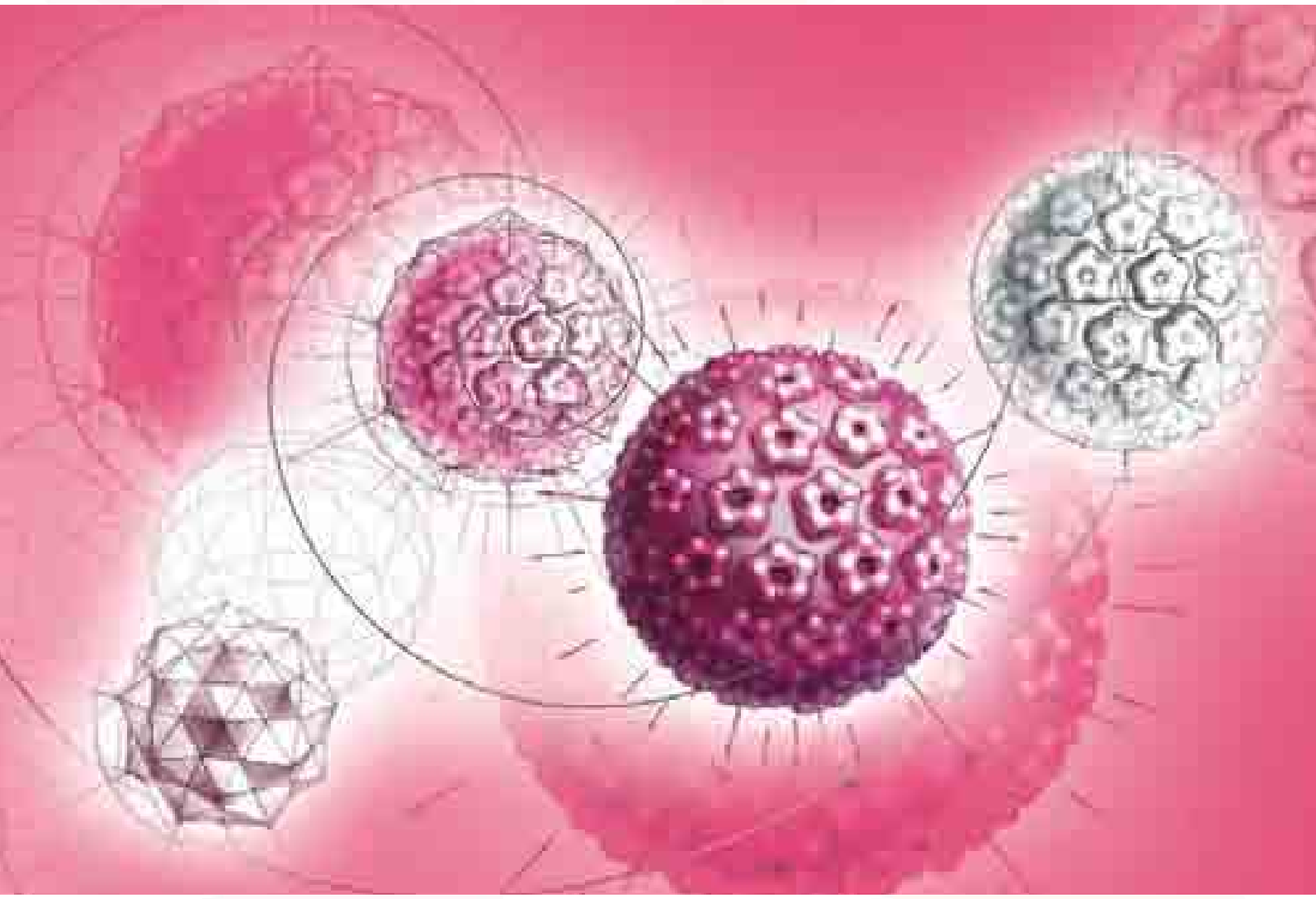
FOGSI FOCUS

December 2008



CERVICAL CANCER
PREVENTION, DETECTION & MANAGEMENT

Editor (s):
Prof. (Dr.) Hiralal Konar
Dr. Partha Basu

The **F**ederation of **O**bstetrics & **G**ynecological **S**ocieties of **I**ndia

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FOGSI FOCUS EDITORIAL

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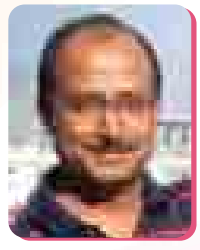

Dr. Partha Basu
DGO, MD, DNB
Head, Dept. of Gynecological Oncology
Chittaranjan National Cancer Institute,
Kolkata

Worldwide, cancer cervix is the second common cause of cancer deaths in women. The major (80%) burden of the disease is in the developing part of the world. Majority of the women are presenting late with advanced stage of the disease. Despite its high prevalence and mortality rate, cancer cervix is a disease that can be prevented and cured entirely by effective screening, early detection and treatment at the pre-invasive (CIN) and even at the early invasive stage. Screening programs successfully brought down the incidence as well as the mortality (7% per year) from cancer cervix in the developed world. Unfortunately due to several reasons, it failed to bring any such positive change in the developing countries.

Recombinant DNA technology and cloning of HPV-DNA confirmed the association between HPV, CIN and the cancer cervix. Well controlled methodological studies have proved that Human Papilloma Virus-DNA (HPV-DNA) is present in 99.7% of all invasive cervical cancers. This signifies that cancer cervix is a disease with infective etiology. Cervical screening by HPV-DNA identification with hybrid capture II method has been found to be cost effective by reducing the referral to colposcopy, biopsy and also increasing the interval of screening. Negative predictive value of HPV-DNA testing is very high. New, low-cost and rapid screening tests based on HPV identification are being developed keeping in view the needs of the low resource settings. We gynecologists have a major role to play to create public awareness and offer cervical screening to the thousands of women who come to our clinics daily.

The latest and the most exciting development in the field of cervical cancer control is the availability of vaccines (bivalent and quadrivalent) that are highly effective and safe. To bring down the incidence of cervical cancer the vaccine should be made available and affordable to all women and adolescent girls in the target age group. For the interest of the women in India, professional organizations like FOGSI have to join hand with the government as we all agree that cervical cancer is a major public health problem in this country.

This volume of FOGSI FOCUS is a unique one. It will disseminate the knowledge what is up-to-date and based on the current research. The contributors to this volume are experts in their field of science, research and work. We express sincere thanks to all the contributors for their whole hearted effort to enrich our knowledge as well as guiding us towards the current management approach.

This is the first time in FOGSI that each of the Vice Presidents is given some responsibility to carry out. Dr. Konar was entrusted with two projects; (i) Maternal Mortality Registry and (ii) Preventive Oncology. We are indebted to Dr. N Malhotra, the present President and Dr. C N Purandare, the President incoming, for their guidance and encouragement. We acknowledge the support from GlaxoSmithkline for providing resources to bring out this publication. We believe this volume of FOGSI FOCUS will be of immense value to all the practicing gynecologists, academicians and the postgraduates.

Prof. (Dr.) Hiralal Konar
Vice President, FOGSI, 2008
In-charge of FOGSI,
Preventive Oncology Initiative

Dr. Partha Basu
Head, Dept. of Gynaecological Oncology
Chittaranjan National Cancer Institute, Kolkata

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President's Message (2008)

Dr. Narendra C. Malhotra
President FOGSI (2008)

This FOGSI FOCUS on Preventive Oncology comes to you today as our endeavor to make a new Indian woman literate and healthier. Preventive Oncology is one of the theme of FOGSI this year.

Cancer in women is a major problem in India with cancer cervix being no. 1 killer

The problem of cancer cervix in India is a high incidence (27% of new cases in world) and a high mortality.

It has now been shown that HPV infection is present in 99.7% of all cases of CA Cervix, hence proving that HPV infection is a significant risk factor for cervical cancer.

A vaccine against HPV infection is now available and is effective. This may be the only preventive vaccine against cancer.

There is a strong argument of a universal recommendation of this vaccine for all school going girls and to include this in the national immunization programme. This may not be possible immediately but whereever possible girls should be immunized with HPV vaccine for prevention of cancer Cervix.

The motto of FOGSI this year has been educate, prevent & eradicate.

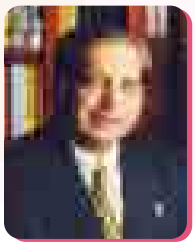
To eradicate cancer we have launched the FOGSI Preventive Oncology Initiative programme and to educate, we bring you this FOGSI FOCUS with a special emphasis on clinical practice guidelines on cancer management.

Happy reading. Do give us your feed back on the FOGSI year 2008

Remember for women welfare only you can make a difference.

Dr. Narendra C. Malhotra
President FOGSI (2008)

President's Message (2009)



Dr. C.N. Purandare
MD(BOM) MA Obst. (Ireland) DGO, DFP
D. Obst. RCPI (Dublin), FICOG, FICMCH, P.G.D.M.L.S.,
Consultant Obst. & Gyn.,
St. Elizabeth Hospital & BSES Hospital,
Ex. Hon. Professor Obst. & Gyn., Grant Medical College, Mumbai - 08

My warm greetings to all of you.

My theme for the year is “SAVING LIVES”.

The last year's logo showed a girl with seven Ribbons, depicting seven problems. I am going to pick up few of those ribbons and concentrate on them. I would like to work hard to save lives. I want all of you to take a vow to make a difference.

We all are aware that the Cervical Cancer is a global public health problem and is the important cause of death from Cancers among women of India, accounting for an estimated 132,000 new cases and 74000 deaths every year. It is unfortunate that though Cancer Cervix is Preventable but Yet not Prevented in our Country. Decision without actions are worthless. Keeping this in mind in the year 2009, we will have about 100 CMEs all over India in our Societies on 'Prevention and Early Detection of Cervical Cancer', to update the knowledge amongst our FOGSI members, which will help to reduce the Cancer burden of the Nation.

I am presenting to all the FOGSI members a 'FOGSI Focus' on “Cervical Cancer: Prevention, Detection & Management”.

This FOGSI Focus is edited by Dr. Hiralal Konar and Dr. Partha Basu, who has carefully selected topics on Cancer Cervix and I must say that the contributors have done a commendable job. Let us join hands and fight against Cancer Cervix and let our dream come true of preventing mortality and morbidity due to Cancer Cervix.

Thank you,

Dr. C. N. Purandare
President - FOGSI (2009)

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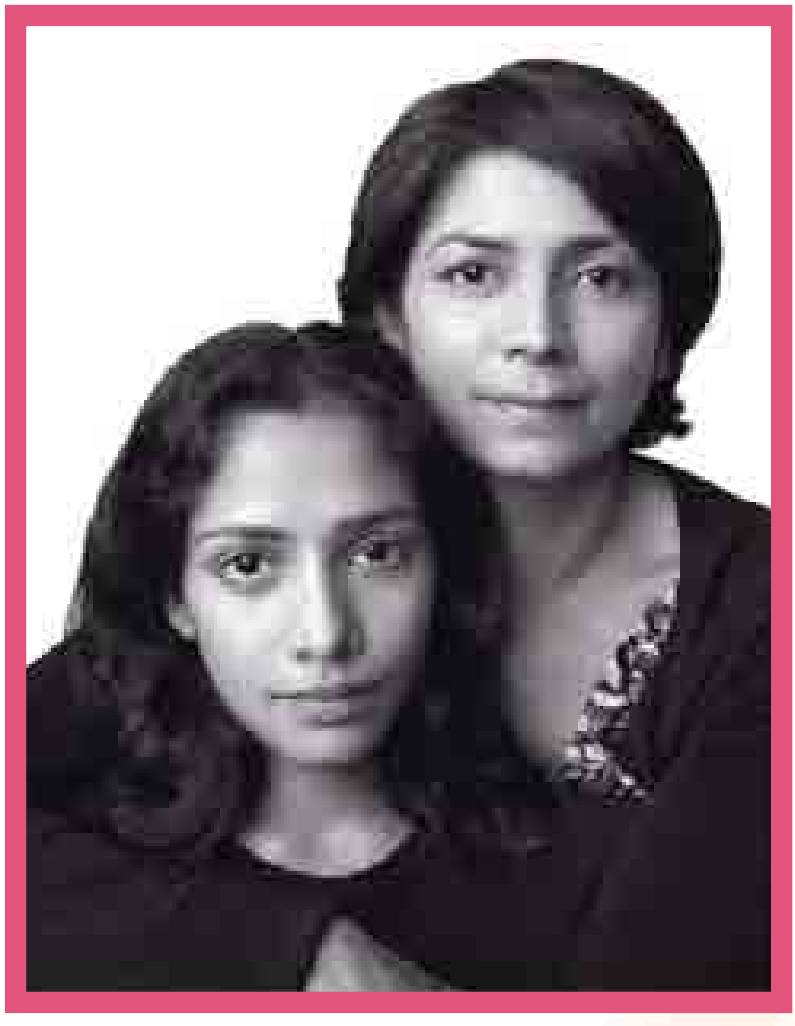

Introducing shortly...
...for protection against Cervical cancer

Cervarix™

Human Papillomavirus Vaccine Types 16 and 18
(Recombinant, AS04 adjuvanted)

Name: Cervarix. Human Papillomavirus vaccine Types 16 and 18 (Recombinant, AS04 adjuvanted). **Composition:** A 0.5 ml dose of the vaccine contains not less than Human Papillomavirus type 16 L1 protein(20 micrograms), Human Papillomavirus type 18 L1 protein (20 micrograms), 3-O-desacyl-4'- monophosphoryl lipid A (MPL) (50 micrograms) and Aluminium hydroxide, hydrated (0.5 milligrams Al^{3+}). **Pharmaceutical form:** Suspension for injection. **Indications:** Indicated in females from 10 to 45 years of age for the prevention of cervical cancer by protecting against incident and persistent infections, cytological abnormalities including atypical squamous cells of undetermined significance (ASC-US) and cervical intraepithelial neoplasia (CIN), CIN 1 and pre-cancerous lesions (CIN 2 and CIN 3) caused by human papillomavirus types 16 and 18. **Posology:** Single 0.5ml dose. The primary vaccination course consists of three doses schedule (0,1 & 6 months). **Method of administration:** For intramuscular injection in the deltoid region. **Contra-indication:** Known hypersensitivity to any component of the vaccine. **Special warnings and special precautions for use:** As with other vaccines, the administration of Cervarix™ should be postponed in subjects suffering from acute severe febrile illness. Should not be administered intravenously, subcutaneously or intradermally. Should be given with caution to individuals with thrombocytopenia or any coagulation disorder since bleeding may occur following an intramuscular administration to these subjects. There are no data on the use of Cervarix™ in subjects with impaired immune responsiveness such as HIV infected patients or patients receiving immunosuppressive treatment. **Interaction with other medicaments and other forms of interaction:** If Cervarix™ is to be given at the same time as another injectable vaccine, the vaccines should always be administered at different injection sites. It may be expected that in patients receiving immunosuppressive treatment an adequate response may not be elicited. **Use during pregnancy and lactation:** Specific studies of the vaccine in pregnant & lactating women were not conducted. **Undesirable effects:** Very common: injection site reactions including pain, redness, swelling; fatigue, headache, myalgia; Common: gastrointestinal including nausea, vomiting, diarrhoea and abdominal pain, itching/pruritus, rash, urticaria, arthralgia & fever (38°C). **Special precautions for storage:** Store in a refrigerator (2°C – 8°C). Do not freeze.

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1. The Federation of Obstetric & Gynaecological Societies of India (FOGSI): Recommendations for Vaccination against Human Papilloma Virus (HPV) Infection for the Prevention of Cervical Cancer

FOGSI Oncology Committee 2008

Preamble

Human papillomavirus (HPV) vaccines are now widely available for the purpose of cervical cancer prevention. The Quadrivalent vaccine (Gardasil) and Bivalent vaccine (Cervarix) have been approved for this purpose.

The objective of these Recommendations is to provide information on the use of HPV vaccine for the purpose of cervical cancer prevention and the need to continue with the current cervical screening programs.

General

The Human Papilloma Virus (high-risk genotypes) is a necessary causal factor of cervical cancers.

The HPV vaccine is a prophylactic vaccine. Both the bivalent and quadrivalent vaccine protects against infection of HPV genotypes (HPV-16 and HPV-18) that account for about 70% of HPV-related cervical cancers. The quadrivalent vaccine also protects against HPV types 6 and 11 that are responsible for about 90% of the genital warts.

The HPV vaccine is not therapeutic. It does not treat existing HPV infection or cervical intraepithelial neoplasia (cervical pre-cancers).

Cervical Cancer Screening

Women who have been vaccinated with the HPV vaccine should continue with the cervical cancer screening as per the recommendations in FOGSI Clinical Practice Guidelines.

Vaccination Target Group

The Bivalent vaccine has been approved for use in females aged 10 to 45 years, whereas the Quadrivalent vaccine has been approved for use in females aged 9 to 26 years.

The vaccine should target females at the most convenient and optimal age (12-16 years old) for vaccination before their first sexual exposure.

Routine HPV vaccination is recommended for females aged 10 to 12 years.

HPV vaccination may be offered to all women- Bivalent vaccine upto 45 years or Quadrivalent vaccine up to 26 years, regardless of sexual activity. The decision is based on the informed discussion between the woman and her health care provider regarding risk of previous HPV exposure and potential benefits from vaccination.

At present, vaccination is not recommended in males. More data is awaited on this issue.

Dosage Schedule

For Bivalent vaccine three doses at 0, 1 and 6 months are recommended intramuscularly. For the Quadrivalent vaccine three doses at 0, 2 and 6 months are recommended intramuscularly. Minimum Intervals between doses are 4 weeks between 1st & 2nd dose and 12 weeks between 2nd and 3rd dose.

At present there is no recommendation for the use of boosters.

Counseling before vaccination

A full explanation of the role, action and usefulness of the vaccine should be provided to the woman or her parent/guardian where applicable, before vaccination.

The explanation should typically include: the role of HPV in cervical carcinogenesis (in particular HPV-16 and HPV-18); trial results and expectations; immunological responses; safety and efficacy; as well as answer queries on issues, as highlighted in this document.

HPV Testing before Vaccination

Testing for HPV is not recommended before vaccination.

