

Review Article

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Cervical cancer prevention & the role of human papillomavirus vaccines in India

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Human papillomavirus (HPV) is a necessary cause of cervical cancer, the leading cause of cancer deaths among Indian women. Current screening and prevention programs based on cytology have not been effective in reducing the disease burden. Two vaccines are now available for primary prevention. They generate neutralizing antibodies to HPV capsid protein. The vaccines have been shown to confer nearly 100 per cent protection against cervical pre-cancers and genital warts caused by HPV types 16/18 in HPV naïve population with few or no side effects. Though there is some cross-protection, around 30 per cent of cervical cancers will not be prevented by the vaccine. Vaccination and screening, which are complementary and synergistic, now constitute the new paradigm for prevention of this disease.

Key words Cervical cancer - human papillomavirus - prophylactic vaccine - virus-like particle

HPV epidemiology in India

A meta-analysis from India showed HPV-16, -18, -6, -11 and at least one other HPV type, with a minimum of 20 cases in each grade of lesion¹. Total as well as type-specific prevalence of various HPV types were estimated, stratified by cervical lesion grade. HPV DNA was present in 94.6 per cent of invasive cervical cancers (ICC). The HPV-16/18 fraction was found to account for 78.9 per cent of ICC, 61.5 per cent with high-grade squamous intraepithelial lesions (HSIL), 30.8 per cent with low-grade squamous intraepithelial lesions (LSIL) and 3.9 per cent in women with normal cytology/histology. The most prevalent HPV types associated with cervical cancer were HPV-16, -18, -45, -33, -35, 58, -59 and -31. This pattern is more similar to the pattern reported from Europe than from south-east Asia².

Implications for early detection and prevention

Knowledge of the HPV types implicated in cervical carcinogenesis paved the way for development of commercial diagnostic tests for HPV DNA as well as a prophylactic vaccine against HPV 16/18. Although HPV genotyping by PCR is necessary for epidemiological studies, the method is expensive, time-consuming and laborious. Development of the Hybrid Capture 2 (HC2) assay system (Digene Corp., Gaithersburg, USA) allowed detection of a cocktail of 13 high-risk HPV types seen most commonly in cervical cancer: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68. With its advantages of convenience and reproducibility, this test was a major step forward for clinicians with three major areas of application: (1) in the triage of cases reported as atypical squamous cells of undetermined significance

(ASCUS) on cytology; (2) in the follow-up of women treated for cervical intraepithelial neoplasia (CIN) by conservative methods like cryotherapy and loop electrosurgical excision procedure (LEEP); and (3) as a primary screening method. However, this method, though cheaper than PCR, is more expensive than cytology. Therefore it has not been possible to consider using HPV tests as a primary screening method even though it has been shown that the sensitivity of HPV DNA testing for detection of CIN2+ disease is far superior to cytology, not only in developing countries but also in the West³. Hope has come in the form of the new cheap and rapid HPV tests that have shown good concordance with HC2⁴. It is expected that these will be commercially available by 2009 to allow universal screening.

Prophylactic HPV vaccines

The knowledge of HPV epidemiology in cervical cancer also formed the basis for deciding that the two most widely prevalent types worldwide, HPV-16 and -18, should be incorporated into the first prophylactic vaccines. In contrast to most viral vaccines that are based on an attenuated form of the virus, the development of an attenuated HPV vaccine was not possible because there is no effective culture system to propagate the virus. The immune response to genital HPV infections is mainly characterized by local cell-mediated immunity that is associated with lesion regression and possibly protection against a further infection with the same genotype of HPV. Humoral immunity in the form of antibody that is virus neutralizing is generated in most, but not all, infected individuals and is directed against conformational epitope(s) on the major coat or capsid protein L1 displayed on the outer surface of the intact virus particle⁵. Serum-neutralizing antibody levels in natural HPV infections, even at peak titres just after seroconversion, are low⁶. This may be because the viral capsid proteins are only expressed in the uppermost layers of the HPV-infected epithelium and are not efficiently presented to the systemic immune system. Despite low antibody levels, however, seropositive individuals are protected against further viral challenge, thus suggesting that vaccines that generate neutralizing antibodies to HPV capsid protein will be effective prophylactically.

