old).[27] Despite this, among a global cohort of 61 million 15-year-old girls in 2018, global HPV vaccination coverage remained very low (12.2%).[28] Moreover, the COVID-19 pandemic had a negative impact on HPV vaccine rollout, causing disruptions in routine immunization, delays in introducing new vaccines, and reduced uptake of HPV vaccination in LLMICs.[29,30] Nearly half of LLMICs failed to introduce HPV vaccination due to low affordability and the health emergency caused by the COVID-19 pandemic.[31] Therefore, global elimination of CC may be better achieved using a single-dose regimen with higher coverage of the target population in LLMICs. Another study suggested similar benefits of a one-dose versus a two-dose regimen, including simplified delivery, reduced costs, and fewer vaccine supply constraints.[32]

The prevalence of high-risk HPV infections in Bangladesh is about 4.2%.[33] To reduce the burden of CC and high-risk-HPV, the Government of Bangladesh (GOB) completed a pilot HPV vaccination program with support from the Global Alliance for Vaccines and Immunizations in Gazipur district during 2016–2018.[30]

Table 3. Relationship between HPV 16 antibodies and characteristics of the participants

Characteristics	HPV 16 antibody		Total N (%)	*p*-value*
	Positive N (%)	Negative N (%)		
Age group (years)				
9–13	401 (75.1)	133 (24.9)	534 (100.0)	0.007**
14–15	71 (62.3)	43 (37.7)	114 (100.0)	
Total	472 (72.8)	176 (27.2)	648 (100.0)	
Participants' educational qualification				
Grades 3–5	192 (75.59)	62 (24.41)	254 (100)	0.050
Grades 6–8	236 (73.29)	86 (26.71)	322 (100)	
Grades 9–10	44 (61.11)	28 (38.89)	72 (100)	
Total	472 (72.8)	176 (27.2)	648 (100)	
Mothers' educational qualification				
No schooling	11 (57.9)	8 (42.1)	19 (100)	0.085
Up to primary	290 (70.4)	122 (29.6)	412 (100)	
SSC	96 (78.0)	27 (22.0)	123 (100)	
HSC	45 (83.3)	9 (16.7)	54 (100)	
Graduate and above	30 (75.0)	10 (25.0)	40 (100)	
Total	472 (72.8)	176 (27.2)	648 (100)	
Mothers' occupation				
Housewife	458 (72.9)	170 (27.1)	628 (100)	0.762
Service holder	14 (70.0)	6 (30.0)	20 (100)	
Total	472 (72.8)	176 (27.2)	648 (100)	
Fathers' educational qualification				
No schooling	19 (70.37)	8 (29.63)	27 (100.00)	0.074
Up to primary	267 (70.68)	114 (29.92)	381 (100.00)	
SSC	83 (77.57)	24 (22.43)	107 (100.00)	
HSC	51 (86.44)	8 (13.56)	59 (100.00)	
Graduate and above	52 (70.27)	22 (29.73)	74 (100.00)	
Total	472 (72.8)	176 (27.2)	648 (100.00)	
Fathers' occupation				
Farming	42 (59.15)	29 (40.85)	71 (100.00)	0.093
Government job	63 (75.90)	20 (24.10)	83 (100.00)	
Private job	108 (71.05)	44 (28.95)	152 (100.00)	
Business	215 (74.91)	72 (25.09)	287 (100.00)	
Living abroad	33 (80.49)	8 (19.51)	41 (100.00)	
Driver	11 (78.57)	3 (21.43)	14 (100.00)	
Total	472 (72.8)	176 (27.2)	648 (100.00)	
Monthly income				
Up to Taka 10,000	255 (75.22)	84 (24.78)	339 (100)	0.093
Taka 10,001–20,000	155 (70.78)	64 (29.22)	214 (100)	
Taka 20,001–50,000	53 (67.95)	25 (32.05)	78 (100)	
Taka 50,001 and above	9 (70.0)	6 (30.0)	20 (100)	
Total	472 (72.8)	176 (27.2)	648 (100)	

*Chi-square test; **significant level $p < 0.01$. HPV, human papillomavirus; HSC, Higher Secondary Certificate; SSC, Secondary School Certificate.