Vaccination of Sexually Active Women

Sexually active women and women with previous abnormal cervical cytology can receive the HPV vaccine. But the benefits may be limited to the protection against infection of HPV genotypes with which they have not been infected.

Women who have been infected with vaccine HPV-type (serologically positive) and have cleared the cervical infection (DNA negative) appears to have similar protective effects as those who are naïve to the same vaccine HPV-type. Further scientific evidence is awaited on this issue.

Special Situations

Women with Previous Cervical Intraepithelial Neoplasia (CIN)

The vaccine can be given to patients with previous CIN, with the protection being offered against infection of HPV genotypes (and related CIN) with which they have not been infected.

It must be emphasized that cervical screening and corresponding management must continue.

Pregnancy and Lactating Women

The use of the vaccine in pregnancy is not recommended, although no teratogenic effect caused by the vaccines have been reported.

There is no evidence to show that the HPV vaccines adversely affect fertility, pregnancy or infant outcome.

Women who are planning to conceive are advised to defer vaccination until after delivery.

Women who become pregnant before completion of vaccination are advised to postpone the remaining dose(s) until after the pregnancy.

Termination of pregnancy is not indicated for women who become inadvertently pregnant during the course of vaccination.

Lactating women can receive the HPV vaccine and still continue breast-feeding because it is a vaccine without live viral DNA.

Immunosuppressed Patients

Immunosuppression is not a contraindication to vaccination. However, the immune response to the HPV vaccine may be less competent in these women compared with a healthy individual.

Contraindications and Precautions

The HPV vaccine is contraindicated for people with a history of hypersensitivity to any vaccine component.

Vaccine should be administered in lying down position and the vaccine should be observed for 15 minutes for possible dizziness/fainting attack

Vaccination of people with moderate or severe acute illnesses should be deferred until after the illness improves.

When the vaccine is administered concomitantly with any other vaccine, it should be at a separate site, with a separate syringe.

The FOGSI Recommendations have been Drafted by the FOGSI Oncology Committee at a Meeting held on 19th October 2008 at Mumbai.

Members of the Committee for Preparation of Recommedations for HPV Vaccine

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This information is provided by GlaxoSmithKline for the Medical Practitioners and is adopted from: Reference - Diane Harper. Prophylactic human papillomavirus vaccines to prevent cervical cancer: review of the Phase II and III trials. Therapy (2008): 5(3), 313-324.		
Summary of the two commercially available Human Papillomavirus Vaccines		
	Cervarix® (GlaxoSmithKline)	**Gardasil® (Merck & Co)**
Vaccine type	HPV 16 and HPV 18 VLP L1 capsid component	HPV 6/11/16/18 VLP L1 capsid component
Concentration	20 µg HPV 16 20 µg HPV 18	20 µg HPV 6, 40 µg HPV 11 40 µg HPV 16, 20 µg HPV 18
Adjuvant	AS04: 500 µg aluminum hydroxide 50 µg 3-deacylated monophosphoryl lipid A	Alum: 225 µg aluminum hydroxyphosphate sulfate
Recombinant technology substrate system	Baculovirus expression system in *Trichoplusnia ni* insect cells	Yeast expression system in *Sacharomyces cerevisiae*
Schedule*	Intramuscular 0,1,6 months	Intramuscular 0,2,6 months
Target Population*	Females 10-45 years	Females 9-26 Years
Safety	Generally safe and well tolerated	Generally safe and well tolerated
Efficacy	100% CIN 2/3 protection caused by HPV 16/18 for at least 5.5 years in a naïve population. —	100% CIN 2/3 protection caused by HPV 16/18 for at least 5 years in a naïve population. 44% CIN 2/3 protection caused by HPV 16/18 over 3 years in a mixed population
	68% CIN 2/3 protection irrespective of HPV type after 5.5 years in a naïve population	17% CIN 2/3 protection caused by any HPV type after 3 years in a mixed population.
	88% HPV 45-related incident infection protection for at least 5.5 years in a naïve population	HPV 45 protection not demonstrated
	54% HPV 31-related incident infection protection for at least 5.5 years in a naïve population	75% HPV 31-related CIN2+ protection for at least 3 years in a naïve population
Immunogenicity	HPV 16 : 100% Seroconversion Seropositivity remains > 98% at 5.5 years Neutralizing antibody titers remain eightfold higher than natural infection titers at 5.5 years	HPV 16 : 100% seroconversion Seropositivity remains > 98% at 5 years Antibody titers remain approximately eightfold higher than natural infection titers at 5 years
	HPV 18 : 100% Seroconversion Seropositivity remains > 98% at 5.5 years Neutralizing antibody titers remain eightfold higher than natural infection titers at 5.5 years	HPV 18 : 100% Seroconversion Seropositivity drops after 2 years Antibody titers approach natural infection titers at 3 years
	HPV 45 : 100% Seroconversion Seropositivity remains > 98% at 4.5 years Type- specific antibody titers remain higher than natural infection titers at 4.5 years	**Some evidence for anti- HPV 45 and 31**
	HPV 31 : 100% Seroconversion Seropositivity remains > 70 % at 4.5 years Type- specific antibody titers remain higher than natural infection titers at 4.5 years	
Cervicovaginal antibody transudation	HPV 16 and 18	

CIN: Cervical intraepithelial neoplasia, HPV: Human papillomavirus

* As per the Prescribing Information in India.

Introducing shortly...
...for protection against Cervical cancer

Name: Cervarix. Human Papillomavirus vaccine Types 16 and 18 (Recombinant, AS04 adjuvanted). **Composition:** A 0.5 ml dose of the vaccine contains not less than Human Papillomavirus type 16 L1 protein(20 micrograms), Human Papillomavirus type 18 L1 protein (20 micrograms), 3-O-desacyl-4'- monophosphoryl lipid A (MPL) (50 micrograms) and Aluminium hydroxide, hydrated (0.5 milligrams Al^{3+}). **Pharmaceutical form:** Suspension for injection. **Indications:** Indicated in females from 10 to 45 years of age for the prevention of cervical cancer by protecting against incident and persistent infections, cytological abnormalities including atypical squamous cells of undetermined significance (ASC-US) and cervical intraepithelial neoplasia (CIN), CIN 1 and pre-cancerous lesions (CIN 2 and CIN 3) caused by human papillomavirus types 16 and 18. **Posology:** Single 0.5ml dose. The primary vaccination course consists of three doses schedule (0,1 & 6 months). **Method of administration:** For intramuscular injection in the deltoid region. **Contra-indication:** Known hypersensitivity to any component of the vaccine. **Special warnings and special precautions for use:** As with other vaccines, the administration of Cervarix™ should be postponed in subjects suffering from acute severe febrile illness. Should not be administered intravenously, subcutaneously or intradermally. Should be given with caution to individuals with thrombocytopenia or any coagulation disorder since bleeding may occur following an intramuscular administration to these subjects. There are no data on the use of Cervarix™ in subjects with impaired immune responsiveness such as HIV infected patients or patients receiving immunosuppressive treatment. **Interaction with other medicaments and other forms of interaction:** If Cervarix™ is to be given at the same time as another injectable vaccine, the vaccines should always be administered at different injection sites. It may be expected that in patients receiving immunosuppressive treatment an adequate response may not be elicited. **Use during pregnancy and lactation:** Specific studies of the vaccine in pregnant & lactating women were not conducted. **Undesirable effects:** Very common: injection site reactions including pain, redness, swelling; fatigue, headache, myalgia; Common: gastrointestinal including nausea, vomiting, diarrhoea and abdominal pain, itching/pruritus, rash, urticaria, arthralgia & fever (38°C). **Special precautions for storage:** Store in a refrigerator (2°C – 8°C). Do not freeze.

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For further information, write to:

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GlaxoSmithKline Pharmaceuticals Ltd.
Dr. Annie Besant Road, Worli, Mumbai - 400 030.

2. The Magnitude of Cancer Cervix in India: A Summary

Dr. A. Nandakumar, Dr. T. Ramnath, Dr Meesha Chaturvedi
National Cancer Registry Programme (ICMR), Bangalore, INDIA

The Indian Council of Medical Research initiated a network of cancer registries under the National Cancer Registry Program (NCRP) in 1981 and data collection commenced in these registries from January 1982. Since then, the registries have provided information on incidence and patterns of cancer that in terms of quality and validity meet international standards. Thus, in India, for cancer, and perhaps for only this disease, we have a systematic program of data collation so as to have reliable incidence and mortality rates, thereby laying a foundation for scientific research whether that research be epidemiological, basic, clinical or in cancer control. However, India being a vast country, setting up of new registries throughout the country as in some Western countries would involve enormous cost in establishing and maintaining the same. Therefore, under a project, on 'Development of an Atlas of Cancer in India' a cost-effective design and plan using advances in modern electronic information technology, was conceived, to collate and process relevant data on cancer so as to fulfill the objectives of obtaining an overview of patterns of cancer in different parts of the country; and, calculating estimates of cancer incidence wherever feasible. The summary given below is based on the reports from both the cancer registries of the NCRP and that from the project on cancer atlas.[1,3]

Cancer incidence is generally expressed as Age Adjusted or Age Standardised Incidence Rates (AAR) according to world standard population per 100,000 persons. Among the older population based cancer registries (PBCR) in India, Barshi and Chennai PBCRs have always recorded the highest incidence rates. The most recent figures on AAR for the PBCRs under NCRP are given in Table 1. The report of the North Eastern PBCRs (NCRP, 2008a) indicate an AAR of 25.4 per 100,000 in Aizawl of Mizoram state with comparable AARs in Imphal west district (20.5) and Kamrup Urban district (17.3) per 100,000.

Cancer of the cervix, is still the most important cancer in women in India, though, in the past two decades, all the older urban Population Based Cancer Registries (PBCR) at Bangalore, Bhopal, Chennai, Delhi and Mumbai have shown a statistically significant decrease in the AARs of this site of cancer. This decline in the incidence of this cancer is, in the absence of any organized screening program. The decline in the AAR varies from 42.3 (in 1982-83) to 22.3 (in 2004-05) per 100,000 in Chennai to a marginal decline in Barshi from 23.5 (in 1988-89) to 22.8 (in 2004-05). As of 2005, cancer of the cervix constitutes 16% of all cancers in women in the urban registries. However, it constitutes 37% of the cancers in females in Barshi. The highest age specific incidence rate of 98.2 per 100,000 for cancer cervix is seen in the 60-64 age group. Since over 70% of the Indian population resides in the rural areas, cancer cervix still constitutes the number one cancer in either sex. Based on the data of the PBCRs the estimated number of new cancers during 2007 in India was 90,708 (NCRP, 2008b). The relative five year survival reported some time earlier averaged 48.7% (IARC, 1998).

In the Hospital Based Cancer Registries (HBCRs), cancer of the cervix is the leading site of cancer in Bangalore and Chennai, the second leading site in Mumbai and Thiruvananthapuram and the third

leading site in Dibrugarh. This site of cancer constitutes between 11.4 (Thiruvananthapuram) to 30.7% (Chennai) of all cancers in women in these five HBCRs. The rise in the occurrence of cancer was at the later age in Thiruvananthapuram compared to the other four HBCRs. Over 63 to 89% of all cervical cancers had regional disease at the time of presentation. Around 40% of all cervical cancer patients in Bangalore, Chennai and Mumbai did not receive treatment at the Reporting Institution despite having had a diagnosis of cervical cancer (NCRP, 2007).

The data from the report of the project on "Development of an Atlas of Cancer in India" shows that at least five districts have even higher incidence rates than that recorded at Chennai. Four of these five districts are concentrated in the north eastern region of Tamil Nadu state and Pondicherry. The atlas has further revealed that this area has also some of the highest incidence rates of penile cancer. There are reports in the literature that the prevalence of Human Papilloma Virus (HPV) is not only high among cancer cervix patients, but also high among patients with penile cancer. This part of Tamil Nadu state has also a high prevalence of Human Immunodeficiency Virus (Nandakumar et al, 2005). We thus have enough descriptive epidemiology pointers on a region of the country to undertake several research studies and control measures in cancer cervix, including population based typing of HPV infection.

Table shows the Rank, Relative Proportion (%) of all cancers in Females, Crude (CR) and Age Adjusted (AAR) Incidence Rates per 100,000 person for Cancer of the Cervix in the Population Based Cancer Registries (PBCRs) under the National Cancer Registry Programme of India

PBCRs	Rank	%	CR	AAR
Bangalore	2	15.7	14.3	18.8
Barshi	1	37.0	20.0	22.8
Bhopal	2	17.9	12.0	17.7
Chennai	2	18.5	20.3	22.3
Delhi	2	14.9	12.3	17.4
Mumbai	2	13.2	11.5	13.4
Ahmedabad	2	18.6	6.9	7.9
Kolkata*	2	15.7	13.2	12.3
Dibrugarh District	5	6.6	3.8	5.1
Kamrup Urban District	2	14.4	12.8	17.3
Silchar Town	1	20.6	10.6	12.1
Imphal West District	1	15.9	17.2	20.5
Mizoram State	3	13.5	13.7	17.4
Aizwal District	1	15.0	20.6	25.4
Mizoram State excl. Aizwal	3	12.0	9.8	12.6
Sikkim State	1	11.1	6.9	10.9

*Based on 2004-2005 data for Bangalore, Barshi, Bhopal, Chennai, Delhi, Mumbai, Ahmedabad 2005 data for Kolkata and 2005-2006 data for Dibrugarh District, Kamrup Urban District, Silchar Town, Imphal West District, Mizoram State, Aizwal District, Mizoram State excl. Aizwal, Sikkim State.

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3. Etiological Role of Human Papilloma Virus in Cervical and other Genital Cancers

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Introduction

Cancer cervix is the second most common cancer in women. Worldwide, nearly 500,000 new cases are detected annually and in India it is nearly 132,000 per year.

In the developed countries, cervical cancer incidence is falling by about 7% annually. This is mainly due to the effective cervical screening procedures. Unfortunately, the scenario is not the same in the developing world, where prevalence of the disease is significantly high. Due to several reasons, mass cervical screening programme in many developing country does not exist. Cancer cervix is the most common cause of death from cancer. Despite its high incidence and mortality rate, cancer cervix is entirely preventable and curable of all major malignancies. This is possible by early detection of the pre-invasive (CIN) and micro invasive stage of the disease. Over a century number of factors have been implicated as the etiology of cancer cervix. Association of cancer cervix and sexual activity is well known. Fortunately it is the advent of recombinant DNA technology and the cloning of human papilloma virus, the etiological role of HPV with CIN and cancer cervix has been confirmed[1]. Several case controlled studies had shown a very strong association between HPV infection and cancer cervix[2, 3]. This association is much greater when compared the association between smoking and lung cancer. The epidemiological evidence that HPV is the main causative agent fulfils the criteria set by Hill[4]. The other risk factors like multiple sexual partners, early marriage, early sex, repeated child birth are all viewed as the association with sexual transmission of HPV[5]. Oral contraceptive pill and smoking may be the true co-factors. It is observed that women with HPV-16 genital infection are 435 times more likely to develop invasive cervical cancer than in women without HPV infection[6]. **Currently the epidemiologic, clinical, molecular and histological data all support HPV as the causative agent for most, if not all, epithelial cancer of the cervix[7].**

The Human Papilloma Virus

Human Papilloma Viruses are the members of the Papillomaviridae. These are non enveloped and have 50 to 55 nm in diameter. They have icosahedral capsid coat. In the center there is a double stranded circular DNA genome. The genome with about 8000 base pairs is divided in to early and late genes. The early genes (E1, E2, E4, E6, E7) are responsible for DNA replication, transcription and transformation. The early gene products E6 and E7 encode the major transforming protein. Over expression of the E6 and E7 oncoproteins interfere the cell cycle regulation by inhibiting the human suppressors p53 and retinoblastoma (RB) protein.

The late genes are L1 and L2 proteins. L1 gene codes for less capsid protein and L2 codes for a major capsid protein. HPV types are distinguished from one another by the degree of nucleic acid sequence homology. Individual types are associated with specific pathology and different clinical manifestation. HPV-1 causes planter warts, HPV-6 and 11 cause an orogenital warts (90%), HPV-16 causes cervical dysplasia and invasive cervical cancer. HPV are highly specific and have not been propagated in common experimental animals. Cross infections between species have not been reported. **Human papilloma virus is an exclusively epitheliotropic virus.** HPV infects virtually all types of surface epitlelia. All types of squamous epithelium (Vulva, vagina anogenital) can be infected by HPV. The histological appearances of individual lesions vary with the site of infection and the types of virus. There is proliferation of epidermal cell layers except the basal layer. Cellular proliferation causes acanthosis, parakeratosis and hyperkerotosis. **Koilocytes** having the large round cells with perinuclear halo are typical of HPV infected cells. Studies have reported that HPV 16 was found in about 60% of all invasive cancers and HPV 18 was found in about 12%[8].

TABLE – 1
CLASSIFICATION OF HPV AFFECTING ANOGENITAL EPITHELIA

A Low oncogenic risk 6,11,40,42,43,44,53,54,61,72,73,81
B High oncogenic risk 16,18,31,33,35,39,45,51,52,56,58,59,68,82
C Possible high oncogenic risk 26,66,73

TABLE – 2
HUMAN BODY CANCERS ASSOCIATED WITH HPV

Type of Cancer	% of cases associated with HPV
Cervical	99.7
Anal	85.0
Vulval, Vaginal, penile	50.0
Oropharyngeal	20.0
Laryngeal	10.0

Over 130 HPV types have been identified. Over 30 of these affect the female and male anogenital epithelia. The types commonly affecting the anogenital region can be divided into three groups, depending on their potential to develop anogenital neoplasia[9] (Table1). Many other human body cancers have been associated with HPV infection[10] (Table-2).