Thus work on HPV prophylactic vaccines has focused on the viral capsid proteins L1 and L2. Recombinantly expressed L1, the major capsid protein of HPV, self-assembles to form empty viral capsids referred to as virus-like particles (VLPs). These L1

VLPs are morphologically and antigenically similar to authentic papillomavirus, but since they contain no DNA they are harmless⁷. Data from animal studies indicate that L1 VLPs are non-infectious and generate protection against a viral challenge^{8,9}. L1 VLPs appear to induce very little cross-neutralization against other genotypes¹⁰. Thus an optimal VLP-based vaccine requires multiple vaccine components to provide good coverage against diseases caused by more than one virus type.

HPV-16/18 prophylactic vaccines

Two HPV L1 VLP vaccines have now been developed commercially that protect against HPV-16 and -18, which are associated with 70 per cent of cervical cancers worldwide.

1. A quadrivalent vaccine (Gardasil®, Merck and Co. Inc., Pennsylvania, USA) that contains purified L1 VLPs of HPV types 6/11/16/18 at 20/40/40/20 µg per dose formulated on a proprietary alum adjuvant. The product is delivered by intra-muscular injection as a 0.5-ml dose in a three-shot immunization protocol at 0, 2 and 6 months. The four HPV types that it protects against are responsible for 70 per cent of cervical cancers and 90 per cent of genital warts.

2. A bivalent vaccine (Cervarix®, GlaxoSmithKline Biologicals, Rixensart, Belgium) that contains purified L1 VLPs of HPV types 16/18 at 20/20 µg per dose formulated on an ASO4 adjuvant comprising 500µg of aluminium hydroxide and 50 µg of 3-deacylated monophosphoryl lipid A. The product is delivered by intramuscular injection as a 0.5-ml dose in a three-shot immunization protocol at 0, 1 and 6 months.

The quadrivalent vaccine was the first to be licensed and is available in the US, Europe, Australia and Asia in over 100 countries. The bivalent vaccine is available in nearly 80 countries. In India, both vaccines have now been licensed. In addition, several candidate vaccines are in the process of development.

When and who do we vaccinate?

The quadrivalent HPV vaccine was the first to be licensed by the Food and Drug Administration (FDA) for use in girls/women aged 9-26 yr. The Advisory Committee for Immunization Practices (ACIP) - a national group of experts in the USA that advises the Centers for Disease Control and Prevention on vaccine issues - has recommended the HPV vaccine for 11-12 yr old girls. The vaccine is also recommended for 13-26 yr old girls/women who have not yet received or

completed the vaccine series¹¹. In India, the quadrivalent vaccine is licensed for use in women up to age 26 yr and the bivalent vaccine up to 45 yr.

Since HPV vaccines are prophylactic vaccines, the most appropriate target population for HPV vaccination will depend on the age at which individuals first get exposed to HPV. This depends largely on the sociocultural behaviour patterns of the region. In a survey among college students in Delhi, the age at sexual debut is earlier than the legal age at marriage, which is 18 yr (unpublished data). Similar results have been reported from the National Family Health Survey also. In order to ensure that recipients receive maximum protection, the target population should be young adolescents (9–13 yr of age). At this younger age the recipients mount a better immune response. Also, it may be feasible to link vaccine delivery with the school health programme and improve coverage. Parental consent will, of course, be a prerequisite regardless of the mode of implementation.

It has been seen that < 3 per cent of women suffer from infection of both HPV types⁴. Thus vaccination of sexually active women of any age is recommended as it will still protect against infection with the other HPV type, and may possibly protect against re-infection with the same HPV type in the future.

