

Original Articles

Evaluation of Human Papilloma Virus 16/18 Antibody Titer Seven Years after Vaccination with Cervarix among Adolescent Girls of Bangladesh

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Abstract

Background: Prevention of HPV infection by prophylactic vaccination is a critical step in reducing the cervical cancer burden. A pilot study was conducted to evaluate the serological immune response to the bHPV vaccine in adolescent Bangladeshi girls at 7th months of vaccination. This is the first in depth follow up study in Bangladesh to directly evaluate the long-term immunogenicity of the bHPV vaccine by measuring sustained HPV16/18 antibody titer seven years after vaccination.

Objective: To determine the long-term immune response against HPV16/18 induced by the immunization with bivalent human papilloma virus (bHPV) 16/18 ASO4 adjuvanted vaccine by measuring the antigen specific antibody titer.

Materials & Method: This was a cross-sectional observational study conducted among 30 adolescent Bangladeshi girls from October 2015 to September 2016 who were vaccinated with 3 doses of bHPV vaccine from December 2008 to June 2009 in the Division of Gynaecological Oncology of BSMMU. Antibody titer against bHPV 16/18 ASO4 adjuvanted cervical cancer vaccine was measured in the serum of the 30 vaccinee in the Virology laboratory of BSMMU using DRG HPV IgG ELISA (REF.EIA- 4907) kit. Data were analyzed by using SPSS version 21.

Results: The mean age of the population was 17.9±1.2 years. Among 30 vaccinated subjects, vaccine-induced long-term anti-HPV ELISA seropositivity was 93.33% (with 95% CI) for both HPV 16/18 types measured at year seven post-vaccination. Immunization with the HPV16/18 L1 virus-like particle vaccine adjuvanted with ASO4 induced sustained high levels of antibodies among adolescent Bangladeshi girls against high-risk HPV genotypes 16/18.

Conclusion: Vaccination with bHPV is predicted to provide long-term protection against HPV infection and subsequent development of high-grade cervical lesions and cancer.

Keywords: Cervical cancer, Human Papilloma Virus (HPV), Bivalent HPV 16/18 vaccine, HPV antibody titer.

Introduction:

Human Papilloma virus (HPV) is accounted for half of the infection-attributable cancers in developing countries¹. Among all HPV-associated malignancies, cervical cancer is the most common

cause of morbidity and mortality worldwide. Most cervical cancers (99.7%) are caused by HPV². Cervical cancer is the 4th most common cancer in women worldwide and is the 2nd most common cancer in women of developing countries³.

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Incidence of cervical cancer is 19.2 per 100,000 women and is the 2nd leading cause of cancer deaths in women aged 15 - 44 years in Bangladesh⁴. Approximately 70% of cervical cancers worldwide are associated with two high-risk HPV types 16 & 18^{5,6}. HPV type 16 was detected in 81.82% and type 18 in 9.09% cases of cervical cancer of the women in Bangladesh⁷.

One of the greatest medical breakthrough in the last century is the development of HPV vaccine against cervical cancer⁸. The bivalent HPV (bHPV) type 16/18 vaccine named Cervarix® is licensed for use in females only, young adolescent girls being the primary target group. Humoral immunity mediated by neutralizing the antibody response to L1 proteins of HPV is considered to be the hallmark of protection induced by the vaccine^{9,10}. Different studies in USA, Australia and Scotland have proved the establishment of herd immunity and cross-protection^{11,12,13,14}. Sustained efficacy, immunogenicity and safety of the bHPV vaccine were observed in the long-term follow-up study¹⁵. The global burden of cervical cancer falls heaviest on the developing countries which haven't introduced the HPV vaccine as part of their national public health strategy.

The duration of protection provided by HPV vaccination is critical to overall vaccine effectiveness¹⁶. Seroconversion is the rule after vaccination. Serum antibody levels are routinely measured to monitor vaccine efficacy¹⁷. As it is neither ethical nor feasible to conduct HPV vaccine efficacy trials in adolescent girls, antibodies against HPV are measured and the principle of immune bridging is used to infer efficacy in younger subjects¹⁸.