Pathogenesis

Human papilloma viruses are epitheliotrophic. Infection is initiated when a virus gains entry to the basal cells of the epithelium. Minor trauma that often occurs during sexual intercourse, allows the virus to access the target epithelial cells at or near the cervical transformation zone. HPV induced neoplastic transformation is associated with the integration of part of the HPV genome into the host cell DNA, leading to over expression of E6 and E7 oncoproteins. There two oncoproteins as stated earlier interfere with cell cycle regulation by inhibiting these tumour suppressors p53 and retinoblastoma (RB) protein. This leads to genetic damage, genetic instability and eventual emergence of a malignant change. HPV and its oncogenic proteins are capable of inducing cellular immortalisation. When a mitotically active immature epithelial cell at the transformation zone adjacent to the squamo -columnar junction is infected, the virus remains in the cell in the latent form. During the **latent phase**, low number of viral genome (about 100 copies) are produced. Clinical diagnosis in this stage is difficult. Only by HPV DNA hybridization, HPV can detected. In majority of cases (80%) host defense mechanism is able to clear the infection spontaneously. In low percentage of women (20%), the virus replicates in large numbers and infect the intermediate and superficial cells. This phase is known as a **productive viral infection**. Both the **latent** and **productive viral infection phases** may remain clinically undetectable.

The progression of infection leading to neoplastic transformation depends predominantly on host immune defense otherwise on the virus-host interaction. Through some ill understood mechanism the circular strand of viral DNA is opened up into the linear and the DNA can be spliced into the host DNA. Then only the virus can use the protein production apparatus of the transformed host cell.

The HPV genome has got three regions. The upstream regulatory region (URR), the early region and the late region. The **URR** mainly regulates the viral replication. The **early region** transcribes proteins that is helpful to program the host cell to produce new viral DNA. The **late region** encodes proteins for the capsid, that surround like DNA core to produce the complete viron molecule. With all these mechanism, the virus takes over the control of the infected epithelial cells. The virus then entirely controls the host cell mechanism to produce new viral DNA and viral proteins. In normal cellular response, p53 being an important protector protein, initiates the process of apoptosis leading to death of the epithelial cell to halt the hijacked DNA synthesis mechanism. On the other hand the HPV E6 protein targets p53 for proteolytic degradation, allowing oncogenic HPV types to overcome the important host cellular defense mechanism.

The L1 encoded capsid protein predominate in the capsid and is similar in all HPV types[11.] On the other hand the L1 encoded capsid protein is responsible for difference in antigenic response of different HPV types[11]. The prophylactic vaccines are targeted against these HPV capsid proteins. Currently available vaccines are HPV type specific depending upon the L1 encoded capsid protein. The quantity of L1 and L2 encoded capsid proteins is directly proportional to the degree of maturation of the epithelium. Well differentiated cells are generally rich in L1 and L2 proteins. Undifferentiated cells in high grade CIN lesions contain only small quantities of capsid proteins.

Host Immune Response:Immunological defense of the host are: A. Humoral and B. Cell- mediated.

A. Humoral : There is development of HPV specific neutralising antibodies (NA) either of immunoglobulin IgA or IgG. These antibodies prevent infection of the anogenital mucosal surface. These are directed against the L1 and L2 HPV capsid proteins.

B. Cell-mediated: Cell-mediated immunity works once the cell is infected with the virus. Specific CD8 + cytotoxic T lymphocytes and CD4 + Helper T lymphocytes are required to clear the infected cells.

The cytotoxic T lymphocytes (CTL) recognize the early proteins in the infected cells in conjunction with host major histocompatibility complex (MHC) Class I molecule. Helper T cells recognize longer peptide fragments complexed with MHC Class II molecules. Both these cytotoxic T lymphocytes (CTL) and Helper T lymphocytes promote specific antibody production through cytokine induction.

Cigarette smoking both active and passive is a known etiologic factor for CIN and invasive cervical cancer. Smoking acts in various ways including modulation of the immune system resulting in a reduction of Langerhans' cells in the cervix.[12]

Telomeres are simple DNA repeats that limit the life of the cell. Telomere shortening occurs with age. This can be prevented by the ribozyme telomerase. **Prevention of telomere shortening** by ribozyme telomerase activity of the HPV infected cells is thought to be the reason for maintenance of telomere length and thus cell immortalisation. E6 and E7 oncoproteins target the enzyme telomerase.[13]

Vaccines against HPV have been introduced mainly to prevent infection targeting against the HPV

capsid proteins by cell-mediated immune response and/or by humoral immune response. Several clinical trials[14, 15] have shown that response varies from 100% protection with prophylactic use to poor response with therapeutic use.

Majority of women (80%) clear the infection over a period of one year. However if the infection is caused by high oncogenic types (see table above), it is either cleared more slowly or not cleared at all. High oncogenic risk HPV are responsible for high grade CIN lesions. About 50% or more of CIN-1 lesions regress over 2 years without any active therapy. Women with persistent infections with high risk type HPV are more likely to develop progressive high grade CIN or invasive Cervical cancer. Host immune defense plays important role in the regression or progression of the pathological process. Immuncompromised women (women living with HIV or otherwise immunosuppressed) have a higher rate of progressive pathology.

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4. HPV Vaccines : Need, Feasibility and Recommendations in India

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Introduction

Cervical cancer, in spite of being a preventable disease, is a significant public health problem in India. The incidence rate of the disease is among the highest in the world (Age-standardized incidence rate 30.4/100,000), primarily due to the lack of population based cervical cancer screening.[1] Cervical cancer affects women at a relatively younger age causing untimely deaths. The social and economic impact of the disease on the individuals, families and the community as a whole is profound. Cervical cancer is responsible for 74,000 deaths per year in India and is the number one cause of 'years of life lost' due to any cancer among Indian women.[2]

The Human Papilloma Virus (HPV) vaccine aimed at prevention of cervical cancer (also ano-genital warts by the quadrivalent vaccine) is a major breakthrough in preventive medicine. The vaccine offers a unique opportunity of saving thousands of lives in a country like India with huge cervical cancer burden.

Cervical cancer control: Current scenario in India

Cervical cancer is preventable. The secondary prevention strategy that achieved more than 60% reduction in mortality in many of the developed countries is population based cervical screening using Pap smear cytology.[3] An organized screening program has to define a target population (every sexually active women belonging to a specified age group), administer the screening test to the target women at a specified interval (1-5 years), achieve a high level of coverage to screening (>70%), establish an effective call-recall system so that the screen-positive women are further investigated and treated and has to ensure appropriate quality assurance at every level of the program. Such organized screening program is non-existent in India.

In spite of being a significant public health problem, cervical cancer control has never received due attention either from the health policy-makers or from the medical fraternity in India. In terms of priority, cancer control is not among the top national health programs. The competing claims for the limited financial and human resources are too many. An expert group formed by Government of India and World Health Organization prepared a guideline for cervical cancer screening in the year 2006.[4] The dissemination of the national guideline and field level implementation is still very poor. The Ministry of Health is yet to come forward with a firm commitment to implement the program with realistic budgetary allocation. As a result, cervical cancer screening of asymptomatic women remains elusive in India barring a few demonstration programs and research studies.

In the recent years a huge momentum has been built to identify low cost alternative screening tests like VIA (visual inspection after acetic acid application) and cost-effective management strategies like use of cryotherapy in single visit approach. Low cost and rapid screening tests based on HPV detection are undergoing evaluation and have shown promising initial results. The new options can

make implementation of cervical screening feasible in a middle income country like India. In spite of these new developments, no concerted effort has yet been made to translate these innovative strategies into action. To implement even a VIA-based national screening program, as suggested by Indian national guidelines, a huge investment has to be made to train different levels of healthcare providers and to get other logistics of population based screening in place.

Scope of HPV vaccine

Availability of the highly efficacious vaccines against Human Papilloma Virus (HPV) has opened up a new vista in cervical cancer prevention. The bivalent as well as the quadrivalent vaccines have nearly 100 percent efficacy to protect against persistent infection of HPV types 16 and 18 and also against the cervical cancer precursors caused by them.[5] In the Indian subcontinent more than 75% of the cervical cancers are attributed to these two HPV types implying that a high level of protection can be offered by the vaccines.[6,7] A successful vaccination program has the potential to reduce the burden of the disease significantly, and is an exciting opportunity to save thousands of lives in a country where women do not have access to any other preventive strategy. The quadrivalent vaccines are also aimed at prevention of the ano-genital warts that are caused by HPV types 6 and 11. The safety issues in terms of local reactions and systemic adverse events of either of the vaccines have been addressed by the clinical trials and the results are very reassuring.

A health and economic impact analysis based on computerized model of cervical carcinogenesis calibrated to the epidemiologic data in India observed that with pre-adolescent vaccination alone, the mean reduction in the lifetime risk of cervical cancer is expected to be 44% if 70 percent coverage can be achieved.[8] Apparently a vaccination based strategy is a simpler option for cervical cancer prevention than the introduction of a new screening program with its many complexities. Administration of effective and safe vaccines through an efficient delivery system against the diseases responsible for significant morbidity and mortality is a highly cost effective public health intervention. India's immunization program is one of the largest in the world in terms of quantities of vaccines used, numbers of beneficiaries, the geographical spread and diversity of areas covered. The results of the 2005-06 National Family Health Survey (NFHS III) of India revealed that 95 percent children aged 12-23 months received at least some of the recommended vaccines. The benefit of vaccination to prevent various infectious diseases is well known to the lay public and is generally well accepted.

Even with this favourable backdrop, introduction of HPV vaccination as a public health program in India will be extremely challenging. Needless to say, the vaccine will remain out of bounds of the public health program in the immediate future due to its prohibitive cost. There are other critical issues that will have impact on the public perceptions about the new vaccine and will affect its acceptability and suitability for a mass vaccination program.

- HPV vaccine is aimed at prevention of cancer that is considered to be a disease of the aged and is not regarded as a health priority of the target age group.
- HPV vaccine is a gender specific vaccine aimed to prevent a sexually transmitted infection (STI).
- The primary targets for the vaccine are pre-adolescent girls who routinely do not receive any other vaccines and are difficult to access by any health facility.
- The long term safety and efficacy data for the new vaccines are currently available up to 6.4 years only.
- The level of awareness about cervical cancer is low in the population

These issues require sensitive handling with appropriate public education and peer group support. The clinicians, specially the gynaecologists, paediatricians and general practitioners have a key role to play. A recent questionnaire survey among the urban, educated parents belonging to the high socio-economic group in eastern India observed that the parents valued the recommendation from the physicians as the most important factor to make decision about their children's vaccination.[9]

Guidelines for use of the HPV Vaccines

Based on the available scientific evidences several guidelines for vaccination against HPV have been formulated by different professional organizations. Such guidelines vary little in their key recommendations. The guidelines of the Indian Academy of Paediatrics (IAP) were formulated and published in August 2008.[10] The IAP guidelines grouped all available vaccines into four categories. The category 1 and category 2 vaccines are the ones that are strongly recommended because of their proven benefit in significant reduction of mortality and/or long term morbidity. Whereas the category 1 vaccines are already in the expanded program of immunization (EPI), the category 2 vaccines need to be inducted in the program once the cost comes down to an affordable range. HPV vaccine has been listed under the second category by IAP.

Target gender for HPV Vaccine

As the vaccine is primarily aimed at cervical cancer prevention, the benefit of vaccinating females is substantial. The incremental benefit of vaccinating the males is very little and is not cost-effective. Stronger herd immunity can be obtained by achieving a high coverage among the female target age cohorts rather than trying to vaccinate males as well as females. HPV has been associated with other cancers like penile cancer, ano-rectal cancer, oro-pharyngeal cancer etc. Once the role of the vaccine in preventing these cancers is established there will be justification of vaccinating males as well. IAP did not recommend administration of the HPV vaccine to males.

Target age for HPV Vaccine

Ideally the vaccine should be administered prior to sexual debut to avoid any possibility of transmission of the virus. IAP recommends that girls aged 10-12 years should be vaccinated. Vaccine can be administered up to the age of 26 years (catch up age) if the individual is not vaccinated earlier. However, women over 26 years of age are also vulnerable to HPV infection and are likely to benefit from vaccination if they are naïve to infection from either of the vaccine subtypes. Each year, approximately one percent of women over 25 years of age acquire a new infection with either HPV 16 or 18.[11] The risk of persistence of HPV infections became higher with age.[12] There is some evidence that women who were previously infected with HPV types contained in the vaccine and subsequently cleared the infection can obtain protection against re-infection and persistence of the same type following vaccination.[13] Further robust evidence is required to recommend vaccination to women above 25 years who are already sexually active. In such women vaccination should be given after one to one counselling and explaining the fact that the vaccine effectiveness may be lower if she is already infected.

Dose of HPV Vaccine

The quadrivalent vaccine is recommended to be administered at 0, 2 and 6 months and the bivalent vaccine at 0, 1 and 6 months. Both the vaccines should be given intramuscularly. IAP recommends that the HPV vaccine can be given in the same setting with Tdap (tetanus, diphtheria and acellular pertussis) and hepatitis B vaccines that are recommended at the age of 10-12 years.

Requirement for booster

Both the vaccines have demonstrated persistently high serum antibody levels and robust immune memories till 5-6 years after initial vaccination. The vaccinated cohorts are still being followed up to see how long the protection lasts without booster doses. Based on the currently available information from the research studies, IAP recommended that no boosters are necessary.

Vaccination of pregnant and lactating women

The vaccine is contraindicated in pregnancy. However, if a woman gets pregnant while undergoing vaccination further doses should be withheld and will be resumed one month after delivery. There is no need to terminate the pregnancy in such an instance or if a women receives the vaccine accidentally during her pregnancy. Vaccine can be administered to lactating women.

Requirement for cervical cancer screening

All vaccinated women should undergo cervical cancer screening as per the country guidelines (from 30 years of age in India) since these women are still susceptible to cervical cancer from the HPV types not included in the vaccines. It is not necessary to do HPV test before vaccination. Women who already have cervical neoplasias may be considered for vaccination after appropriate explanation of the fact that they will not get full benefit of vaccination and need to continue with the screening.

Introduction of HPV Vaccine into national health care system

For effective disease control, the HPV vaccine should be accessible to all girls belonging to the target age through a national program. The girls between early childhood and sexual debut consist one of the most difficult cohorts to reach for healthcare. The Universal Immunization Program (UIP) was launched in India in the year 1985. Subsequently it became an integral part of the Reproductive and Child Health Program. Adding the HPV vaccine to the already existing vaccination program will be most cost-effective. The incremental programmatic cost associated with adding the pre-adolescent vaccine will depend on the cost of the new vaccine and the strategies adopted to reach the target population. Innovative service delivery models need to be developed depending on the social, cultural and economic background of the population under consideration. School based vaccination is an attractive strategy because parents usually trust health-care recommendations made in a school setting and children are already gathered in one place. Unfortunately approximately 40 percent of first grade entrants never complete primary schools in India and the rate is higher among girls in rural areas.[14] By the age of 10 years only a minority of the girls are still in school. Reproductive and family planning services will not be the ideal contact point for HPV vaccine administration as these services are almost exclusively accessed by women during or following first pregnancy. There is a great social barrier for the adolescents to access such services.

Adolescent health programs like Kishori Shakti Yojana (to improve the health and nutritional status of girls) have poor uptake. A comprehensive adolescent health program that provides attractive and user-friendly services including counseling on sexual health can be a good platform to introduce the HPV vaccine.

Besides delivery strategy a well-coordinated vaccination program needs to address the following critical issues as well:

- Training of health professionals and other service providers
- Ensuring regular supply of vaccine based on the realistic assessment of need
- Developing mechanisms for procurement and supply chain maintenance
- Enforcing sustained monitoring and evaluation of the program

Cost and financing of the new vaccine

The costs of the HPV vaccines are much higher than the traditional EPI vaccines. The highest UNICEF Weighted Average Price per dose for any of the EPI vaccines is $ 0.18 (for DTPw vaccine).[15] At the current cost of approximately $ 60 per dose the quadrivalent vaccine is unaffordable to the public sector in India even though the price is substantially lower than that in United States. The price of the bivalent vaccine is yet to be announced but is likely to be at par. The demand for the vaccine initially will be in the private sector and the population coverage will be very modest. The private spending will raise awareness among the medical fraternity as well as the general population, stimulate broader demand and generate confidence in the efficacy and safety of the new vaccine. Going by the experience with other vaccines it will take at least one or two decades for the price of the HPV vaccine to come down to a level when it will be affordable to the public health program of a middle income country like India. Meanwhile generous contributions or purchasing commitments from GAVI Alliances, UNICEF, World Health Organization, World Bank, Bill and Melinda Gates Foundation, national government and nongovernmental organizations can encourage the manufacturers to bring down the price and ensure availability of the vaccine to the poorer section of people at least for some demonstration projects.

Conclusions

India needs a framework of comprehensive cervical cancer control that should focus on phased introduction of cervical screening program along with sustained advocacy at the highest level for making the HPV vaccines available through the national immunization program when they become affordable. India is a very diverse country and the resource and logistic requirements for screening and vaccination are different. Depending on the local infrastructure, resources and socio-cultural milieu, the coverage with screening and vaccination will vary widely within the country. Setting up or augmenting cervical cancer screening facilities should be a priority under the National Cancer Control Program. Gradually the efforts can be expanded to introduce preadolescent vaccination. In regions where screening is not likely to be feasible, focused efforts to achieve high coverage of HPV vaccination in preadolescent girls would be the most meaningful investment. The advocacy for such a preventive strategy must come from those who understand the disease and its societal and population-based burden. A partnership has to be developed between professionals and organizations engaged in immunization, sexual and reproductive health, community-based health education and public health initiatives with the common long-term goal of eliminating cervical cancer.

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5. Efficacy and Safety of Prophylactic HPV Vaccines

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Introduction

Cervical cancer prevention has been a major challenge in India because of the lack of systematic screening programs. An understanding of the causal role of human papillomavirus (HPV) in cervical carcinogenesis has lead to the development of HPV-based diagnostic tests for screening and early detection and also the development of HPV vaccines. There are primarily two types of anti-HPV vaccines:

- Prophylactic - to prevent primary infection in susceptible population
- Therapeutic - to eliminate infections in infected populations.