The question of male vaccination is still debated. Some countries have licensed the vaccine for use in boys as well. The case for male vaccination against HPV relies on the establishment of herd immunity, thus reducing the chances of an infected individual transmitting the virus to a susceptible person. However, this increases the cost of vaccination two-fold. Since the quadrivalent vaccine immunizes against HPV types that cause genital warts as well, it is more likely to be taken up by teenage boys.

Immunogenicity and efficacy

Peak antibody titres are achieved 1 month after dose 3, *i.e.*, at month 7, after which detectable titres decline until about month 18, when the rate of decline decreases considerably and titres appear to stabilize over the next few months at or above titres observed in women with naturally acquired and cleared infections (*i.e.*, those positive for type-specific anti-HPV serum antibodies and negative by PCR-based assay for the same type of HPV-DNA at enrolment)¹²⁻¹⁵.

The aim of the HPV vaccines is to prevent cervical cancer but because of the long natural history of this

disease, it may well be a couple of decades before this difference can be documented. Therefore, it has been agreed that efficacy can be measured by surrogate markers, namely, the occurrence of new HPV infections and development of high-grade cervical intraepithelial neoplasia (CIN 2+) disease. In this respect, both the bivalent and quadrivalent HPV vaccines have demonstrated truly remarkable efficacy in Phase II and Phase III trials. Protection against new HPV infection and development of CIN is observed among women with a wide range of antibody titres.

The Phase II trial of the bivalent HPV-16/18 vaccine was divided into an initial follow-up period that had a median follow-up of 2.2 yr and a subsequent follow-on study of a subset of the original enrollees with a median follow-up of 4.0 yr¹³⁻¹⁵. More than 98 per cent seropositivity was maintained for HPV-16/18 antibodies during the extended follow-up phase of 4.5 yr¹⁵. They noted significant vaccine efficacy against endpoints: incident infection, 96.9 per cent (95% CI 81.3-99.9); persistent infection: 6 month definition, 94.3 (95% CI 63.2-99.9); 12 month definition, 100 per cent (95% CI 33.6-100). In a combined analysis of the initial efficacy and extended follow-up studies, vaccine efficacy was 100 per cent (42.4-100) against CIN lesions associated with vaccine types. Broad protection against cytohistological outcomes beyond that anticipated for HPV-16/18 and protection against incident infection with HPV-45 and HPV-31 was also noted. This has been attributed to the fact that they belong to the same phylogenetic clade as the vaccine types.

When the quadrivalent vaccine was administered to subjects not been previously exposed to either HPV-16 or 18, the vaccine was 98-100 per cent effective in preventing HPV-16 and -18 related CIN 2 or 3 and adenocarcinoma *in situ* (AIS)¹⁶⁻¹⁸. Efficacy was lower (36-44%) when the entire population who had undergone randomization was considered, since this also included HPV-16/18 non-naïve subjects before enrollment¹⁶. Seroconversion occurred in virtually 100 per cent of subjects¹⁷. Efficacy persisted for at least 5 yr following vaccination. In an intention to treat analysis, protection against CIN2+ or AIS associated with any HPV type among women who were baseline HPV-16/18 negative was seen in 17 per cent (95% CI 4-44)¹⁶. The percentage of cases HPV positive among CIN 2+ (but not invasive cancer) is lower, as also the fraction of cases attributable to HPV-16/18, as described above¹. Since the proportion of cases of

adenocarcinoma attributed to HPV 18 is higher than in squamous carcinoma, and in fact almost entirely associated with HPV 16/18/45. Therefore the degree of protection in these cases is estimated to be higher. This is of special significance since these cases are more difficult to detect than squamous carcinoma, since they are harboured within the endocervical canal and likely to be missed even in women who are regularly screened.