S. Khatun .et al. (2011)¹⁹ published an article on a pilot program on bHPV vaccine in Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from March, 2008 to July, 2009 to evaluate the serological immune response to the bHPV vaccine at the 7th month of vaccination. Currently, there is no study to assess the long-term immunogenicity of the bHPV vaccine in Bangladesh. So, this is the first study to directly evaluate the long-term immunogenicity of the bHPV vaccine in adolescent Bangladeshi girls.

Materials and Methods:

This was Cross-sectional observational study. The study population was 30 adolescent Bangladeshi girls who were vaccinated with bHPV vaccine in 2008. The place of study was at the Division of Gynecological Oncology, BSMMU and the period of study was from October, 2015 to September, 2016. Ethical clearance was obtained from Institutional Review Board of BSMMU. Informed written consent was obtained from all participants or legal guardian or both. A structured data collection sheet was used to collect data.

After counseling, 3cc of blood sample was collected from ante-cubital vein in a sterile vacuum container with proper labeling and sent to Virology laboratory of BSMMU. The samples were allowed to clot at room temperature for 30 minutes, serum was separated by centrifugation for 10 minutes, taken in an Eppendorf and labeled. Specimens were stored at -20°C before assay. Antibody titer against HPV16/18 was measured by Enzyme Linked Immunosorbent Assay (ELISA) with DRG HPV IgG ELISA kit (REF.EIA- 4907) as per manufactures instruction.

All the data were entered into computer database, organized and analyzed using Statistical Package for the Social Sciences (SPSS) software version 21. Categorical data were presented as frequency and percentage and the continuous variables were expressed as Mean and Standard Deviation. Vaccine-induced long-term serum anti-HPV responses at 7 years of vaccination were evaluated by the Binomial (Clopper-Pearson exact) test and 95% confidence intervals (CI). Data and results were presented by tables and figures.

Results:

A total 30 vaccinee who received first dose of bivalent human papillomavirus- 16/18 ASO4 adjuvanted cervical cancer vaccine on 27th December, 2008 were enrolled. In this study their seven years of post-vaccination responses (Seropositivity) to bivalent HPV VLPs were assessed by ELISA method.

Socio-demographic characteristics of the girls are presented in table-I. The mean age were 17.9 years with SD \pm 1.2. The marital status of study subjects showed that most of them (73%) were unmarried.

Fifty three percent of the vaccinee were in the lowest economic strata of monthly income. Seventy three percent of the study subjects were student.

Distribution of residence of the study population showed that most of them (83%) reside in Dhaka. Fifty three percent of the vaccinee were the students of secondary school certificate level.