Therapeutic vaccines are still under research. Two prophylactic HPV vaccines have already been developed commercially. Both vaccines contain hollow virus-like particles (VLPs) assembled from recombinantly expressed major capsid L1 proteins of HPV. The L1 VLPs are morphologically and antigenically similar to HPV, but they are harmless as they contain no DNA.[1] These protect against HPV-16 and -18 which are the high risk types associated with 70 percent of cervical cancers worldwide and in India.[2-5]

Currently available prophylactic HPV vaccines

- **Gardasil®** is a quadrivalent HPV-16/18/6/11 L1 VLP vaccine developed by Merck and Co. Inc., Pennsylvania, USA that protects against four HPV types: HPV-16 and -18 are high risk types that together cause 70 percent of cervical cancers, while HPV-6 and -11 are low risk types that are responsible for 90 percent of genital warts. It has also been shown to protect against vaginal and vulvar intraepithelial neoplasia caused by HPV-16 and 18. It is formulated on an alum adjuvant, AAHS.
- **Cervarix®** is a bivalent HPV-16/18 L1 VLP vaccine developed by GlaxoSmithKline Biologicals, Rixensart, Belgium that protects against HPV-16 and -18. It has also been shown to have some cross-protection against the related phylogenetic high risk types HPV-45 and -31. It is formulated on an ASO4 adjuvant comprising 500µg of aluminium hydroxide and 50µg of 3-deacylated monophosphoryl lipid A (**MPL**) for an exaggerated immune response.

The efficacy of the prophylactic vaccines

The two important parameters to judge vaccine efficacy are immunogenicity and clinical efficacy, which have been assessed for both the bivalent and quadrivalent HPV vaccines in extensive Phase II and Phase III trials.

I. Bivalent vaccine (Cervarix®) :

Immunogenicity: 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 years and a subsequent follow-on study of a subset of the original enrolees with a median follow-up of 4.0 years.[6,7] Seroconversion occurred in virtually 100 percent of subjects and more than 98 percent seropositivity was maintained for HPV-16/18 antibodies during the extended follow-up phase of 4.5 years.[7] The same has now been noted in the follow-up to 6.4 years, with antibodies maintained at nearly 11-fold the level of natural infection.[8]

Immunobridging studies in 10-14 year age group: Efficacy studies have been done in older, sexually active girls, however the proposed age for vaccination is preadolescence onwards. Phase III clinical trials have compared antibody titres at 0 and 7 months in girls aged 10-14 years and compared them with those in the age group 15-25 years. It was observed that antibody levels were nearly twice as high in the younger age group, which is as expected since the immune response is more robust at a younger age.[9]

Immunobridging studies in >25 year age group: Similarly, immunobridging studies have been carried out in the older age group from 25-55 years and the efficacy evaluation is ongoing. It has been seen that irrespective of whether the women were initially seronegative or seropositive for HPV-16 and 18, there was a significant response to vaccination with antibody titers nearly 8-fold of those for natural infection.[10]

Immunobridging trial in India: A Phase IIIb, multicentric, double-blind, randomized, controlled study to evaluate the immunogenicity and safety of the bivalent HPV-16/18 VLP/AS04 vaccine administered intramuscularly according to a 0, 1, 6 months schedule was conducted in healthy Indian female subjects aged 18-35 years. The antibody response with both HPV-16 and -18 compared well with that reported in previous trials.[11]

Efficacy : In phase II trials, significant vaccine efficacy was noted against the following endpoints: protection from incident infection - 96.9 percent (95% CI 81.3-99.9); protection from persistent infection (HPV test positive at 6 months interval) - 94.3 percent (95% CI 63.2-99.9); protection from persistent infection (HPV test positive at 12 months interval) 100 percent (95% CI 33.6-100). In a combined analysis of the initial efficacy and extended follow-up studies, vaccine efficacy was 100 percent (95% CI 42.4-100) against cervical intraepithelial neoplasia (CIN) lesions associated with vaccine types.[7]

The observed broad protection against CIN 2/3 by the bivalent vaccine was beyond that anticipated by its prevention of HPV-16/18 infection alone. It was found that there was cross-protection against incident infection with HPV-31 and HPV-45 as well, which are phylogenetically related with HPV-16 and -18 respectively. In the ongoing Phase II study, 78 percent cross-protection (range: 39-93%) against incident infection with HPV 45 and 60 percent cross-protection (range:20-81%) against incident infection with HPV 31 has been demonstrated.

II. Quadrivalent Vaccine :

Immunogenicity : The first trials were with HPV-16 and these were followed by phase II trials of the quadrivalent HPV-6/11/16/18 vaccine, in which subjects have been followed up for 5 years.[12,15] Seroconversion occurred in virtually 100% of subjects. Seropositivity for HPV -6, -11 and -16 were maintained at a level several fold higher than that of natural infection during this period. However,

HPV-18 antibody levels dropped to about 65% in a period of 18 months.[16,17]

Immunogenicity in 9-15 year age group : Seroconversion occurred in all girls. Antibody titers were higher in this population.[18]

Immunogenicity in older women 35-45 years old: Comparable safety and immunogenicity of HPV vaccines have been reported in women aged 35 to 45 years.[19]

Efficacy : In studies evaluating efficacy, when the quadrivalent vaccine was administered to subjects not previously exposed to either HPV-16 or -18, the vaccine was 98-100 percent effective in preventing HPV-16/18 related CIN 2/3 and adenocarcinoma in situ (AIS).[13,15] Efficacy remained high for at least 5 years following vaccination. However, the entire population of women who had undergone randomization (who may or may not have received vaccines as per protocol) included HPV-16/18 non-naïve subjects (already exposed to either or both types). Efficacy was lower 44% (95% CI 31-55) in this intention-to-treat cohort. [14]

The efficacy of the quadrivalent vaccine in preventing genital warts due to HPV-6/11 was 100 percent.[15] Protection against vaginal and vulvar intraepithelial neoplasia has also been reported.

Cross protection has been reported against other high-risk HPV genotypes.[20] Vaccination reduced the rate of HPV-31/33/45/52/58 persistent infection by 19.5 percent (95% CI 6.5, 30.7). Vaccination also reduced the rate of HPV-31/45, -56 and -59 related diseases by 13.8 percent (95% CI- 8.2, 31.4), 23.2 percent (95% CI - 2.3, 39.7) and 47.5 percent (95% CI- 16.2, 67.7) respectively.

Comparable efficacy has been reported in women aged 24 to 45 years.[19]

Requirement of boosters

As discussed above, antibody levels fall from the peak levels after immunization to a plateau level that is about 11 times higher than those detected in natural infections and that have persisted so far for 6.4 years post-vaccination in the case of the bivalent vaccine.[8] In the case of the quadrivalent vaccine, HPV-16 levels are maintained but HPV-18 levels drop. Data correlating antibody persistence with protection is yet awaited. The antibody levels have been shown to rise again with a booster dose but there is no evidence yet whether exposure to virus post-vaccination will act as a natural booster. So far it is seen that efficacy is maintained beyond 5 years and it is thought that there may be methods of memory cells and cell-mediated immunity that are active as well.

The longest follow-up results available from the vaccine trials are for up to 6.4 years. The total duration of protection is not yet known. Mathematical modeling suggests that the protection may last for over 30 years. At present there are no data to support the use of boosters.

Safety of the vaccines

Data are available from a large safety database with nearly 16,000 women vaccinated with each vaccine and a broad age range starting from 9-10 years age. The vaccines have a favourable safety profile similar to that of other currently licensed vaccines in general use.

Reported adverse events are generally mild or moderate in intensity. 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 (93% versus 87%)[6,13,21.] However, grade III symptoms were reported in only 6-8% cases.[21] Headache, fatigue and myalgia are the most commonly reported systemic adverse events. Gastrointestinal complaints and itching were

frequently reported. Temperature elevations were reported in about 15% of women.[14,15]

Serious adverse events were reported with similar frequency in vaccine and control groups and did not lead to any increase in withdrawal rates. There was no increase in the incidence of new onset of chronic or autoimmune disease in any age group. Though the vaccines were not intended to be administered to pregnant women in the trials, some of the women who received the vaccine got pregnant. The pregnancy outcomes in the vaccinated women were similar to the women in the placebo group. No additional fetal anomalies were observed in the vaccinated women.

In the Indian trial of the bivalent vaccine, pain was reported in only 56% of subjects vs 41% in placebo. Fever was reported in 12% of cases in both groups. There was no significant difference in adverse events or unsolicited symptoms between the groups. Compliance was good and the vaccine was well accepted and tolerated.[11]

Vaccination of women already naturally infected with vaccine HPV types has not been associated with any adverse effects in clinical trials. Overall, both vaccines appear to be safe and well-tolerated.

Summary

The prophylactic HPV vaccine is one of the greatest advances in recent times. There are some differences in the profile and coverage with the two currently available vaccines. Further studies will elucidate the immune response and efficacy in special populations, e.g., immunosuppressed persons, the duration of protection, etc. In general, both vaccines are highly immunogenic, safe and efficacious and provide a hope for primary prevention of cervical cancer and other HPV-related diseases.

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6. Cervarix, the AS04 Adjuvanted HPV 16 and 18 Vaccine Providing for Strong and Lasting Cervical Cancer Protection

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Introduction

The GSK HPV vaccine contains 20 µg each of human papillomavirus (HPV) types 16 and 18 virus-like particle (VLP) L1 protein in combination with a proprietary ASO4 adjuvant system comprising 50 µg monophosphoryl lipid A (MPL) absorbed upon 500 µg aluminium hydroxide. Recognising the increased complexity of infectious diseases being prevented, the ASO4 adjuvant was developed to provide innovative ways to control the quality and/or quantity of vaccine antigen-specific immune responses. ASO4 represents a new generation of vaccine adjuvants that activate innate immunity to potentiate protective and adaptive immune responses, through mediation by antigen presenting cells (APCs). In targeting cervical cancer prevention with a vaccine, it is recognised that the contribution of HPV 16 and 18 to the oncogenesis of cervical cancer far exceeds that of other benign and malignant conditions and this vaccine was thus developed with the vision in order to provide the best protection against cervical cancer.

The evasive nature of the HPV infection in the cervix

Antibody responses to natural genital HPV infections are characterized as neutralizing serum antibody responses specific to the HPV type of L1 protein. This natural antibody response is very slow and weak, because there is no viremia and the HPV infection stays entirely within the epithelial surface, with no blood or lymphatic system contact. In addition, the virus does not kill the keratinocytes that it infects, and there is no inflammation or cascade of pro-inflammatory cytokines, only poor activation of epithelial antigen presenting cells (APC). Free viral particles are shed from mucosal surfaces with poor exposure to APCs, leaving natural infections with little ability to stimulate an anamnestic response due to prior low antibody titer induction from an initial infection and most importantly very poor and very slow immune recognition of a new infection within the epithelium. In addition, natural infection antibody titers are known to be insufficient to provide long-term protection; and these weak titers do not transude neutralizing antibodies at the cervical mucosal surface.

Vaccines prevent HPV infection by transuding antibodies through the cervical basement membrane to protect not only against initial infections, but also against persistent/latent HPV re-infections over time. This is important, as antibody levels from natural infections have been shown to be insufficient to protect against new HPV infections of the same type.[1]

Hepatitis B is dissimilar from Human Papillomavirus infection

An attempt to make inferences to the HPV infection mechanism of disease causation using Hepatitis B as a comparator does not apply for several reasons.

Hepatitis B is a blood borne infection and causes a viraemic state whereas as described before, HPV does not cause viraemia and is a localized infection of the cervix.

This recently published Taiwanese study[2] showed that anti-HBs was below the protective level in 63.0% of 5981 students who were born after 1986, the year when universal HB vaccination was instituted in Taiwan. They were vaccinated with plasma-derived HB vaccine (Hevac B; Pasteur-Mérieux), as neonates, 15 years or more previously.

After a booster in the seronegative individuals aged 15 to 21 years, overall 29.2% did not seroconvert. Among the ones who underwent full course vaccination (4 doses) 28.7% continued seronegative and 27.9% of the ones who had received < 4 doses also continued seronegative.

What is more important is that 27.2% of the study subjects had no detectable HBsAg-specific memory T cells. This is evidence that these subjects truly had no immunity against HBV infection. Hence the argument of immune memory, even if applied to the natural history of HBV, does not guarantee protection.

Development of the GSK cervical cancer vaccine and the AS04 adjuvant system

Giannini et al (2006)[3] investigated whether the quantity of the antibody response to the HPV 16/18 L1 antigen could be increased by use of the ASO4 adjuvant system, compared with 16/18 VLP L1 proteins formulated with aluminum hydroxide alone. This hypothesis is based on the concept that the more antibodies that are able to cross the cervical basement membrane, the greater the likelihood of neutralizing the new HPV infection.

Female subjects were given an intramuscular injection with 20 µg of HPV types 16 and 18 VLPs combined with either 500 µg aluminum hydroxide alone, or with 500 µg aluminum hydroxide plus 50 µg MPL (AS04) at 0, 1 and 6 months. Specific antibody responses to HPV 16 or 18 L1 VLPs were then assessed over a 4-year follow-up.

Significantly higher levels of antibodies to HPV types 16 and 18 were achieved in subjects receiving VLPs given in combination with the ASO4 adjuvant system than in women given types 16 and 18 VLPs plus aluminum hydroxide (Figure 1). Furthermore, the enhanced immunogenicity associated with the ASO4 adjuvant was sustained throughout the 48-month study period.

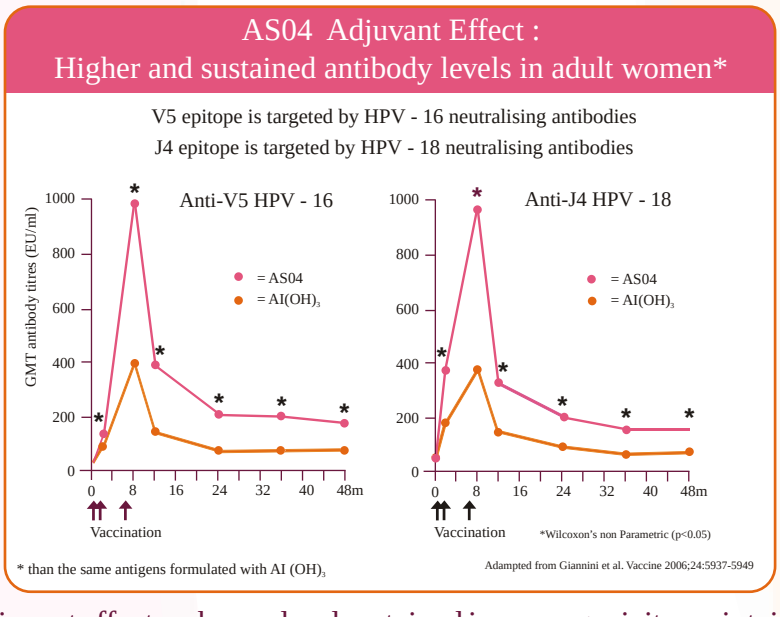


Figure 1. ASO4 adjuvant effect: enhanced and sustained immunogenicity maintained over 4 years.[3]

B-memory cell responses against HPV types 16 or HPV 18 L1 VLPs were also assessed among the two groups at 0, 2, and 7 months. Induction of B-memory cells is essential for a sustained immune response to vaccine antigens, which can be maintained for decades. Figure 2 shows the frequency of HPV 16 or HPV 18-specific B-memory cells per 10^6 human peripheral blood mononuclear cells, with the number of participants shown in parenthesis. These data clearly show the increase in frequency of HPV L1 specific B-memory cells with the As04 formulated vaccine versus that with aluminum hydroxide alone, providing evidence that AS04 can induce a long-term memory response to HPV.

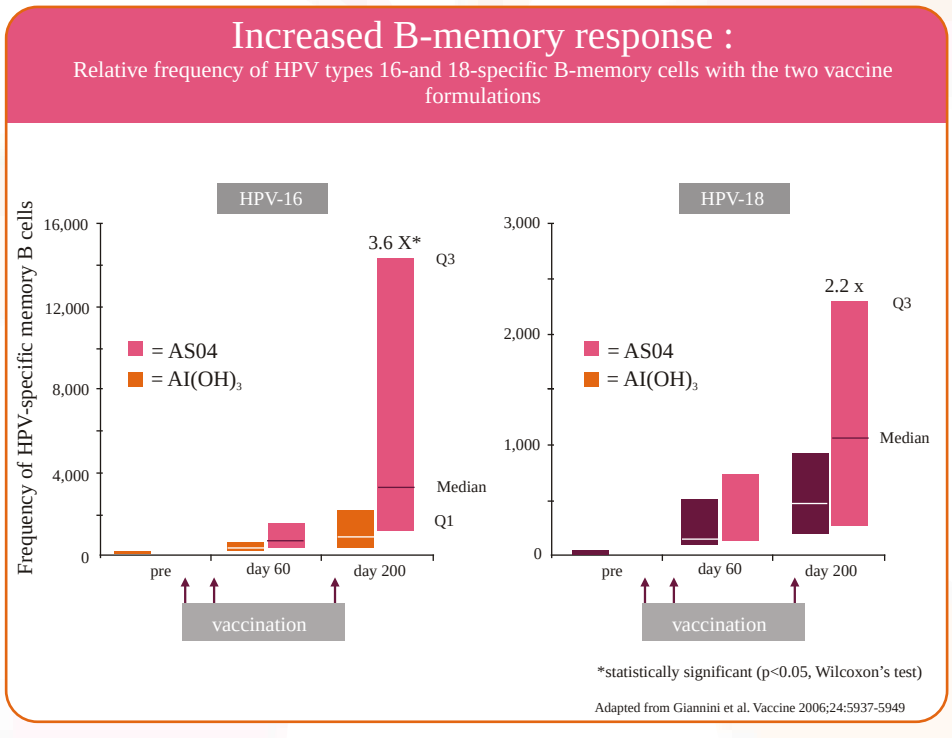
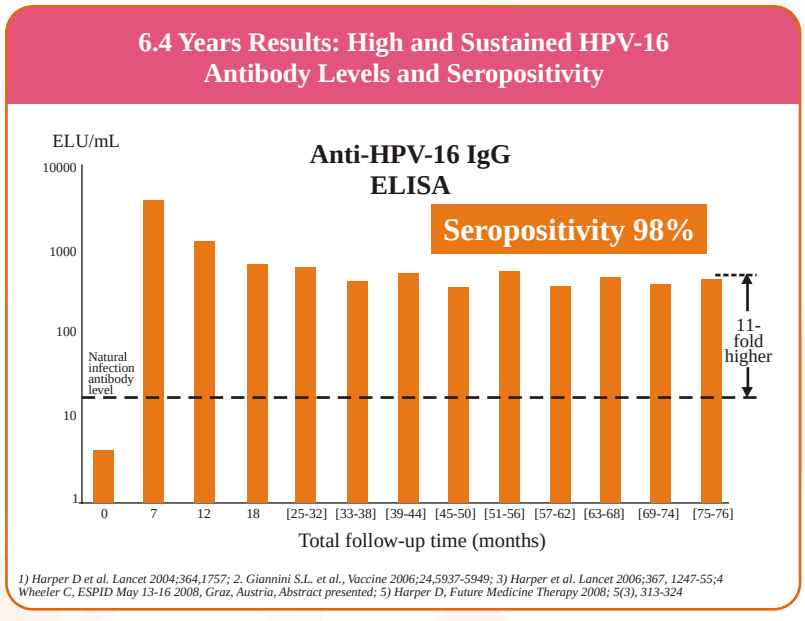