Cross protection has also been reported against other high-risk HPV genotypes for both vaccines. The bivalent vaccine has demonstrated 78 per cent cross-protection (range: 39–93%) against incident infection with HPV-45 and -60 per cent cross-protection (range: 20–81%) against HPV-31, which are phylogenetically related to HPV-18 and -16 respectively. The quadrivalent vaccine reduced the rate of HPV-31/33/45/52/58 persistent infection by 19.5 per cent (95% CI 6.5, 30.7) and disease by 15.7 per cent (95% CI 2.3, 27.3)¹⁹. Vaccination also reduced the rate of HPV-31/45, 56 and 59 related diseases by 13.8 per cent (95% CI -8.2, 31.4), 23.2 per cent (95% CI 2.3, 39.7) and 47.5 per cent (95% CI 16.2, 67.7). Vaccine efficiency in preventing genital warts was 100 per cent^{17,18}.

Previous studies focussed on the younger age group only but recent reports have demonstrated comparable safety and immunogenicity of HPV vaccines in women aged 24 to 45 yr, with good efficacy in protecting against new infections and CIN, as was previously seen in the case of the non-naïve population, since most women are infected with one or the other type only²⁰. In India, a phase IIIb multicentric randomised controlled immunobridging study in 330 subjects found the antibody levels for both HPV-16 and -18 compared well with those from previous studies²¹.

Duration of protection

The available data from HPV vaccine trials indicate that antibody levels fall from the peak levels after immunization to a plateau level that is at least a log higher than those detected in natural infections and that persists for at least 48 months post-vaccination¹²⁻¹⁵. In the case of the quadrivalent vaccine there is a drop in the titer of the HPV-18 antibody by the end of 3 yr but there is no loss of efficacy. Moreover it has been shown that there are adequate memory cells. There is no unequivocal evidence from the trials whether exposure to virus post-vaccination will act as a natural booster. So far, the follow-up in the vaccine trials has been for a period up to 6.4 yr. The total duration of protection

is not yet known. Data correlating antibody persistence with protection is also awaited. At present data do not support the role of a booster dose.

Adverse effects

Reported adverse events were generally mild and moderate. The most commonly reported adverse events are reactions at the injection site including pain, redness, or swelling, which were reported more often among vaccine recipients than among placebo recipients in the quadrivalent vaccine trial (86% versus 77%) as well as in the bivalent vaccine trial (94% versus 88%). Headache, fatigue and myalgia are the most commonly reported systemic adverse events. Gastrointestinal complaints and itching were also frequently reported, and temperature elevations were reported in about 15 per cent of women¹⁶⁻¹⁸. Serious adverse events were reported with similar frequency in the vaccine and control groups. Vaccination of women already naturally infected with vaccine HPV types has not been associated with any adverse effects in the clinical trials. Overall, both vaccines appear to be generally safe and well-tolerated. Pregnancies have been reported in equal numbers in both groups and there is no increase in the incidence of congenital malformations, pregnancy loss or prematurity.

In the Indian immunobridging study with the bivalent vaccine, the vaccine was well accepted and tolerated, with only minor side effects²¹.

What does the vaccine not protect against?

Even after vaccination programs have been instituted and reasonable levels of coverage obtained, cervical cancer screening programs cannot be discontinued for a number of reasons. One is that the primary target population for vaccination is 9-13 yr old females. Although some “catch-up” vaccination of older, sexually active women may occur, much lower rates of coverage will likely be achieved though “catch-up” vaccination efforts compared to a targeted cohort vaccination of young adolescents.

Secondly, vaccination will not protect against HPV types not included in the vaccines. Although there is evidence that some cross-protection against other high-risk types from the same phylogenetic clade is achieved by vaccinating against HPV-16 and -18, the extent and duration of cross-protection is currently unclear. Since the vaccine does not protect against all types of HPV, it will not prevent all cases of cervical cancer or genital warts. As many as 30 per cent of cervical cancers will

not be prevented by the vaccine. Thus if women who were previously in a screening program discontinue screening after vaccination, they will actually increase their risk of developing cervical cancer. Also, the vaccine does not prevent about 10 per cent of genital warts nor will it prevent other STIs.