Table 4. Relationship between HPV 18 antibodies and characteristics of the participants

Characteristics	HPV 18 antibody		Total N (%)	*p*-value*
	Positive N (%)	Negative N (%)		
Age group (years)				
9–13	442 (82.8)	92 (17.2)	534 (100.0)	0.596
14–15	92 (80.7)	22 (19.3)	114 (100.0)	
Total	534 (82.4)	114 (17.6)	648 (100.0)	
Participants' educational qualification				
Grades 3–5	212 (83.46)	42 (16.54)	254 (100)	0.352
Grades 6–8	265 (82.30)	57 (17.70)	28 (100)	
Grades 9–10	57 (79.17)	15 (20.83)	35 (100)	
Total	534 (82.4)	114 (17.6)	648 (100)	
Mothers' educational qualification				
No schooling	15 (78.9)	4 (21.1)	19 (100)	0.252
Up to primary	335 (81.1)	77 (18.9)	412 (100)	
SSC	99 (80.5)	24 (19.5)	123 (100)	
HSC	50 (92.6)	4 (7.4)	54 (100)	
Graduate and above	35 (87.5)	5 (12.5)	40 (100)	
Total				
Mothers' occupation				
Housewife	519 (82.5)	110 (17.5)	629 (100)	0.419
Service holder	15 (78.95)	4 (21.05)	19 (100)	
Total	534 (82.4)	114 (17.6)	648 (100)	
Fathers' educational qualification				
No schooling	20 (74.07)	7 (25.93)	27 (100)	0.071
Up to primary	307 (80.58)	74 (19.42)	381 (100)	
SSC	87 (81.31)	20 (18.69)	107 (100)	
HSC	55 (93.22)	4 (6.78)	59 (100)	
Graduate and above	65 (87.84)	9 (12.16)	74 (100)	
Total	534 (82.41)	114 (17.59)	648 (100)	
Fathers' occupation				
Farming	55 (77.46)	16 (22.54)	71 (100)	0.093
Government job	69 (83.13)	14 (16.87)	83 (100)	
Private job	124 (81.58)	28 (18.42)	152 (100)	
Business	239 (83.28)	48 (16.72)	287 (100)	
Living abroad	36 (87.80)	5 (12.20)	41 (100)	
Driver	11 (78.57)	3 (21.43)	14 (100)	
Total	534 (82.41)	114 (17.59)	648 (100)	
Monthly income				
Up to Taka 10,000	301 (88.79)	38 (11.21)	339 (100)	0.007**
Taka 10,001–20,000	170 (77.63)	49 (22.37)	219 (100)	
Taka 20,001–50,000	53 (67.9)	25 (32.1)	78 (100)	
Taka 50,001 and above	10 (83.33)	2 (16.67)	12 (100)	
Total	534 (82.4)	114 (17.6)	648 (100)	

*Chi-square test; **Significant level $p < 0.01$; ***Significant level $p < 0.05$. HPV, human papillomavirus; HSC, Higher Secondary Certificate; SSC, Secondary School Certificate.

Table 5. Association between the characteristics of the participants and the presence of HPV 16 antibodies

Characteristics of the participants	Crude OR (95% CI)	Adjusted OR* (95% CI)	*p*-value
Age group (years)			
9–13	Ref		
14–15	0.55 (0.36–0.84)	0.61 (0.39–0.95)*	0.020***
Participants' educational qualification			
Grades 3–5			
Grades 6–8	0.89 (0.61–1.29)	0.88 (0.60–1.29)	0.510
Grades 9–10	0.51 (0.29–0.88)	0.50 (0.28–0.89)	0.004**
Mothers' educational qualification			
No schooling			
Up to primary	1.73 (0.68–4.40)	2.40 (0.66–8.68)	0.182
SSC	2.59 (0.95–7.07)	3.37 (0.82–13.76)	0.090
HSC	3.64 (1.14–11.58)	4.33 (0.91–20.73)	0.057
Up to primary	1.73 (0.68–4.40)	2.40 (0.66–8.68)	0.176
Graduate and above	2.18 (0.69–6.95)	3.73 (0.79–17.61)	0.096
Mothers' occupation			
Housewife			
Service holder	1.05 (0.37–2.95)	0.94 (0.33–2.67)	0.903
Fathers' educational qualification			
No schooling			
Up to primary	0.99 (0.42–2.32)	0.55 (0.17–1.72)	0.302
SSC	1.46 (0.57–3.74)	0.69 (0.20–2.42)	0.556
HSC	2.68 (0.88–8.17)	1.14 (0.28–4.67)	0.854
Graduate and above	1.00 (0.38–2.61)	0.44 (0.12–1.64)	0.223
Fathers' occupation			
Government job	2.18 (1.09–4.34)	1.99 (0.96–4.13)	0.056
Private job	1.69 (0.94–3.05)	1.57 (0.85–2.88)	0.146
Business	2.06 (1.20–3.55)	1.93 (1.10–3.39)	0.023***
Living abroad	2.85 (1.15–7.05)	2.26 (0.89–5.70)	0.076
Driver	2.53 (0.65–9.88)	2.85 (0.67–12.07)	0.154
Monthly income			
Up to Taka 10,000			
Taka 10,001–20,000	0.80 (0.54–1.17)	0.77 (0.52–1.15)	0.201
Taka 20,001–50,000	0.70 (0.41–1.19)	0.65 (0.36–1.17)	0.145
Taka 50,001 and above	0.99 (0.26–3.74)	0.73 (0.19–2.91)	0.654