Figure 2. Increased B-memory response: relative frequency of HPV types 16- and 18-specific B-memory cells with the two vaccine formulations (*P<0.05).[3]

Efficacy data and duration of protection

The long-term efficacy and immunogenicity of Cervarix was investigated in a Phase II clinical trial in women aged 15 to 25 years.[4] High initial peaks were seen in HPV type 16 antibody levels, with seropositivity being sustained at levels that were >11-fold higher than antibody titers in response to natural infection up to 6.4 years (Figure 3)[5,6]. Importantly, the same initial high antibody levels and sustained seropositivity at a similar level to HPV type 16 were seen with HPV type 18 (Figure 4).[5.6]

Figure 3. High and sustained HPV type 16 antibody levels and seropositivity up to 6.4 years.[5,6]



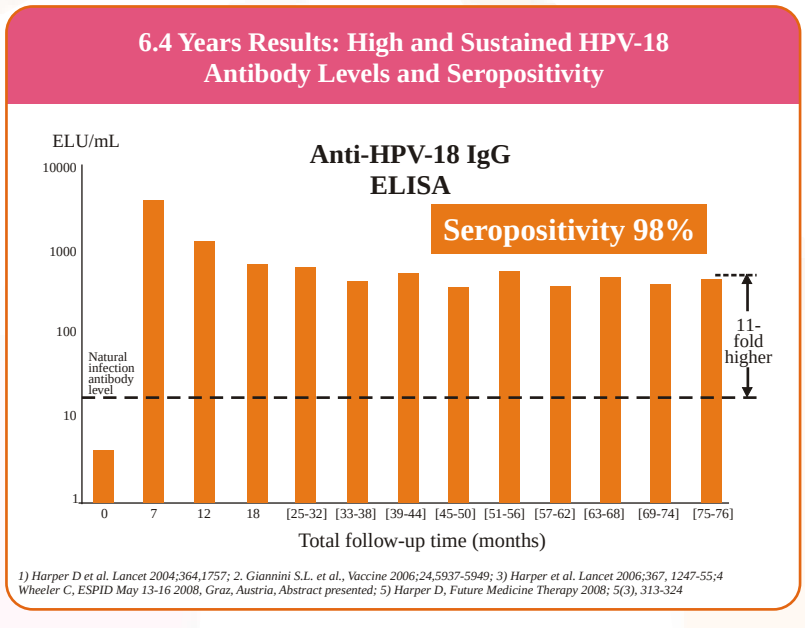


Figure 4. High and sustained HPV type 18 antibody levels and seropositivity up to 6.4 years.[5,6]

The main reason VLP vaccines such as Cervarix elicits such high levels of antibody to VLP L1 is that they are delivered intramuscularly in the deltoid muscle, giving rapid access of VLPs to blood vessels and local lymph nodes, thereby inducing antibodies which protect against HPV 16 and/or 18 cervical infections. An additional aspect of protection may be the detection of an effective immune reaction at disease-relevant sites, i.e. the cervical mucosa, with a cervical protection occurring by transudation of IgG into the cervical secretions.

These antibody profiles were observed in both the ELISA as well as the Pseudovirion Neutralising assays and there has also been a correlation performed between these two assays.[7] The acknowledged “gold standard” is still the PBNA assay which the NCI uses to assess the levels of neutralizing antibodies in HPV infections.[8]

Vaccinating adolescent girls

HPV-12 was the immunobridging study that studied the immunization for early adolescent girls aged 10 to14 years[9] as it would have been unethical to perform efficacy trials on such a young female population. There was 100% seroconversion for both HPV 16 and 18 and geometric mean titres were twice that of the efficacy data in the 15 to 25 years comparator group. These data provided support to the approved age indication for Cervarix in many countries for females aged 10 to 25 years.

Vaccinating older women

Women of all ages need to be protected against HPV, because there is a continued risk of infection, regardless of age or lifestyle, with infections continuing to occur over time. In addition, persistent infections, which are the most likely to progress to CIN III or cancer, are more common in older than in younger women, so the time taken to see a reduction in cancers is accelerated by vaccinating women of all ages.

In a study of 7,600 women treated for cervical intraepithelial neoplasia grade I (CIN I) and followed for 27 years, the incidence of CIN I, CIN II or cervical cancer was nearly three-fold that seen in the normal population.[10] This suggests there is re-infection with HPV, either from new infections but more likely by autoinoculation from already infected basal cells. Importantly, this also shows there is no lifetime protection from natural HPV infection.

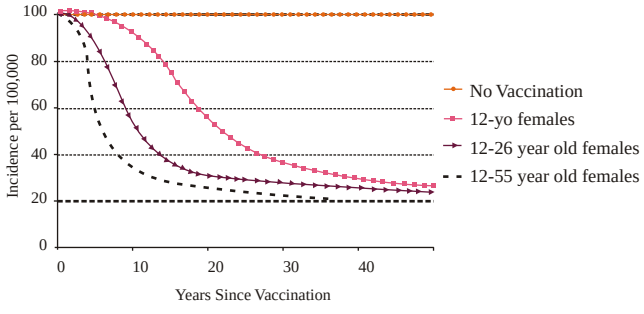
Swedish researchers using national registration data followed 3.7 million women with a history of treated CIN III versus those with no CIN III disease for 37 years to see if there was any difference in incidence of HPV-associated cancers.[11] There was a marked increase in vaginal and vulval cancers in those treated for CIN III after the first year and these remained significantly higher than the normal population even 10 years after the initial treatment for CIN III. There were also more anal cancers among the treated women, but these developed much more slowly. HPV infections at any point in life therefore continue to put women at risk for ano-genital cancers long after the initial infection.

Robust antibody responses to this HPV candidate vaccine have also been reported for HPV types 16 and 18 among older women up to age 55 years.[12-13] While antibody titers were slightly lower than those observed in the efficacy study, they were nevertheless at least nine-fold higher than natural infection titers.

For example, a UK cohort study followed more than 20,000 women for at least 10 years. Of those women 30 years and older who had normal cytology at enrolment and developed persistent HPV 16 or 18 infections, 20% of the women with persistent HPV 16 and 15% of the women with HPV 18 progressed to CIN III or cancer within 10 years. [14]

Figure 5 shows a mathematical extrapolation of the effects of the HPV 16/18 vaccine on the burden of cervical cancer presuming the vaccine has lifetime protection or continued lifetime boosters are given starting at age 12, in which case it would take approximately 35-40 years before the number of cervical cancers was halved.[15]

Impact on HPV 16/18-related CIN 2/3 INCIDENCE with 16/18 vaccine

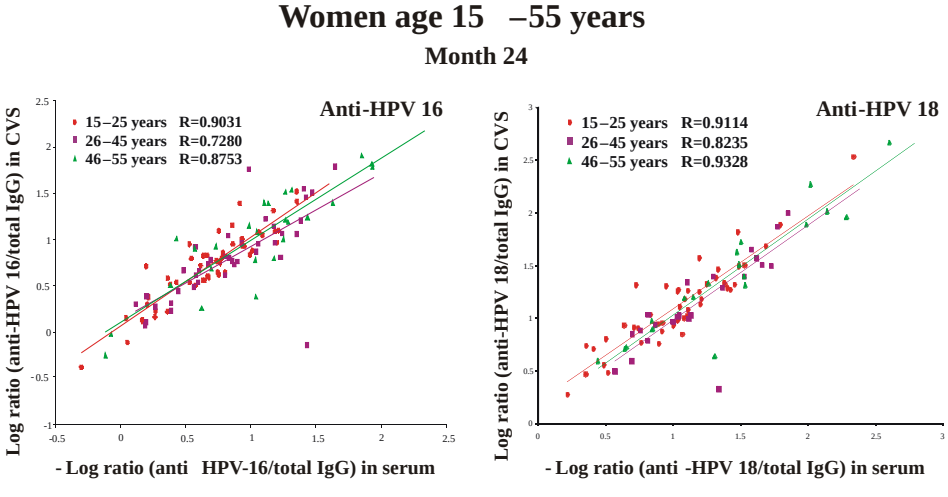


Courtesy of Prof D. Harper
Elbasha EH, Dasbach E J, Insinga RP. Emerg Infect Dis. 2007 Jan;13(1):28-41.

However, if the age at which women are vaccinated is increased up to age 26 years, this time is reduced to about 25 years. Moreover, if the age of vaccination is increased further to 55 years, the time to halving of the cervical cancer burden is reduced to around 10 years. Comparable reductions are also seen in incidence of CIN II/III by vaccinating older women. There is therefore a major advantage associated with the inclusion of older women in HPV vaccination programs.

The relevance of Cervico vaginal secretion data

Cervarix® - Strong correlation between serum & cervical mucosal antibody levels



Higher serum Ab levels → Higher Ab levels at the mucosa/in the servix - where they are most needed

Poncelet et al. 24th International Papillomavirus Conference and Clinical Workshop 2007;PS19-25.
Stanley M et al.Vaccine 2006;24:Suppl 3,S106. Giannini et al.Vaccine 2006;24:5937.

A study of the relationship between serum antibody titers and those in cervical/vaginal secretions Fig 6 showed there was an almost perfect linear correlation between the two in combined age groups of women aged 15 to 55 years for both HPV types 16 and 18. These high cervical mucosal and serum antibody responses were similar, regardless of age.[3,16,18]

Antibodies are required to neutralise the virus and prevent entry into the cell[17]. Several studies[19, 21] had reported that the uptake and internalization of HPV occurs within 30 minutes to 4 hours and usually a memory response only occur after 2 days[22] and in some cases up to 7 days. Clearly the reliance on immune memory to combat this infection in the absence of antibodies would not be sufficient, hence the need for antibodies at the site of infection, i.e. the cervix.

Safety of the GSK cervical cancer vaccine

The safety of the AS04 adjuvanted vaccine have been reviewed and analysed in two studies. The first is an integrated analysis of subjects examining the potential risks of autoimmune disease. This was performed given the background incidence of autoimmune disorders in some of the groups targeted for immunisation with these vaccines and it being likely that autoimmune events will be reported in temporal association with vaccination, even in the absence of a causal relationship.

All randomised, controlled trials of HPV-16/18, HSV and HBV vaccines were analysed in an integrated analysis of individual data amounting to more than 68,000 subjects. A separate analysis of the HPV-16/18 vaccine trials alone was also undertaken having greater than 39,000 subjects. Reporting rates of overall autoimmune events were around 0.5%, not being any different between the AS04 and control groups. The relative risk (AS04/control) of experiencing any autoimmune event was not statistically significant difference in event rates between the AS04 and control groups.[23]

The second analysis was on the pooled safety data for eleven of GSK's HPV trials. Rates of solicited local and general symptoms were higher in the HPV-16/18 vaccine group than in the control groups. However, compliance with the 3-dose schedule was high and did not differ between groups. There was also no clinically relevant differences were seen between the HPV-16/18 vaccine and pooled control groups in rates of SAEs, MSCs , NOCDs or NOADs. Similarly, no differences in pregnancy outcomes or rates of withdrawals due to AEs or SAEs were observed between groups.[24]

The AS04 adjuvant system will continue to be monitored in post marketing studies and the safety database for Cervarix will only increase as more females are vaccinated.

How long can we estimate the antibody levels will last?

A common concern among doctors is the duration of the vaccine's protective effect. As HPV vaccines have only entered the realm of recommended vaccines about 3 years ago, we have to rely on modeling studies which are based on the antibody kinetics as previously studied with other vaccines like Hepatitis A and B. Also efficacy trials are still ongoing and it is the development plan for Cervarix to continue its phase IIb efficacy trials till 10 years and its phase III trial (HPV 008) is still continuing, being an event driven analysis.

It has been predicted based on modeling that the antibody levels of Cervarix can last up to 20 years.[25]

Conclusion

HPV 16 and 18 L1 VLPs with the ASO4 adjuvant system induce higher antibody levels than are seen with an aluminum hydroxide adjuvant alone, long-term B-cell memory, and sustained robust antibody titers well above natural infection titers in all ages of women that are more than ten fold higher than natural infection titers for more than 6,4 years. [3, 5, 6, 18]

At present 90 countries have approved the use of Cervarix globally. There has been an unprecedented velocity in which vaccination authorities have reviewed and also drawn up recommendations to the use of HPV vaccination, as a recognition of the urgency of the need to acknowledge the heavy burden of cervical cancer, especially in resource challenged countries and also those where screening is virtually non existent.

The United Kingdom National Health Trust and Department of Health has in June 2008 chosen Cervarix as the national immunization vaccine of choice and implemented a comprehensive three programme for vaccination of girls aged 12 to 14 with a catch up for 16 to 18 years. The choice of vaccine was determined on a 15 point plan looking at the quality of the scientific data e.g. duration of protection and also that cervical cancer will be the chief focus of the vaccination strategy as well as cost effectiveness.[26]

Cervical cancer protection can further be accelerated when mature women are also vaccinated against HPV. Already in place in the age indication in some countries eg. Australia, Malaysia, India and the Philippines this will be the next paradigm in HPV vaccination as experts recognise that there is a persistent infection risk throughout these women's lives and they stand to benefit from this intervention as well.

Ongoing trials will further refine the approach to cervical cancer prevention. It is also worth concluding that screening should continue as these are not therapeutic vaccines and that cross protection will emerge as an added bonus to the types included in the vaccine as the data matures.

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7. Cervical Cancer Screening by Pap Smear Cytology

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Dr. Radhika N. Joshi *MBBS, DGO, DFP*

Introduction

Cervico-vaginal Cytology or Pap Smear as it is popularly known is a technique used successfully in the early detection of pre and early cancers of the cervix. Cytology is, by definition, the study of cells, (CYTOS in Greek means cells). It was introduced in clinical practice by George Papanicolaou[1], a Greek scientist, settled in USA. In 1943, he described "The presence of abnormal cells in the vaginal smears of asymptomatic women, which was exfoliated from an early carcinoma in situ".

Since 1950, it has been accepted that an annual "Pap Smear" done on all women will control cervical cancer by diagnosing it early at a stage when treatment can give 100% cure rate. When this is done on a large scale it is called a Screening Program.

Screening is defined as presumptive identification of unrecognized disease by application of diagnostic procedures which are reliable, safe and rapidly applied. There are different types of screening-

- **Mass screening:** It means an entire at risk population must be screened. It is difficult and expensive to implement.
- **Selective screening:** One can set up criteria to select the high risk population and screen only those. For cervical cancer the age group of 35-64 years is considered as most suitable.
- **Multiphasic screening:** Means screening for more than one condition. One can combine breast & full genital tract screening. Even medical conditions like anemia and diabetes can be added.
- **Opportunistic screening:** This is relatively easy to implement. Any women attending a medical facility for any service like health check up, family planning, delivery, abortion can be screened for cancer. Such a strategy does not achieve significant reduction in disease load as the test is repeatedly administered on a low risk population and the population coverage achieved is minimal.

The liberal use of Pap smear and Colposcopy in the developed countries has caused a shift from invasive to preinvasive disease of cervical cancer at the time of diagnosis, thereby reducing mortality.

It was Papanicolaou & Traut in the early forties who undertook the first screening programme by using cytology. There has been no turning back since then. Soon after the World War II ended many countries introduced mass cytology screening as a public health measure to control cervical cancer.

Technique of Cytology

Smear taking is very simple. After introducing a speculum, a smear is taken from the squamo-columnar junction with an Ayre's spatula or a cotton swab. It is smeared on a clean glass slide which is immediately dipped into fixative consisting of equal parts of Ether and 95% alcohol. A spray fixative

is also equally effective. Endocervical cells can be collected by introducing an endocervical brush into the cervical canal and rotating it 360°. The content of the brush can be spread on the same slide on which the ectocervical smear has been taken.

These slides are taken to the laboratory where they are stained using Papanicolaou staining technique & then mounted in DPX and covered with a coverslip. The slide is then studied under a binocular research microscope under low power and then high power.