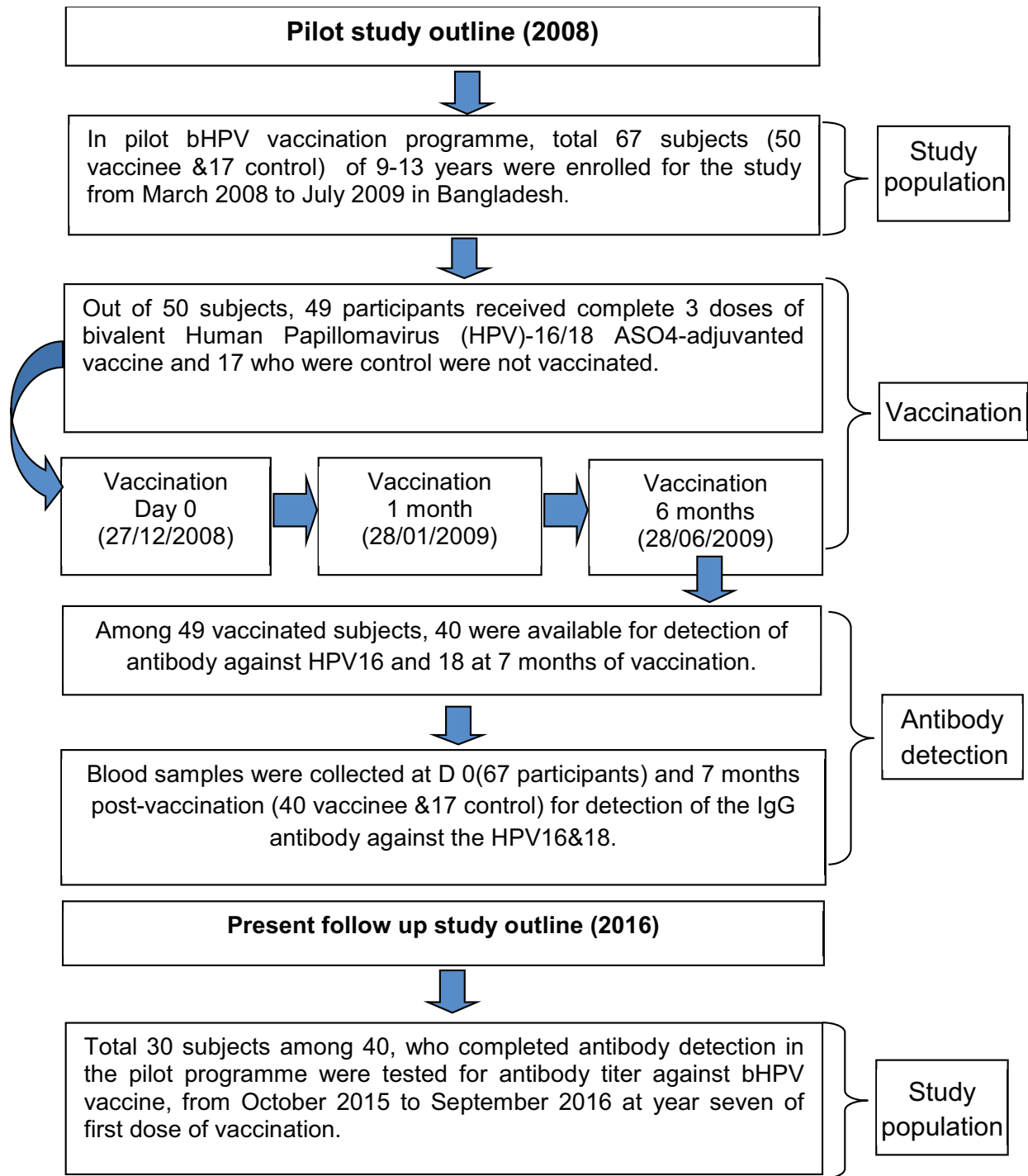


Fig.-1: Outline of the pilot study and present follow-up study

Table-I
Socio-demographic characteristics of the vaccinated girls(n=30)

Characteristics	Frequency (n)	Percentage
Age (years)		
<17	04	13
17-19	23	77
>19	03	10
Marital status		
Unmarried	22	73
Married	08	27
Socio economic status		
Tk. <20000/month	16	53
Tk. 21000-30000/month	08	27
Tk. >30000/month	06	20
Occupation		
Student	22	73
House wife	05	17
Service holder	03	10
Residence		
Dhaka	25	83
Outside Dhaka	05	17
Educational status		
Primary education	06	20
SSC	16	53
HSC	08	27

Immunogenicity:

Vaccine-induced long-term antibody responses to bHPV VLPs were assessed in the vaccinated population in percentage. Among 30 vaccinated subjects, vaccine-induced sero-conversion was found in 93.33% for both 16 and 18HPV types. Vaccine-induced long-term serum anti-HPV responses at 7 years of vaccination with three-dose regimen of bivalent HPV vaccine was evaluated by the Binomial (Clopper-Pearson exact) test and 95% confidence intervals (CI) were calculated (Table III).