*Adjusted for all variables listed above. **Significant level $p < 0.01$; ***Significant level $p < 0.05$. CI, confidence interval; HPV, human papillomavirus; HSC, Higher Secondary Certificate; OR, odds ratio; SSC, Secondary School Certificate.

The GOB decided to scale up a school-based vaccination program through EPI in collaboration with Global Alliance for Vaccines and Immunizations and development partners.[34] Still, after completing the HPV vaccination demonstration program, Bangladesh had been awaiting the rollout of HPV vaccination since 2018. The long delay in scaling up vaccination may be related to vaccine manufacturing shortages, costs, and supply-related problems. Eventually, the GOB introduced a single-dose bivalent HPV vaccine into the national program on 2 October 2023, with a rollout plan to cover all eight divisions for girls aged 10 to 14 years.[35] Recent advances in HPV vaccine development reinforce the viability of single-dose regimens as practical strategies for CC prevention in low- and middle-income countries. A preclinical study from Tianjin, China, demonstrated that a single-injection bivalent HPV vaccine using

Table 6. Association between the characteristics of the participants and HPV 18 antibodies

Characteristics of the participants	Crude OR (95% CI)	Adjusted OR * (95% CI)	*p*-value
Age group (years)			
9–13	Ref		
14–15	0.87 (0.51–1.45)	1.07 (0.62–1.85)	0.801
Participants' educational qualification			
Grades 3–5			
Grades 6–8	0.92 (0.59–1.42)	0.94 (0.60–1.48)	0.782
Grades 9–10	0.75 (0.38–1.45)	0.74 (0.37–1.50)	0.402
Mothers' educational qualification			
No schooling			
Up to primary	1.16 (0.37–3.59)	0.71 (0.14–3.45)	0.661
SSC	1.10 (0.33–3.61)	0.59 (0.10–3.38)	0.550
HSC	3.33 (0.74–14.95)	1.75 (0.24–12.73)	0.584
Graduate and above	1.86 (0.43–7.93)	1.26 (0.17–9.34)	0.821
Mothers' occupation			
Housewife			
Service holder	0.79 (0.25–2.44)	0.77 (0.25–2.38)	0.649
Fathers' educational qualification			
No schooling			
Up to primary	1.45 (0.59–3.56)	1.17 (0.35–3.90)	0.801
SSC	1.52 (0.56–4.09)	1.46 (0.39–5.54)	0.573
HSC	4.82 (1.27–18.21)*	4.02 (0.67–24.28)	0.124
Graduate and above	2.52 (0.83–7.65)	2.16 (0.51–9.09)	0.293
Fathers' occupation			
Farming			
Government job	1.43 (0.64–2.57)	1.29 (0.52–3.19)	0.589
Private job	1.28 (0.64–2.57)	1.23 (0.59–2.56)	0.576
Business	1.44 (0.76–2.74)	1.39 (0.71–2.74)	0.339
Living abroad	2.09 (0.70–6.22)	1.75 (0.57–5.34)	0.329
Driver	1.06 (0.26–4.29)	1.05 (0.23–4.81)	0.951
Monthly income			
Up to Taka 10,000			
Taka 10,001–20,000	0.43 (0.27–0.70)	0.41 (0.26–0.67)	0.000**
Taka 20,001–50,000	0.26 (0.15–0.48)	0.21 (0.11–0.40)	0.000**
Taka 50,001 and above	0.63 (0.13–2.98)	0.29 (0.06–1.39)	0.123

*Adjusted for all variables listed above; **Significant level $p < 0.01$; ***Significant level $p < 0.05$. CI, confidence interval; HPV, human papillomavirus; HSC, Higher Secondary Certificate; OR, odds ratio; SSC, Secondary School Certificate.