Cells which are normally exfoliated from the genital tract are shown in Figure 1. In the presence of a precancerous condition, abnormal cells are detected in the smear. These are shown in Figure 2[2]

Figure 1: Normal cells

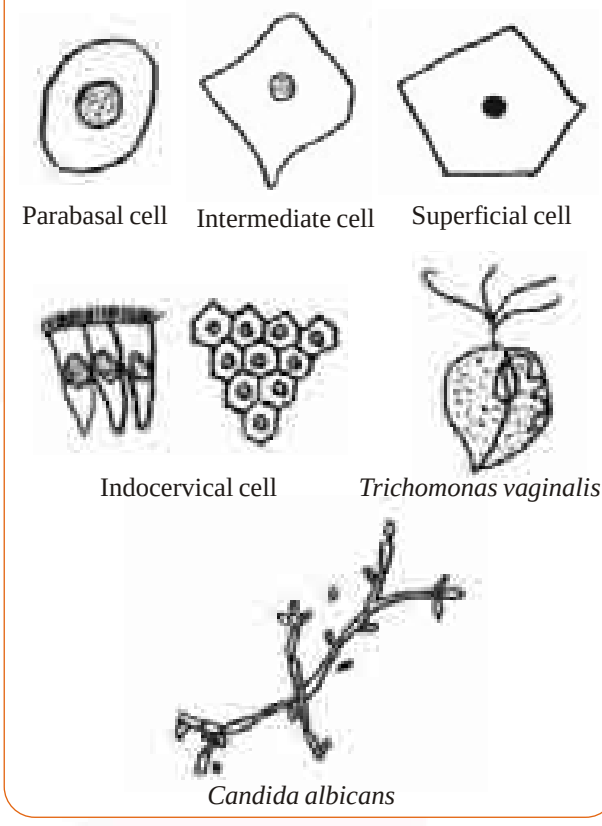
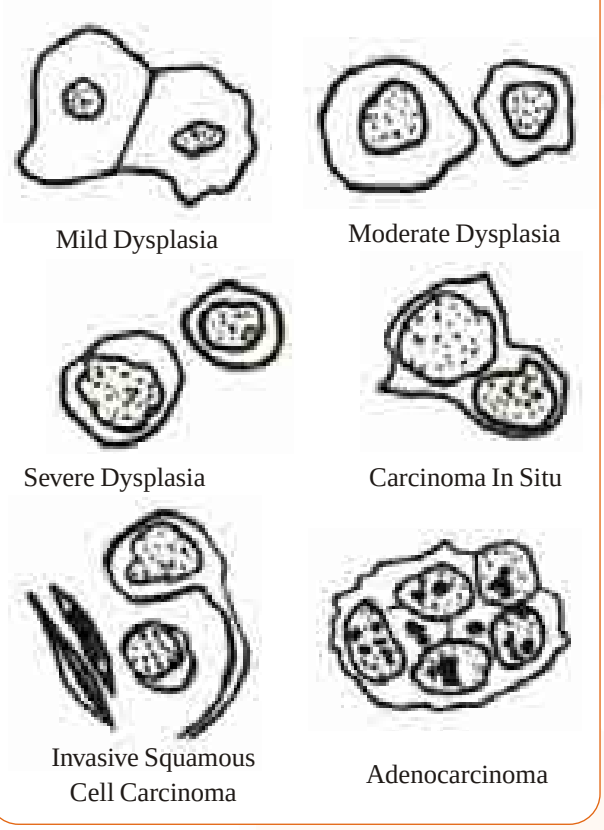


Figure 2: Abnormal cells



Clinical Application of Cytology

If an annual Pap smear is done on every woman after the age of 30 years it is expected that all cases will be diagnosed at pre-cancerous stage. This will lead to a good control of cancer cervix and mortality will come down. This has been achieved in many developed countries and planned for India since the 7th five year plan (1984). Unfortunately, in India, nearly 80 percent of the cervical cancers are detected at Stage III and IV when curative treatment is not possible and mortality is very high.

All women who have an abnormal smear on cytology have to be evaluated further by Colposcopy, biopsy and other modalities. They also have to be treated properly. Long term follow-up is mandatory.

Reporting of the smear

There are many classifications which are used to report the smear. The Clinicians must understand these and interpret the report correctly.

a) Papanicolaou's Classification:

This is the oldest. The smears are classified from I to V.

- Class I is negative
- Class II is negative but atypical cells due to infection are seen
- Class III is doubtful
- Class IV is positive but isolated atypical cells are seen
- Class V is positive with numerous atypical cell groups present.

b) WHO Classification:

This was introduced in 1962[3]. Here the smears are classified as normal, inflammatory, mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ, invasive cancer.

c) CIN Classification of Richart[4]

- The smears are classified as normal.
- Cervical intraepithelial neoplasia CIN I
- Cervical intraepithelial neoplasia CIN II
- Cervical intraepithelial neoplasia CIN III
- Invasive Carcinoma

d) Bethesda Classification [5]

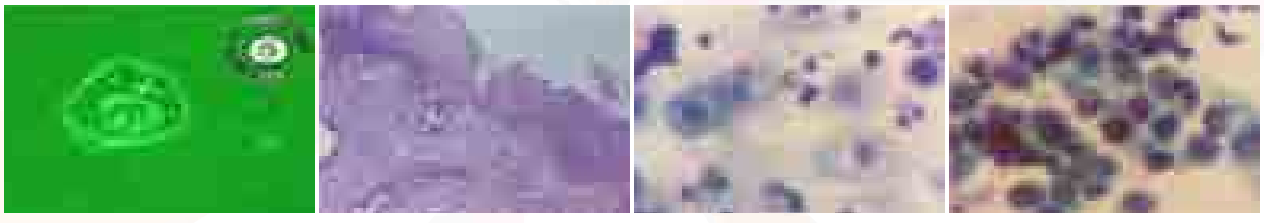
Introduced in 1991 & revised again from time to time. Currently all over, Bethesda 2001 is used. It is a descriptive detailed study of the smear and the abnormalities are classified as follows:

- ASCUS Atypical Squamous Cells of Unknown Significance
- ASC-HSIL can not be excluded
- AGUS - Atypical Glandular cells of Unknown Significance
- LGSIL - Low Grade Squamous Intraepithelial Lesions
- HGSIL - High Grade Squamous Intraepithelial Lesions
- Invasive Squamous cell Carcinoma
- Adenocarcinoma

Table 1 gives the comparison of all the classifications and may be used as a reference table. It is advisable to have a discussion with the Pathologist regarding which classification is being used & the significance of the findings.

PAPANICOLAOU CLASSIFICATION	WHO	RICHART	BETHESDA
CLASS 1	NORMAL INFLAMMATORY		ASCUS
CLASS 2	MILD DYSPLASIA	CIN I	LGSIL
	MODERATE DYSPLASIA	CIN II	HGSIL
CLASS 3	SEVERE DYSPLASIA	CIN III	HGSIL
CLASS 4	CARCINOMA IN SITU	CIN III	HGSIL
CLASS 5	INVASIVE	INVASIVE CANCER	INVASIVE CANCER

Table 1. Test characteristics compiled from IARC multi-centric studies



Koilocyte | Koilocytotic changes breaks in the basement membrane | Low Grade SIL | High Grade SIL

Cytology-based Screening - Indian Perspective

Cytology Screening in India was started in 1970 under the guidance of Dr. P. N. Wahi. Many centers were set up mostly in urban areas. Some excellent departments of Cytopathology were set up through The Indian Academy of Cytologists. However, these services remain inadequate for the huge population. Although the National Cancer Control Programme was officially launched in 1984, cervical cancer screening as a population based program has not received its due support from the national or state health services.

Cytology services are however available in most metro cities and in Medical Colleges. They should be fully utilized and expanded to meet the demands. There are many organizations in India which offer full medical facilities to their employees & they are more than capable of offering Pap smear & early detection facilities to al the women under their care. Some of these organizations are Armed Forces, Railways, BARC, CGHS and Police Personnel. Out of these BARC has been successful in their screening programme.

In the current situation we can recommend the following:

1. All FOGSI members must offer opportunistic screening to all women under their care.
2. All medical colleges must develop a full fledged modern Cytopathology Departments & participate in Screening.
3. Close links should be formed between the clinicians & Cytopathologists for follow-up and data management.
4. Teaching & Training of Personnel should be encouraged by all FOGSI members.
5. Pap smear should become a backup for all VIA (visual inspection after acetic acid application) positive women. Correct treatment must be given.

Screening for Cervical Cancer - Global Perspective

One of the earliest and most effective cytology based screening programs is the one implemented at British Columbia in Canada[6]. This programme began in 1949-and gradually expanded to population screening by 1960s. It is against this programme that the achievements of other programmes in Canada and elsewhere tend to be measured. This program has shown a dramatic fall in the incidence and mortality of invasive cervical cancer (Ref Table 2). An initial shift was noted from invasive to micro invasive to pre-invasive disease. Further, the staging of the invasive disease showed a marked downward trend. Whereas initially only 41% had stage I disease, at the end of a 20 year period most had stage I disease. The mortality rate fell remarkably to 2.0 in 2005.

Year	Incidence per 1,00,000 women	Mortality per 1,00,000 cases
1950	28.4	21.2
1974	8.6	11
2003	7.2	2.5
2005	6.7	2

Table 2: Reduction in cervical cancer incidence and mortality in British Columbia following the introduction of cytology based screening program

In the United Kingdom the Cervical Cancer Screening program is continuously reviewed and updated[7]. UK National Health Services (NHS) guidelines[8] advocate 3 yearly screening by Pap smear for women between 25 to 50 years of age. The interval is expanded to 5 years for the age group 50 to 64 years. Patients are registered with General Practitioners who collect the smears. Success is due to a very effective call/recall system that ensures that all the women with abnormal Pap smear results undergo colposcopy and further management as appropriate. The age-adjusted incidence of cervical cancer in UK in 2004 was 8.9/1,00,000 women. In the year 2003, there were 2,726 cases of invasive cancer in UK and as many as 24,105 of CIN III cases treated and were prevented from progression to invasive disease. Due to the organized screening program the mortality from the disease has also decreased significantly. It has been reported that NHS spends 150 million pounds per year on the program. The cost incurred is at the rate of £ 37.50 per woman screened. Recently Liquid Based Cytology has been introduced to improve the efficiency of the test.

In the United States of America it is still an opportunistic program. Education intervention to all Physicians has strengthened the screening. To reduce the false negative rates in conventional cytology there is a change to liquid based cytology, which is much more expensive.

Smaller countries in Europe have shown that program can be made successful. In Netherlands, only two percent of all newly diagnosed malignant tumors in women are cancers of the uterine cervix, corresponding to about 700 new cases of invasive carcinoma per year[9]. Putting this in perspective, cervical cancer is not among the top ten of most frequent cancers in the Netherlands and the mortality from the disease is extremely low. Such a success story is entirely due to the consistent and effective screening program. This is facilitated by the fact that it is a small country with a small population and a good health infrastructure.

Conclusion

An effective cervical cancer prevention program will have to ensure the following.

1. The test facilities must reach significant number of women approx 80 percent of the target population.
2. Screening must be linked to appropriate treatment. It is no use detecting abnormality if suitable treatment is not given. All screen +ve women must undergo further evaluation with colposcopy.
3. Effective follow up of the treated cases and of the low grade abnormalities is an essential component of organized screening program.
4. Periodic monitoring and evaluation of the programme and its impact on incidence and mortality due to the disease must be studied.

It takes time, resources and consistent planning on the part of health services managers, public health departments and professional bodies for the cervical cancer screening program to succeed. The education and motivation of women play important role.

Initiating an organized cervical screening program in India in the model of western countries will be extremely challenging. Financial constraints are the main stumbling block. Lack of qualified personnel and lack of health awareness need to be overcome. Competing priorities in women's health namely safe motherhood and family planning continue to dominate our health scenario.

However, as Jawaharlal Nehru said in 1949

"We cannot allow tomorrow

to slip out of our hands,

because of petty problems of today".

The responsibility lies with the practicing Gynaecologist and General Practitioners. With their whole hearted support, we may be able to say "Good Bye to Invasive Cervical cancer" just as we have been able to say that to small pox and polio.

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8. Cervical Screening with Visual Inspection after Acetic acid Application (VIA)

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Introduction

Cancer of cervix uteri is the second most common cancer among women worldwide, with an estimated 493,000 new cases and 274,000 deaths each year. About 80 percent of the cases occur in developing countries.[1] The Pap smear cytology has been recognized widely as the most effective cancer screening test in the history of medicine. Introduced by Dr. George Papanicolaou into clinical practice in 1940, it is widely believed that the use of this test in the organized cervical screening programs with systematic call and recall facilities for every woman in the screening age range has been responsible for the drastic reduction in the incidence and mortality of cervical cancer in the United States, Canada, and much of Western Europe in the past fifty years.

In India, like most other developing countries, there is no nationwide organized screening program. Pap smear facilities available in a few metro cities are ineffective due to low population coverage and poor quality cytology. The barriers to organize screening programmes in developing countries include competing health needs, limited human and financial resources, poorly developed health care services, sociocultural factors etc. The challenges and failure in introducing cervical cytology screening in low resource settings has stimulated the search for exploring alternative methods of cervical screening tests during the past decade.

VIA Test

In 1982, Ottaviano and La Torre published a study where women who were examined visually and colposcopically after a cervical wash with acetic acid were found to have equal detection rates of cervical abnormalities by both the techniques.[2] This important observation made researchers to use visual inspection after application of acetic acid (VIA) as a low technology screening test that can be used for low resource settings.

VIA, also known as direct visual inspection, or cervicoscopy, involves naked eye inspection of the cervix under bright light at least one minute after the application of 35% dilute acetic acid using a cotton swab or a spray. The test provider has to look for the appearance of acetowhite areas in the transformation zone (TZ), close to the squamo-columnar junction (SCJ) or the os.

Patho physiological basis

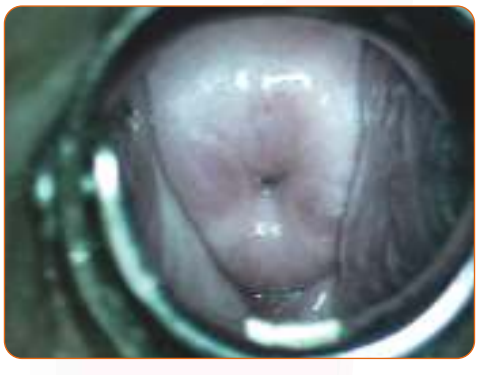
Application of 3-5% acetic acid is believed to cause a reversible coagulation of the intracellular proteins. Due to the precipitation of the coagulated proteins the cells become opaque and appear white. The normal squamous epithelium appears pink and the columnar epithelium red even after acetic acid application as none of them contain much of protein. In areas with cervical intraepithelial neoplasia (CIN) there are increased number of actively proliferating cells that contain a lot of cellular proteins (increased nuclear chromatin material, intact cytoplasm). Acetic acid coagulates these proteins obliterating the colour of the stroma and the abnormal areas appear as acetowhite areas.

Reporting of VIA

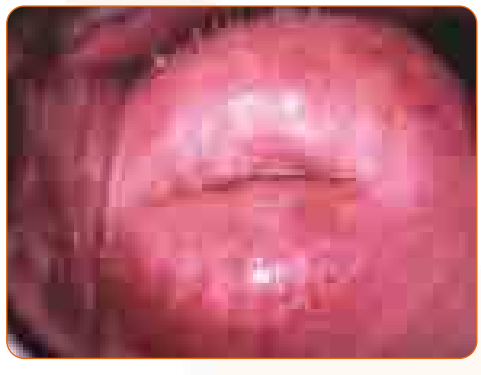
The tests results are categorized in to the following three groups.[3]

1. Negative
2. Positive
3. Suspicious of cancer.

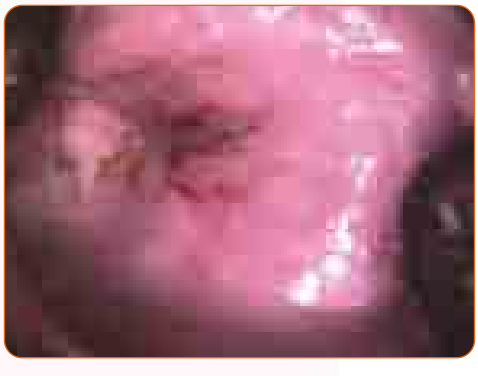
The test should be reported 'positive' when there are distinct, well-defined, dense (opaque, dull or oyster-white) acetowhite areas with regular margins close to or abutting the squamo-columnar junction in the transformation zone. VIA is reported as 'suspicious of invasive cancer' when there is a clinically visible ulcero-proliferative growth on the cervix that bleeds on touch and may or may not turn densely white after acetic acid application. The test is categorized 'negative' when there are no acetowhite lesions observed on the cervix. Nabothian cysts that appear whitish, dot like white areas on columnar epithelium, patchy white, ill defined white areas with indefinite margins, acetowhite areas away from the squamo-columnar junction (satellite lesions) are also categorized as 'negative'. Proper categorization of the negative test is important to improve the specificity of VIA. The test is not meant to identify the nature of the abnormality (grade of CIN etc.). An abnormal test signifies that the woman should be referred for colposcopy just as is recommended for abnormal Pap smear.



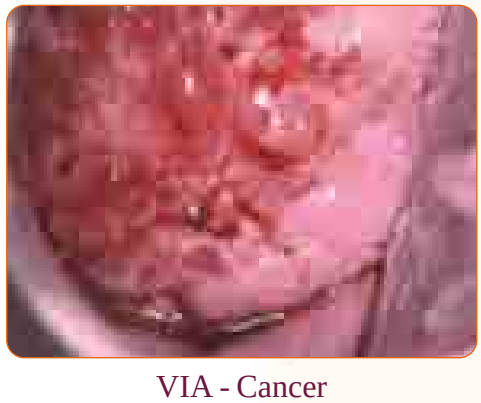
VIA Negative



VIA Negative



VIA Positive



VIA - Cancer

Accuracy of VIA

A range of multi-centric cross-sectional studies conducted by International Agency for Research on Cancer (WHO) in India and Africa in the recent past have proved that VIA performed by trained paramedics has higher test accuracy compared to Pap smear cytology performed in the same settings. The sensitivity of VIA to detect high grade CIN varied from 58% to 94% and the specificity ranged from 75% to 94%.[4] The specificity of VIA has been shown to be increased by adding adjunctive tests like VILI (visual inspection after application of Lugol's Iodine).[6] (Table 1) Addition of magnification (VIAM) does not improve the performance of VIA.

Screening Test	Number of Participants	Sensitivity % (range)	Specificity % (range)
Cytology	22633	**58** (29-77)	**95** (89-99)
HPV	18065	**67** (46-84)	**94** (92-95)
VIA	54981	**77** (58-94)	**86** (75-94)
VIAM	16900	64 (61-71)	87 (83-90)
VILI	49080	**92** (76-97)	**85** (73-91)
VIA(+) or VILI (+)	49080	94	81
VIA(+) and VILI(+)	49080	79	89

Table 1. Test characteristics compiled from IARC multi-centric studies

The comparative efficacy of visual inspection with acetic acid, HPV testing (Hybrid Capture II) and conventional cytology in cervical cancer screening was evaluated in a randomized intervention trial in Maharashtra, India.[7] The study observed that the detection rates of CIN 2 and worse lesions were similar with all the methods. In contrast to the other laboratory based tests, VIA provided immediate result based on which colposcopy and management could be done at the same visit, making the test very cost-effective.