Among 30 vaccinated subjects, vaccine-induced anti-HPV ELISA seropositivity percentages along with 95% CI before vaccination, at 7 months of vaccination and at 84 months (7 Years) of vaccination were 0%, 97.5% and 93.33% respectively (Table II). In the study population anti-HPV antibody response (Seropositivity %) observed at 7 months was 97% and at 7 years was 93%. Out of 30 vaccinee, one girl remained seronegative at 7 months post vaccination whereas two girls were seronegative at 7 years post vaccination (Fig 2). Maximum levels of anti-HPV IgG concentration were found at 16 and 19 years (Fig 3).

Table-II
Summary of anti-HPV seropositivity in the study population at different phases of vaccination

Antibody type	Point of Time	No. of subjects	Seropositivity (%)	95% CI (%)
	0 month	50	0	0
Anti-HPV16/18	7 months	40	39(97.5%)	86.8-99.9
	84 months	30	28 (93.33%)	77.9-99.2

Table-III
Summary of Anti-HPV16/18 antibody titer at 7 years of vaccination

Antibody type	No. of subjects	Serostatus	Percentage (%)	95% CI
Anti-HPV16/18	30	Seropositivity	28/30 (93.33)	77.9-99.2
		Seronegativity	2/30 (6.67)	0.01-15.10

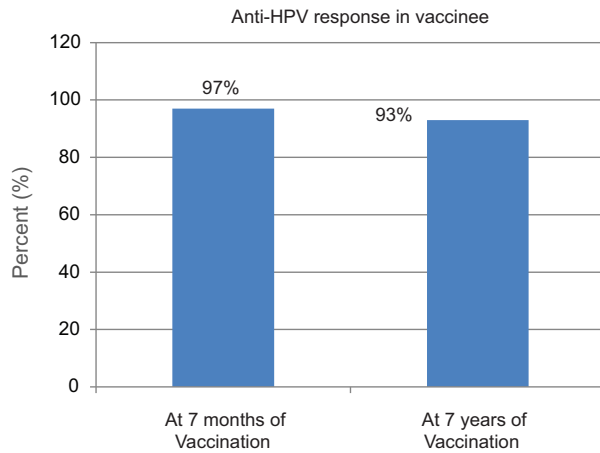


Fig.-2: Comparison of anti-HPV antibody seropositivity of vaccinated girls (n=30)

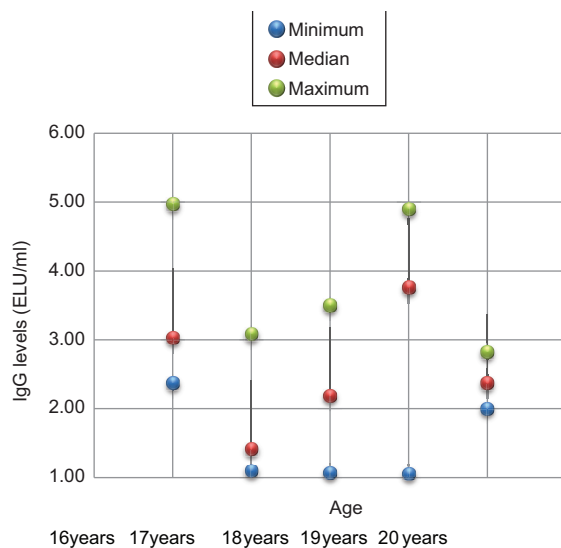


Fig.-3: Age-specific trends in anti-HPV IgG concentration among study subjects

Discussion:

About 85% of the global burden of cervical cancer are in the developing countries, where it accounts for almost 12% of all female cancers³. Prophylactic vaccination against HPV infection is expected to offer a major advance in the prevention of cervical cancer. Vaccines are highly efficacious in preventing infection with HPV types 16 and 18 and precancerous cervical lesions caused by these virus types²⁰. Serum neutralizing antibody titer is several-fold higher after vaccinations with bHPV than natural infection by HPV-16 and 18.²¹

Present study is a follow-up study of S. Khatun et al. (2011)¹⁹, where the immunogenicity and safety

profile of human papillomavirus-16/18 AS04- adjuvanted cervical cancer vaccine given in 9–13 years healthy Bangladeshi girls were studied. In the present follow up study among 40 vaccinee only 30 could be contacted and were available for the detection of the IgG antibody against the HPV 16/ 18, seven years after the last dose of vaccination.