HPV16/18 VLPs delivered via an erodible tannic acid/polyethylene glycol film elicited robust humoral and cellular immune responses comparable to multi-dose regimens.[36] Another 2025 study using sustained-release poly lactic-co-glycolic acid microparticles loaded with HPV VLPs showed prolonged antigen retention and potent immunogenicity in animal models.[37]

Global policy shifts further support single-dose strategies. In October 2024, the WHO prequalified Cecolin—the fourth HPV vaccine—for use as a single dose, endorsing the 2022 recommendation to transition from two-dose to more flexible single-dose schedules to alleviate global supply constraints.[38] Additionally, real-world data from Thailand demonstrated 90.6% effectiveness of a single-dose bivalent HPV vaccine four years post-vaccination, comparable to 95.4% effectiveness of the two-dose regimen.[39]

These findings, along with the observed high seroprevalence of HPV16 (72.8%) and HPV18 (82.4%) antibodies 36 months post-vaccination, reinforce the durability and practicality of single-dose HPV vaccination. The accumulating global and local evidence strongly supports a shift toward single-dose schedules, especially in resource-constrained settings like Bangladesh, where such an approach could significantly improve coverage and advance CC elimination efforts.

To maximize impact, Bangladesh should rapidly expand its single-dose HPV vaccination program, targeting a broader cohort of girls within a shorter timeframe. This strategy can address challenges related to supply, logistics, and cost, ultimately enhancing vaccine uptake.

Our study also found similar antibody persistence across age groups up to 15 years, with no significant differences by age, suggesting the vaccine's applicability to girls aged 14 and 15. Incorporating this older age group into the existing school-based program could increase coverage. Future research on antibody and DNA presence in a broader female population will further inform population-level vaccine impact.

The study has limitations. Being limited to Dhaka, the findings may not fully represent rural or underserved populations. It also excluded out-of-school girls, potentially missing important subgroups. The cross-sectional design limits causal inference, and the absence of a control group makes it difficult to attribute antibody presence solely to vaccination. However, the high seroprevalence in a largely HPV-naïve population still indicates a likely vaccine effect.

Despite these limitations, the study's strengths include a large sample size, standardized antibody testing, and high relevance to public health planning in low-resource settings. These findings contribute to the growing body of evidence supporting simplified, cost-effective HPV vaccination strategies.

Conclusions

This study demonstrates sustained immunogenicity three years after a single-dose bivalent HPV vaccination among adolescent girls in Bangladesh, with high seroprevalence of both HPV16 and HPV18 antibodies. Seropositivity for HPV16 is significantly lower among older adolescents and those in higher grades. In contrast, lower household income shows a strong association with higher HPV18 antibody prevalence, suggesting that socioeconomic status may influence immune response. The GOB should consider rapidly scaling up this cost-effective, single-dose HPV vaccination strategy within the national immunization program to increase coverage among girls.

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Conflict of interest

The authors declare no competing interests.

Author contributions

Conception and design (AN, SAB, JF, FH), data collection and coordination of the field activities (NF, SJS, MFI, FH), arranged and supervised laboratory work (AN, SUM, JF), acquisition, analysis, and interpretation of data (AN, MFI, SUM, SJ), manuscript drafting and revising it critically (AN, MFI, SAB, NF, JF, SUM, SJS, FH), and approval of the final version of the manuscript (AN, MFI, SAB, NF, JF, SUM, SJS, FH).

Ethical statement

Ethical clearance was received from the Institutional Review Board of the BMU Ethics and Scientific Review Committee (Ref: No. BSMMU/2020/686, dated 16/01/2020). This study was performed following the principles of the Declaration of Helsinki (as revised in 2024). All participants and their parents/guardians provided written informed consent.

Data sharing statement

Most of the data are contained within this published article. To preserve data privacy, the data used are not freely available. The authors confirm that the data will be shared upon a justified request.

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