Similarly a cluster randomized trial conducted in Tamil Nadu, India aimed to evaluate the effect of visual screening on cervical cancer incidence and mortality after a single round of VIA screening followed by cryotherapy.[8] Follow up of the entire cohort after 7 years showed a significant 25 percent reduction in cervical cancer incidence (hazard ratio 0·75), a significant 35 percent reduction in cervical cancer mortality (hazard ratio 0·65) and a 27 percent reduction in the incidence of stage II or advanced cancers (Hazard Ratio 0·73) in the intervention group as compared with control group. This study indicates that VIA screening, in the presence of good training & sustained quality assurance, is an effective method to prevent cervical cancer in developing countries.

In terms of cost effectiveness, a computer based model analysis using many of the Indian study data showed that the most cost effective strategy could be screening women once in life time at the age of 35yrs, using VIA or HPV DNA testing (single or two visits).[9] With such a strategy the life time risk of cervical cancer can be reduced by 25-36% at a cost less than $500 per year of life saved.

Conclusion

The main advantages of VIA are that it is a simple, inexpensive, does not require sophisticated equipment, can be done by trained health workers and the results are available immediately making it an attractive approach for use in low-resource settings. The limitations of this test are that this has got moderate specificity resulting in resources being spent on unnecessary treatment of women who are free of precancerous lesions in a single-visit approach. It is a provider-dependent and is less accurate among post-menopausal women and women with endocervical disease.

The management options of positive VIA test are either to refer the woman for confirmatory diagnosis with colposcopy or to treat immediately using conservative approaches like cryotherapy, loop electrosurgical excision procedure (LEEP) as in single visit approach or to refer for further adjunctive test (Pap / HPV DNA screening). Ministry of Health, Government of India has released guidelines for cervical cancer screening programme under the National cancer control programme. The recommendation is to provide VIA based screening at the primary health centers by the trained nurses/health workers and then a single visit approach for colposcopy and management at the district hospital.[10]

Thus, although VIA is a useful alternative to cytology in low-resource, the test positivity and detection rates of lesions have to be carefully monitored to maintain satisfactory performance. Regular training of healthcare providers is an important component. As VIA is an entirely provider-dependent screening method, clear standards for identifying precancerous lesions that should be treated are essential.

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9. Human Papilloma Virus Testing in Screening for Cancer of the Cervix.

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Introduction

India with one sixth of the world's population (over one billion) also has one fourth of the world's burden of cancer of the cervix. It is estimated that nearly 132,000 women are newly diagnosed with cervical cancer and around 75,000 women die each year. It is projected that these figures will double in India by 2020 if no action is taken.[1] Lack of effective programs for early detection and treatment is the main reason for this burden of suffering. India, as most developing countries, lacks the infrastructure and trained personnel needed to replicate the successful cytology-based (Pap) multi-visit approach traditionally used in developed countries for cervical cancer prevention.

It is well established that persistent infection with at least one of the 15 cancer-related, high risk, human papillomavirus (HPV) is a necessary cause for cancer of the cervix; hence the use of tests to detect these high risk HPV types and making it available as a primary screening test for cervical cancer is becoming more important. Over the past decade it has become apparent, that molecular testing for human papillomavirus (HPV) is a highly sensitive primary screening method.[2] In 2004, the International Agency of Research on Cancer (IARC) working group of experts in the field of cervical cancer prevention and control examined the published evidence and declared that there is sufficient evidence to support the use of HPV-DNA testing for primary screening. The working group also observed that an appropriate test is needed for low-resource settings and recommended that any such test should be carefully evaluated in demonstration projects.[3]

High Risk HPV Hybrid Capture 2 DNA Test

Much of the currently available information on the clinical usefulness of HPV testing is based on experience with Digene Hybrid Capture 2® HPV-DNA test (HC2, Qiagen Inc, Gaithersburg, MD, USA formerly Digene Corporation) assay which tests for 13 oncogenic HPV types (16,18,31,33,35,39,45,51,52,56,58,59 & 68) in vaginal or cervical samples.[4] However type specific HPV testing for clinical practice is not currently available commercially but it is expected to be available soon. A new generation of HC2 with some modifications such as the inclusion of HPV 6[6], being completely automated, and the capability for running bigger batches of samples is also in the pipeline. Unfortunately, as it is further explained below, the cost of this technology is a limitation for its introduction nationwide in India and other developing countries.

Sampling & Lab processing: HPV test sample can be either a vaginal or a cervical sample. Higher sensitivities are obtained with a cervical sample collection that involves speculum examination and the specimen is taken using a brush or Dacron® swab. Collection of vaginal samples is done, without a speculum, by the woman herself (self collected) or a trained health care provider. The sample is then transported to the laboratory in a special liquid medium where they are processed in batches of about 90 samples using an automated system taking about seven hours (see Table 1 and Figure 2).

The test findings are expressed as relative light units (RLU) and compared with the mean RLU from a minimum positive control set at 1pg/ml referred to as cut off.

Tables & Figures:

Table I. Comparison of the test specifications of the Hybrid Capture 2 (hc2) and the new HPV DNA tests

Characteristics	QIAGEN's hc2 (existing test)	QIAGEN's new HPV-DNA test (Care HPV test)
Detects	HPV-DNA	HPV-DNA
Number of oncogenic HPV types	13 types	All 13 + type 66
Test format	Batch	Rapid-batch
Laboratory Processing Time	7 hours	Less than 2.5 hours
Setting	Lab Refrigeration needed	Static or mobile clinic No refrigeration needed
Power supply	Mains	Mains or battery
Laboratory bench space	3.5 linear meters	1.5 linear meters
Number of samples	96 well batch	24 or 48 well batch
Target price per specimen	Substantially more than US$5	Less than US$5*
*Pricing for the new HPV-DNA test refers to government and not for-profit organizations		

Test Characteristics: HPV testing is an objective test and identifies both women with precursor lesions and women with a greater risk for cervical disease in the future. Cuzick and colleagues published report on meta analysis of HPV tests in different settings has shown the sensitivity of HPV DNA testing for the detection of moderate or severe cervical intra epithelial neoplasia (CIN) as 96.1% (CI 94.2 -97.4) substantially higher than that of cytology.[2] In addition the sensitivity of HPV test was similar in all studies carried out in different settings compared to the sensitivity of cytology which was variable. However data shows that HPV test is less specific than cytology though the specificity increases in women over the age of 35 years. The major advantage of using HPV-DNA testing for primary screening is its very high negative predictive value. This characteristic gives the clinicians the confidence that a woman with negative result has minimal, almost null, risk for developing cervical cancer within the next 10 years; therefore, the inter-screening period could be extended, making a better use of the very limited resources.

Even though the positive predictive value (PPV) of the test is low when it is seen with a cross sectional point of view; the risk of developing cervical disease in women testing positive for high risk HPV and a normal initial colposcopy is something that need to be considered very carefully. The risk for developing cancer of the cervix is much higher in women with positive HPV test than general population with negative results.

Rapid HPV Testing

Advances in molecular technology and the need for a rapid more affordable HPV-DNA test provided the impetus for PATH's Screening Technologies to Advance Rapid Testing (START) project.[5] A new rapid test has been co-developed by PATH and QIAGEN Inc. (Gaithersburg, MD) and detects the presence of oncogenic types of HPV DNA and is promising for primary screening in low-resource settings. This new rapid HPV test - *Care*HPV test (formerly, Digene's FastHPV test)- is a signal amplification assay and detects 14 high-risk HPV types (16,18,31,33,35,39,45,51,52,56,58,59,66 and 68) i.e. HPV type 66 in addition to the other 13 also detected by HC2. *Care*HPV is run in batches of 18 or 88 samples and it is very suitable for low-resources settings because it does not need electricity or running water (Table 1).

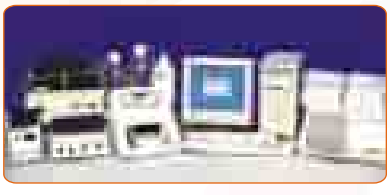

Figure 1: Hybrid Capture 2 (HC2) Equipment

Figure 2: *Care*HPV test equipment (Courtesy of Qiagen, Inc.)

The test runs on two pieces of portable equipment, each about the size of a shoe box, weighing less than 5kg, and using a rechargeable battery for power (Figure 2). The person in charge of running the test does not need to have extensive experience in lab-related work and the training is much simpler than other tests. The basic requirements of the lab are 1.5m of bench space and a ventilated (no air-conditioned) environment.

Collection of cervical and vaginal samples is similar to HC2 and sent to the laboratory using a medium containing non toxic surfactants and is formulated for solubilization of the cervical specimen without the need for extended mechanical shaking thus simplifying the lab processing procedure and time. Refer to Figure 2 for the C*are*HPV and Table1 the comparison of the test specification.

A clinical study in China, enrolling 2500 previously un-screened women, reports the performance of *Care*HPV test as comparable to HC2 (QIAGEN Inc.) and better than VIA.[6] The sensitivity and specificity of the *Care*HPV for CIN 2+ was 90% and 84% respectively (using a cut-off of 0.5) in clinician-collected cervical samples, compared to 97% and 86% for HC2. However, it is more important to compare *Care*HPV to other testing methods already available in low-resource settings.

Addressing Programmatic Needs

An affordable and well-accepted screening test with high sensitivity that can provide results within a few hours can address many of the cervical cancer prevention programmatic challenges in low resource settings. Success of a cervical cancer prevention program is dependent on providing appropriate treatment for women who screen positive. Loss to follow up of women who need treatment is a major challenge and a rapid and highly sensitive test providing results within a couple of hours can significantly reduce lost to follow up and treatment.

Evidence from a study in South Africa, in which women were randomly allocated to screening by visual inspection with acetic acid (VIA) versus HPV-DNA testing, showed that cryotherapy based on results of HPV-DNA testing would be approximately twice as effective in eliminating high-grade cervical neoplasia.[7]

Self sampling: An additional advantage of a screening approach based on HPV-DNA testing is the programmatic flexibility it provides by allowing women to obtain their own vaginal samples. This has the advantage of collecting samples in areas where there is a lack of trained providers for performing speculum examination; thereby having the potential to increasing participation in screening programs. Published studies report that HPV test self collection is both acceptable to women and also feasible in several settings.[8] Qiao et al in their study reported a slightly lowered sensitivity for detecting CIN 2+ compared to cervical sampling (81.4% compared to 90.0% for cervical samples)[6]

Public health program planners in countries without existing cervical cancer prevention programs would benefit from having local evidence on how the various screening and treatment methods compare on feasibility, effectiveness, acceptability, and affordability; given their geographical, cultural, and economic circumstances. These issues will be addressed through demonstration projects to be implemented in two sites in India - Uttar Pradesh and Andhra Pradesh. (Sellors et. al. 2008 submitted for publication in IJMR).[9]

What does the future hold for HPV & screening?

HPV DNA testing opens the possibility for making some changes in the future screening programs by providing more sensitive tests and reducing the frequency of screening visits for women at risk age with significant impact in the incidence and mortality due to cervical cancer.[10] As it was mentioned above, there are several studies showing a consistently low cumulative incidence of CIN 3 for up to 6 to 10 years in women who tested negative for oncogenic HPV, suggesting that the screening interval can be prolonged and still be highly beneficial to women and screening programs.

The advent of HPV vaccines has certainly brought hope to cervical cancer prevention. The currently available vaccines only target the two most common oncogenic types (16 & 18) associated with 70% of cervical cancers. Even in settings where the HPV vaccine is currently widely available, screening and treatment of cervical precancer will be required to provide equitable prevention services for those who are infected with HPV types other than HPV 16 & 18 and for women who have not been vaccinated. Evidence shows that precancerous lesions caused by HPV types other than HPV 16 & 18 are more difficult to discern and likely to be missed by visual methods, hence HPV testing will play a bigger role in detection of cervical lesions.

Conclusion

The spectrum of possibilities for having a comprehensive cervical cancer prevention program has expanded in recent years. Now we have the option for primary prevention using HPV vaccines in combination with a more strengthened secondary prevention using a highly sensitive, affordable and acceptable HPV-DNA test with the potential to significantly reduce the burden of cervical cancer.

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10. Role of Colposcopy to Evaluate Screen Positive Women

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Introduction

The key role of colposcopy in the algorithm of cervical cancer screening is to ensure that unnecessary radical procedures are not applied to a relatively trivial lesion and that inadequate therapy is avoided for lesions that turn out to be more sinister and extensive than originally suspected.

To avoid these pitfalls of over and under diagnoses and management, Colposcopy is the preferred diagnostic method to be applied to all screen positive cases.

History

The first account of Colposcopy was published by Hans Hinselmann, a German scientist in 1925. His original idea was that the earliest cancers of cervix must occur as a minute ulcer or tumor that could be recognized by means of suitable magnification and illumination. He designed an instrument using sharply focused lights and binocular magnification that he called a 'Colposcope'. Thus a new field of clinical investigation was invented called 'Colposcopy'.

Initially colposcopy was widely used in the continent of Europe and in some Latin-American countries and made little impact on the “english speaking part of the world” with the exception of Australia. The delay in its adoption in Great Britain and the United States was probably due to the language difficulties. Hinselmann and his pupils published their work in German and introduced a terminology that was difficult to translate in other languages.

Appearance on scene of exfoliative cytology to diagnose early cervical neoplasias gave a setback to Colposcopy for sometime. For many years colposcopy and cytology were considered as competing methods to detect cervical neoplasm. Gradually it was realized that a combination of the two methods improved diagnostic accuracy and they were complementary to each other.

Instrumentation

The colposcope is used to visualize the lower genital tract with the help of a bright light and magnification. The magnification can be stepwise increased from 6x to 40x. The focal length of the lens system varies from 200 to 300 mm and determines the distance at which the colposcope should be placed from the object (cervix). The head containing the optics is balanced on a central axis

attached to 3-4 wheeled base. Usually a variable intensity light source is attached to the central axis and a fibre-optic cable is used to transmit the light from there to the optics. Green filters are used for better visualization of the vascular patterns. Colposcopes are also available with facilities for transmitting digitized images to TV monitors or to computer screens from where the images can be captured and stored. Such digital video colposcopes have auto-focus facilities.

Indications of colposcopy

1) Abnormal Cytology or other screening tests
2) Abnormal per speculum findings despite normal cytology
3) Persistent symptoms like chronic leucorrhoea, post menopausal bleeding, menorrhagia.
4) Post-treatment follow up of CIN by ablative or excisional techniques

Steps of colposcopy

- History should be reviewed and screening test reports should be checked.
- Patient is to be positioned in lithotomy or dorsal position.
- Cusco`s or Graves self-retaining specula should be used to expose the cervix. Cervix should not be held with volsellum.
- Characteristics of vaginal discharge, if any, should be noted. Cervix should be examined for any obvious abnormality (polyp, growth and ulcer).
- Cytology if required, should be obtained before applying any solutions to the cervix.
- Colposcope should be focused on the cervix. To obtain a panoramic view of cervix lower magnification should be used initially.
- Cervix should be examined after cleaning with normal saline and effort should be made to identify the squamo-columnar junction (SCJ).
- Green filter may be used after application of normal saline.
- 3 - 5 percent acetic acid solution should be applied to cervix liberally with a swab for one minute and the changes should be noted. Identification of SCJ becomes easier after application of acetic acid.
- Cervix is to be painted with Lugol's iodine and examined before completion of the procedure.

Principles of Colposcopy

Transformation zone

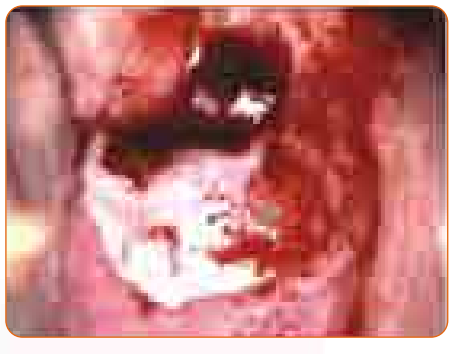
Due to the effect of estrogen the columnar epithelium, normally confined to endocervix, comes out on the ectocervix giving rise to a condition called ectropion or ectopy. Subsequently this columnar epithelium is replaced by newly formed squamous epithelium called metaplastic epithelium. The area on the ectocervix where all these changes take place is called transformation zone (TZ). The inner margin of the TZ is squamo-columnar junction and the outer margin is the junction between the new squamous epithelium and the original squamous epithelium. It is essential to evaluate the TZ meticulously during colposcopy as neoplastic changes invariably start in this area.

Role of acetic acid

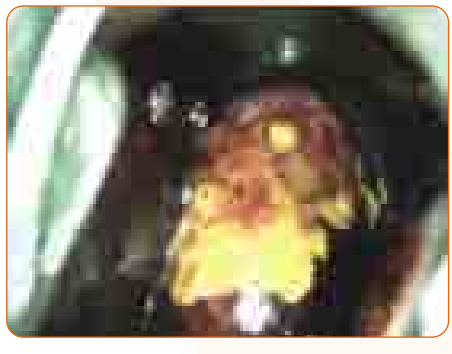
- Acetic acid helps to coagulate mucus that can then be easily removed. Areas of columnar epithelium stand out as typical grape like structures that become white for a transient period due to the blanching of the underlying blood vessels.
- Acetic acid is absorbed in the superficial layers of the squamous epithelium and coagulates protein in nuclei and cytoplasm of cells. Normal mature squamous epithelial cells have very little protein either in the nuclei or in the cytoplasm (replaced by glycogen) and remains transparent after the application of acetic acid. In contrast, neoplastic epithelium has much more protein (greater number of cells, increased chromatin in nuclei, intact cytoplasm) that is coagulated and precipitated by acetic acid. This precipitated protein gives rise to the opaque acetowhite patch and the density of aceto-whitening depends on the grade of neoplasia.

Observing the density of aceto-whitening, margin of the acetowhite area, relationship of the patch with squamo-columnar junction and the rapidity of onset of the acetowhite change can help differentiate the aceto-whitening due to non-neoplastic conditions (metaplasia, inflammation, condyloma) from that due to cervical intra-epithelial neoplasias (CIN) or invasive cancers. The CIN lesions are usually opaque acetowhite patches with well-defined smooth margins and arising from squamo-columnar junction. The density of acetowhite area depends on the grade of CIN. The denser is the patch, the higher is the grade of disease.