Demographic characteristics of the study population (Table-I) shows that age distribution of the subjects ranged from 16-20 years with a Mean±SD of 17.9±1.2 years while the age range was 9-13 years with 11.1±1.4 (SD) years at the entry of the study in 2008. While the study conducted by Romanowski et al (2014)¹⁸ age range was 15-25 years with 19.7± 3.0 (SD) years in 3 dose group which is almost comparable to present study. Most of the vaccinee belonged to low socio-economic class. Benard et al. (2008)²² also found lower education and higher poverty among their study population with cervical cancer.

Current study showed sustained HPV antibody titer in vaccinated girls at 7 years of vaccination with bHPV vaccine. Vaccine-induced long-term anti-HPV seropositivity was found in 93.33% subjects for both HPV 16 and 18 types (with 95% CI) (Table- III). Similar long-term immunogenicity against the bHPV vaccine was observed by many researchers in different follow-up studies. Harper et al (2006)²³ in their study observed seropositivity in > 98% and Carvalho et al (2010)²⁴ observed seropositivity in ≥96% for HPV-16/18 antibodies. Similar results were observed in Roteli-Martins et al (2012)²⁵ and Naud et al. (2014)¹⁵ follow up studies. The present study showed a low seropositivity may be due to a small sample size and a different HPV antibody assay technique.

As the present study is a follow-up study of the pilot randomized controlled trial (RCT) conducted by S. Khatun et al. (2011)¹⁹, direct comparison of IgG levels measured for both 16 and 18HPV types in 28 seropositive subjects were not possible. Present follow up study conducted by using same semi quantitative ELISA kit (EIA-4907) for better comparison of antibody titer with the previous study. But the other studies showed the trend of antibody titer as rising, declining & maintaining a plateau level with time^{15,18,23,24,26}.

In this study, anti- HPV IgG showed maximum peak at 16 and 19 years old subjects (Fig 3) while the study conducted by Lupi et al. (2014)²⁷ maximum antibody

peak was detected at 17 years while Schwarz et al. (2011)²⁶ showed higher anti-HPV-16/18 antibody titers in the 15–25 years' age group. So, current study finding is compatible with above studies.

Several folds higher antibody titers for total IgG and neutralizing antibodies above natural infection levels is desired for any vaccine immunogenicity and efficacy study. In all the above follow-up studies seropositivity was maintained between 96% to 100% by the bHPV vaccinee. So, the study findings are almost consistent with many previous studies. But there were

some limitations of this study like-

1. Low seropositivity level is reflected due to small sample size.
2. Cross-sectional observational study and lack of a direct control group.
3. The Kit used in the study can perform only semi-quantitative assay of bHPV antibody.
4. The kit used for bHPV serological analysis is not yet widely used to monitor HPV vaccine immunogenicity and comparison with other studies was difficult and may not reflect the actual protective status.

Conclusion:

Immunization with the bHPV-16/18 L1 virus-like particle vaccine adjuvanted with AS04 induces sustained high levels of antibodies against high-risk HPV genotypes 16 and 18 even after seven years post-vaccination. The durable immune response induced by the HPV16/18 vaccine is predicted to provide long-term protection against HPV16/18 infection and subsequent development of high-grade cervical lesions and cancer. Therefore, a National HPV Vaccination Program seems to be the window of opportunity to reduce the mortality and morbidity from cervical cancer in Bangladesh.

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