Given that screening will need to continue after the introduction of HPV vaccination programs it will be important to eventually re-evaluate how we screen²². The recommended approach of frequent rounds of screening utilizing cytology has not been possible in India. Pap smear anyway has a low sensitivity. After the introduction of the vaccine, there will be a decrease in the number of cytological lesions and the positive predictive value of cytology is likely to drop even further. The rapid HPV DNA test or visual inspection methods hold the most promise in this regard. Using HPV testing for screening coupled with HPV genotyping will provide a simple strategy to monitor long-term protection among vaccinated women, but this may be possible only in research settings in India.

Obstacles and concerns

The major obstacles to implementation of HPV vaccine programs include cost, acceptability, lack of public awareness and infrastructure, concern about unknown side-effects and social and religious barriers.

Cost is a major factor. The Australian government was the first to provide free vaccination to all girls in the target age group. In India and other developing countries, this may be difficult in the face of other competing priorities. GAVI has now approved the HPV vaccine on its list of priority vaccines²³. Since there is already a delivery system for vaccines that includes the cold chain down to the grass roots level²⁴, it is anticipated that linking this vaccine with the EPI program may lead to good acceptance. In general, people in India accept vaccination well²⁵. However, overall coverage by the EPI program has not exceeded 50-60 per cent²⁶.

Parental concerns include the possibility of change in sexual behaviour of teenagers due to a false sense of security against STIs which may lead to an increase in other STIs. Since HPV vaccines target cancer prevention it may be difficult for parents to understand the role of vaccinating 9-13 yr old girls for a cancer that they are unlikely to develop for at least two to three decades. This problem is exacerbated by the fact that the duration of protection afforded by vaccination is not yet known. However, preliminary surveys in India

have revealed that in general parents are accepting of this vaccine²⁵.

Concern has also been expressed about the possibility of replacement of HPV-16/18 related lesions with other high-risk types. Again, the view is that in the case of the HPV virus it is unlikely that this may happen based on the fact that it is a DNA vaccine and from epidemiological data which compare the proportion of types seen in multiple vs single infections.

Second generation vaccines

The current VLP vaccines have fundamental weaknesses for achieving their goal, particularly for widespread distribution in developing countries, where most cervical cancers occur. Attempts are being made to overcome these shortcomings. Some of the new generation of vaccines under study are - additional VLP types (HPV-31, -45, -33, -52, etc. in nonavalent formulation)²⁷, heat stabilized VLPs²⁸, slow release formulations²⁹, oral vaccines³⁰ and chimeric VLPs³¹. Chimeric VLPs have been shown in preclinical studies to elicit both neutralizing antibodies to the VLP and T-cell responses to L1 and oncogenic protein E7, thus offering better protection. Polynucleotide vaccines are being considered because of the ease of production and delivery in the developing world, and the ability to generate both B and T-cell responses³². Capsomere vaccine may offer a simplified, economical alternative to VLPs that is particularly suited to the developing world where the burden of cervical cancer is greatest³³.

Therapeutic vaccines

Therapeutic vaccines are aimed at eradicating or reducing infected cells. They are based on the generation of cell-mediated immunity. Agents that are in, or nearing clinical trials are HPV peptides, fusion protein of HPV-16 L2/E7 and dendritic cells. The main difficulty in evaluating the efficacy of therapeutic vaccines is the known pattern of natural history of cervical neoplasia, since it is known that a large majority of CIN lesions can regress spontaneously as well.

Conclusion

In developing countries, where screening services are sporadic because of unpredictable funding and poor infrastructure, HPV vaccination represents a great hope in the fight against cervical cancer. The exact point at which vaccination supersedes screening will depend upon the percentage of cancers preventable by the vaccine, the population coverage by the vaccine,

the percentage being prevented by existing screening programs, the attitude of women to screening and the attitude of the society in which they live to vaccination. This will vary considerably in time and place. Cervical cancer screening strategies that will be cost-effective for proper surveillance of women protected by HPV vaccination should be encouraged.

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