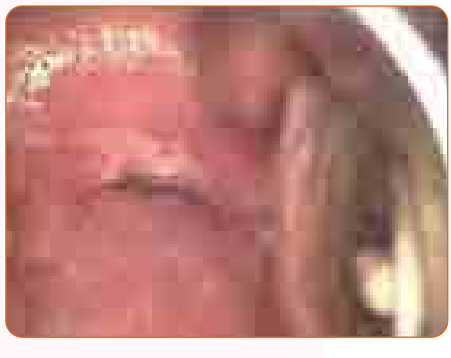
Vascular patterns of **punctation** and **mosaic** indicate adaptive vascular hypertrophy of connective tissue vessels which run perpendicular and parallel to basement membrane respectively. Atypical vessels occur due to neo-vascularisation in high grade neoplasias and are seen as coarse punctations, coarse mosaics and irregular vessels. They are the hallmark of high grade CIN or in invasive lesions.



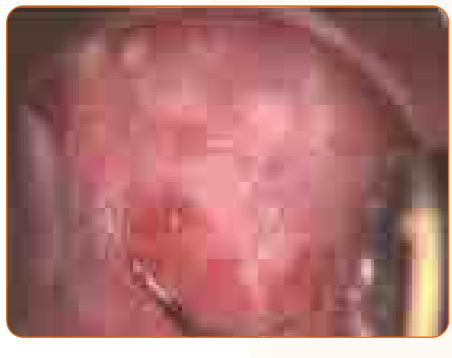
Large acetowhite area on posterior lip of cervix



Lugol's Iodine Negative area



Squamo-columnar junction fully visible with features of metaplasia



Nabothian cysts and metaplastic changes in anterior lip of cervix

Reverse mosaic is a sign of immature squamous metaplasia red islands represent loops of former grape like papillae each are surrounded by white metaplastic squamous epithelium which has filled the clefts and folds of columnar epithelium.

A white patch seen before the application of acetic acid is called leukoplakia or keratosis. This white patch is due to accumulation of keratin in the epithelial cells and shows hyper-keratosis or para-keratosis on histology. Such white patches usually have a raised margin and may become more white after acetic acid application. Rarely a leukoplakia may have an underlying high grade CIN. So any leukoplakia seen over the transformation zone should be biopsied.

Role of Lugol's Iodine

Lugol's iodine stains the glycogen in the cells. The mature squamous epithelium containing glycogen becomes mahogany brown after application of Lugol's iodine and the neoplastic areas without glycogen remain iodine-negative. Iodine non-uptake can be observed in metaplasia, inflammation, condyloma and post-menopausal cervix.

Colposcopic Terminology

Classification of Colposcopic findings

A. Normal Colposcopical findings
    a. Original Squamous Epithelium normal
    b. Columnar Epithelium normal
    c. Transformation zone normal
B. Abnormal Colposcopic findings
    a. Atypical transformation zone
        1. Keratosis
        2. White epithelium
        3. Mosaic
        4. Punctation
        5. Atypical Vascular Pattern
    b. Suspect occult invasion
    c. Unsatisfactory (indecisive) colposcopical findings - Squamo-columnnar junction not visible in totality
    d. Miscellaneous Colposcopical findings
        1. Inflammatory changes, atrophic changes, true erosion, condylomata, papilloma etc

Based on the abnormal findings it is often possible with experience to predict underlying histological picture.

Methods of Recording Colposcopic Findings

1. Diagrammatic representation: This type of recording saves time and it is useful for follow up and also to study sites of Biopsies
2. Description of the lesion
3. Colpo-photography: Colpo-photography offers an excellent and objective method of recording appearances and can be of great value in observation of changes which take place over a period of time (for follow up)

Grading of Colposcopic findings (Coppelson and Reid's Classification)

Grade I

Atypical colposcopic appearance of minor significance compatible with an overlapping histologic series ranging from normal metaplastic epithelium to minor dysplasia

Grade II

Atypical Colposcopic appearance compatible with major dysplasia or carcinoma in situ (CIS)

Grade III

Atypical Colposcopic appearance of major significance compatible with CIS or invasive cancer

With the introduction of Bethesda Classification for reporting cytology, Colposcopic terminology has also been revised as follows:-

- Colposcopic findings suggestive of LSIL include lesions with indistinct borders, fine punctations, small size mosaics, smooth surface with absent or few atypical vessels.
- Colposcopic findings suggestive of HSIL include lesions with distinct borders, coarse punctations, large mosaics, atypical vessels with surface contour variations.

Advantages of Colposcopy

- Precise and accurate diagnosis of nature and extent of neoplstic lesion of cervix may be made with the help of colposcope in patients with suspicious looking cervix and abnormal cytology
- Guided biopsy can be obtained from abnormal area and definitive therapy may be instituted with an assurance of accurate diagnosis
- Useful adjunct technique in follow up of cervical lesions
- Useful for diagnosis and follow up of vaginal and vulval lesions
- Therapeutic procedures like cryotherapy, Laser vaporization or laser conization and Large Loop Excision of TZ (LLETZ) can be carried out with precision.

Limitations of Colposcopy

- Endocervical canal cannot be visualised. Lesions within the endocervical canal, specially adenocarcinoma-in-situ or adenocarcinoma can be missed
- Colposcope gives only surface representation of lesion
- Low grade abnormalities are sometimes indistinguishable from metaplasias
- Increased vascularity with contact bleeding of acute cervicitis tends to mask the true nature of underlying epethelium
- Sensitivity of colposcopy in the detection of CIN is around 80 percent

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11. Treatment of Cervical Precancer

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Principles of Treatment of Cervical Intraepithelial Neoplasia (CIN)

The management of cervical intraepithelial neoplasia is based on the understanding of the natural history of the disease. CIN 1 is mostly detected in younger women and is often transient. Only 10-15 percent of CIN 1 will progress to higher grade and the possibility of developing invasive disease is very low. Such lesions need not be treated and the patient should be kept under follow up. High grade lesions (CIN 2 and CIN 3) are true cancer precursors as they have very high possibilities of progressing to invasive cancer. Conservative management involves either destroying the abnormal epithelium (ablation) or removal of the diseased part (excision). The entire affected area of the cervix including the extension into the crypts (average depth 5mm) should be treated leaving a healthy margin of about 2 mm. CIN II and CIN III being true cervical cancer precursors should always be treated. Most of the CIN I lesions are transient. Only 10-15% will progress to higher grades. CIN I should be treated if follow up can not be ensured (as in most low-resource settings) or the lesion persists for 2 years or worsens in grade or size.[1] Treatment of CIN (any grade) is deferred if the patient is pregnant or is having acute pelvic inflammatory disease.

Ablative techniques

Ablative therapy can be done by cryotherapy, carbon dioxide laser or cold coagulation. Adequate punch biopsies should be obtained from the abnormal area prior to ablative therapy. Any grade of CIN can be treated by these techniques. Suitability of a particular lesion for ablative therapy is decided based on the following criteria.

- The entire lesion is visible and does not extend beyond 2-3 mm in the endocervix.
- There is no suggestion of micro-invasive or invasive disease.
- There is no suspicion of glandular disease on cytology or histology

Cryotherapy is simplest, safest and most widely practiced among the ablative procedures. We will limit our discussion of ablative techniques to cryotherapy only.

Cryotherapy

The cryotherapy unit consists of a compressed gas cylinder (nitrous oxide or carbon dioxide), cryo-gun with circular metal probes, pressure gauge and gas transmitting tube. Carbon dioxide or nitrous oxide gas freezes when released to the atmospheric pressure. An ice-ball is formed over the area of contact that brings down the temperature of the tissue to 20^{o} C. This results in crystallization of the intracellular water. The spear-like crystals of water pierce the cell membrane and cause irreversible damage and cell death (cryo-necrosis). The cell damage is accentuated by associated protein coagulation.

The eligibility criteria for cryotherapy are same as for any ablative treatment. The lesions that can not be adequately covered by the largest available cryo-probe and lesions that occupy more than 75% of the transformation zone are not suitable for cryotherapy.

No anesthesia is required for cryotherapy. The lesion is re-evaluated by colposcopy and a probe with appropriate diameter is selected to cover the lesion completely. The cryo-gun along with tube is attached to the gas cylinder. The pressure of the gas cylinder should be between 40 to 70 kg/cm^2 when the gas is flowing. Cryo-probe surface is wiped with saline to ensure adequate thermal contact. The probe-tip is then firmly applied to the cervix with the center of the tip on the external os. The vaginal walls should not come in contact with the probe-tip. As the gas starts flowing, ice-ball is seen forming at the tip of the probe. Freezing should be done in two cycles of three minutes with five minutes of thawing in between. When adequate freezing has been achieved the margin of the ice-ball extends 4-5 mm beyond the outer edge of the cryo-tip. Some authors observed that single cycle of 3 minutes of freezing is as effective as double cycles of freezing.[2] However, a randomized controlled trial by Schantz et al demonstrated that the double-freeze technique had a lower probability of residual disease after treatment of CIN I and CIN II (Odds Ratio: 2.93; 95% CI: 2.93-8.60).[3]

Cryotherapy is quite safe with no significant operative morbidity. During the procedure some patients may feel a little discomfort or cramp in the lower abdomen. Less than 5% of women experience significant pain. Most of the women will have a watery discharge per vagina for 2 to 3 weeks after the procedure. Excessive bleeding requiring hospital admission or blood transfusion is extremely rare. A small number of patients may develop infection, requiring antibiotics. Cervical stenosis is an infrequent late sequel of cryotherapy that does not have any adverse impact on fertility or obstetric outcome.[4]

A number of randomized controlled trials observed the cure rates following cryotherapy to be 86.0-94.6% for all grades of CIN, 90.9-100.0% for CIN I, 75.0-95.9% for CIN II and 71.0-91.7% for CIN III lesions.[4] A meta-analysis of such trials comparing laser ablation and cryotherapy failed to demonstrate any significant difference in the frequency of residual disease (Odds Ratio: 0.96; 95% CI: 0.67-1.36)5 Nuovo et al concluded in their meta-analysis that there was no statistically significant difference in cure rates among cryotherapy, laser ablation, LEEP and cone biopsy at a median follow up of 12 months.[6]

Large Loop Excision of the Transformation Zone (LLETZ or LEEP):

A wire loop electrode powered by an electro-surgical unit is used to remove the entire transformation zone along with the lesion. The heat from a high voltage electrical arc between the operating electrode and tissue allows the operator to cut by vaporizing the tissue. The excision of transformation zone treats the abnormality effectively and provides a specimen for detailed histological evaluation. The width of the loops ranges from 10 to 20 mm and the depth (the height from the cross bar to the farthest point of the wire arc) ranges from 8 to 15 mm. The appropriate size of the loop is chosen to achieve adequate depth and width of cut depending on the size and position of the lesion. All CIN lesions including the glandular abnormalities can be treated by LLETZ.

Most of the cases can be done under local anesthesia. General anesthesia is required if the lesion is very extensive or if the patient is unlikely to cooperate. Before the procedure colposcopic assessment is repeated and Lugol's iodine is applied to delineate the margin of the lesion. Local anaesthetic agent (5-10ml of 1% xylocaine with adrenaline) is injected into the stroma of the ectocervix (just beneath the epithelium) in a ring pattern at the periphery of the lesion. A smoke evacuation system or an ordinary suction machine is required to remove the smoke generated during the procedure.

The power setting of the electrosurgical unit depends on the size of the electrode being used. The most commonly used power setting is a blend of 50 watts of coagulation and 50 watts of cutting currents.

An appropriate sized loop is selected depending on the size and endo-cervical extent of the lesion so that the entire abnormality can be removed in a single pass. If the diameter of a lesion exceeds the width of the largest loop, it has to be removed with multiple passes. The loop is introduced into the tissue 5mm outside the outer margin of the lesion. The loop is directed gradually into the cervix until the cross bar nearly comes in contact with the epithelial surface. Then the loop is guided along parallel to the surface till the opposite outer margin of the lesion is reached and is gradually withdrawn. The operator should simply provide directional guidance and allow the loop to cut its own way without pushing it. Once the specimen has been removed and placed in formalin, the defect is carefully fulgurated using a ball electrode.

Excessive bleeding may infrequently occur during or immediately after surgery. Usually such bleeding can be controlled by diathermy fulguration or by applying Monsel's paste. Rarely placement of lateral sutures is required. The randomized trials by Alvarez, Gunasekera and Mitchell did not observe any significant difference in the frequencies of primary or secondary hemorrhage following LLETZ and laser ablation.[7,9]

The chance of post operative infection can be reduced by delaying treatment till complete resolution of any existing PID, cervicitis, trichomoniasis or bacterial vaginosis. Cervical stenosis is a long term sequel of the procedure seen in less than 2% of the cases.

Up to 20% of the post-LLETZ biopsy specimens may have disease at the margin. Murdoch et al in a follow up study of such incompletely excised lesions did not observe higher failure rates compared to the completely excised ones.[10] They concluded that such cases with positive margins can be followed up without resorting to repeat treatment. The chance of recurrence is higher if the endocervical margin has residual disease. The failure rate of LLETZ varies between 4-10% in various studies.[10] The randomized trials that compared residual disease at follow up did not observe any significant difference between LLETZ, laser conization or knife conization.[5]

Cold Knife Cone Biopsy:

Currently cold knife conization is reserved only for the treatment of micro-invasive cancer where evaluation of the margin is of prime importance. Excising the abnormality with a knife avoids the thermal artifact of diathermy excision and allows proper pathological evaluation.

The procedure has to be performed under regional or general anesthesia. The cervix is exposed by a Sim's speculum after putting the patient in the lithotomy position. The extent of lesion is confirmed by colposcopy. To reduce bleeding, the two descending branches of cervical arteries are occluded by lateral stitches. With a pair of tissue forceps the cervix is grasped peripheral to the lesion and drawn down, the ectocervical incision is made with a pointed knife completely circumscribing the transformation zone and the lesion. It is often easier to begin the incision posteriorly and then carry it on anteriorly. Manipulating the cone with a tissue forceps, a vertical incision is made into the cervix, angled towards the upper part of the endocervical canal so as to remove a conical block of tissue. A depth of cut between 15 and 20 mm will clear most endocervical lesions. Local haemostasis can be achieved by cauterizing the base or by applying a few stitches at the margin.

The most common complications of knife conization are per-operative and post-operative hemorrhage. Strumdorf sutures are no longer routinely used as there is no evidence that it reduces the risk of primary or secondary hemorrhage. Such sutures increase the risks of dysmenorrhoea and inadequate colposcopy at follow up.[5]

Hysterectomy:

Hysterectomy should be reserved only for a select few cases of CIN. The indications of hysterectomy are as follows:

- Associated gynecological conditions requiring removal of uterus
- Persistent abnormal smear following excision or ablative treatment
- Positive endocervical margin after conization (LLETZ or Knife)

Management of cervical intra-epithelial neoplasia in pregnancy:

The occurrence of cervical precancer in pregnancy is relatively rare. Figures from larger centers in UK suggest a prevalence of one case of CIN III per 750 pregnancies.[11] During pregnancy, it is often a practice to rely on colposcopy diagnosis alone, without biopsy confirmation unless an invasive lesion is suspected. If the colposcopic features are suggestive of low-grade disease only, a repeat assessment is advised at 12 weeks post partum, by which time the cervix is completely involuted. If a high grade lesion is suspected on colposcopy, the woman is recalled 6 weeks after delivery for repeat colposcopy and biopsy. If there is suspicion of invasion at any point of time, a colpscopically directed punch biopsy should be obtained. Treatment of CIN should be deferred till 12 weeks after child birth.

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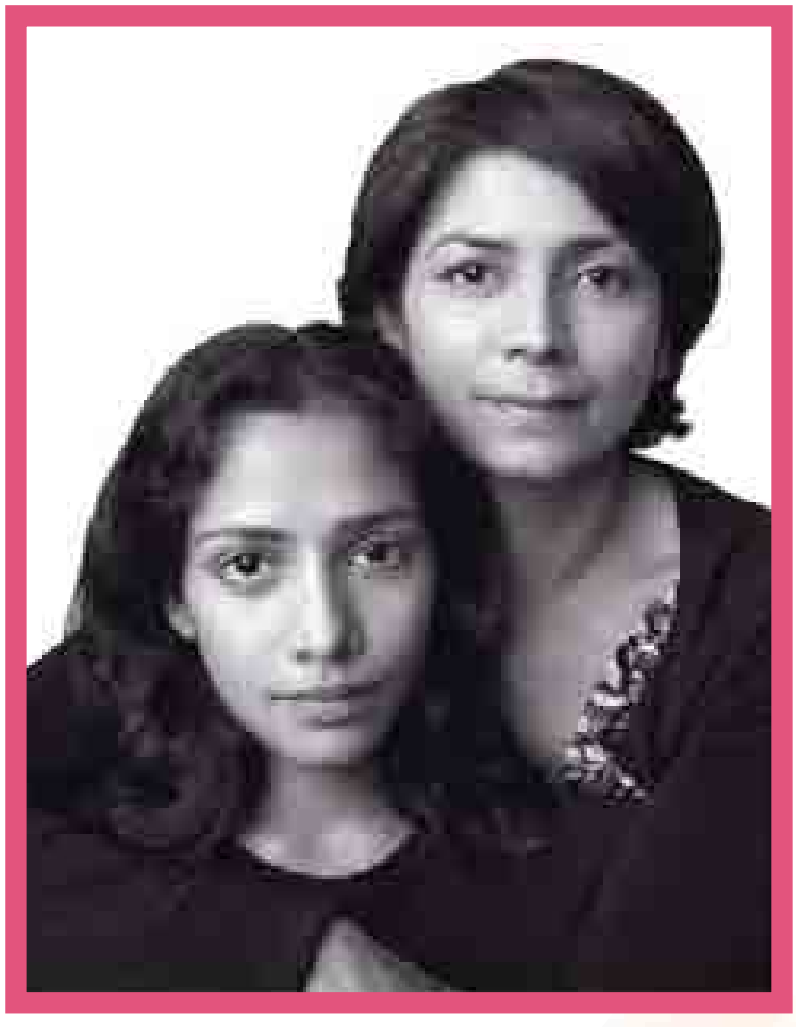

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