Annexure XX

Disease Specific Documents for XII Plan

# Human Papilloma Virus (HPV)

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# Human Papillomavirus Virus

## ICMR institutes working on Human Papilloma Virus

1. Institute of Cytology and Preventive Oncology, NOIDA (Lead Institute)
2. National AIDS Research Institute (Pune)
3. National Institute for Research in Reproductive Health (Mumbai)

Cancer of the uterine cervix is the second most common cancer among women worldwide. According to global cancer statistics, in 2002 there were approximately 493,000 new cases and 274,000 deaths, with more than 80% of them in developing countries. In these regions, cervical cancer generally affects women in the prime of their lives with multiple school-age children, and their deaths have a major negative impact on the societal life of the community. In India, cervical cancer is a leading cancer among women, with an annual incidence of approximately 132,000 and mortality rates of approximately 74,000. As India contributes approximately 17% of the world's population, it disproportionately shares more than a quarter of the global cervical cancer burden. Ironically, despite a cancer control program being an integral component of the national health policy of India, there have been meagre improvements on cancer prevention programs such as screening and early detection. The emphasis is primarily on the establishment of basic cancer treatment facilities, which are expensive and highly inadequate for the large population. Unlike other cancers, cervical cancer is potentially preventable as well as curable if detected early by effective screening, but the disease is far from under control owing to the lack of organized screening programs, suitable health infrastructure and trained manpower.

It is now well established that specific types of oncogenic human papillomavirus (HPVs) are the major etiological agents associated with the development of cervical cancer. In addition, various epidemiological risk factors such as early age of marriage, multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking, malnutrition, use of oral contraceptives and lack of awareness, including specific religious practices, may modify the risk of developing cervical cancer in women following HPV infection. Epidemiological estimates suggest that the worldwide prevalence of HPV infection is between 9 and 13%, which equates to approximately 630 million infected women. Although much less studied, a recent report estimates that the prevalence of HPV infection in men is similar to that of women. This further increases the prevalence, making HPV the most common sexually transmitted infection, with no specific treatment.

The major reason for high mortality from cervical cancer in India is late stage of diagnosis owing to the lack of a cervical cancer screening program. Moreover, most genital HPV infections are asymptomatic and unapparent. In the past three and a half decades, an enormous amount of studies indicate that nearly all cervical cancer cases are caused by genital infection with specific high-risk HPV types. The association of viral infection provides a unique opportunity to detect HPV infection and associated cellular changes using molecular techniques, such as low- or high-grade lesions at an early stage of the disease. With the cytology-based simple Papanicolaou smear test (Pap-smear test) or visual inspection with acetic acid (VIA), as well as the recent development of effective HPV detection procedures coupled with early and regular screening programs for disease precursors, cervical cancer can be prevented and successfully treated at an early stage. Apart from screening, which is

relatively cost effective, primary prevention by vaccination against HPV is also gaining ground. Currently, two prophylactic HPV vaccines, Gardasil® (Merck, USA) and Cervarix® (GlaxoSmithKline, Belgium), have been approved by several countries, including India, for clinical use. However, because of the high cost of these vaccines, the benefits are not reaching the people who need it the most.

Organized population-based screening using Pap-smear cytology for cervical precancer and cancer in developed countries such as the USA had a vast impact, reducing the incidence and mortality of cervical cancer by 70%. In developing countries such as India where cervical cancer is a major public health problem in women, no such organized screening program is implemented at a national level. This is partly due to the large population, high cost, lack of trained manpower and inadequate infrastructure, but mainly due to lack of proper planning, awareness and political interest to develop an organized screening program. Moreover, the Pap-smear procedure itself has several limitations, such as high false-negative rates, low sensitivity (only 45%, vs 96% for HPV DNA testing), poor quality control, subjective interpretation, inability to identify latent HPV infection and low predictive value because one third of women who progressed to cervical cancer had a normal Pap-smear. In addition to this, demands for competing health needs, particularly for other infectious diseases such as HIV/AIDS, TB and hepatitis, offer additional hurdles to establish cervical cancer screening as a priority program in India.

Despite all these limitations, there has been a renewed public health initiative under pressing demands from Indian as well as global health authorities. Taking the importance of HPV into consideration, an effective national screening program should be developed for successful control and management of cervical cancer. Here we, review the current status of HPV research in India and the possibility of its integration in population screening and cervical cancer control programs.

The Papillomaviridae family represents a heterogeneous group of viruses with a remarkable diversity of different genotypes. These viruses are epitheliotropic in nature; infect mucosal and cutaneous epithelial tissues, and preferentially localize to the anogenital tract. To date, more than 110 human and animal papillomavirus genotypes have been characterized and sequenced. Of the approximately 30 HPVs that infect the anogenital tract, 15 HPV types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) have been found to be associated with high grade lesions and invasive cervical cancer and hence are classified as 'high-risk' types. Molecular and clinico epidemiological studies have demonstrated that HPV types 16 and 18 are the two most common highly oncogenic HPV types found in invasive cervical cancer and high-grade cervical intraepithelial neoplastic (CIN) lesions. Prevalence of other high-risk types is very low and rarely high in specific endemic populations, or they appear as co infections to HPV16 and 18. On the other hand, 11 different HPV types that have been mainly associated with genital warts and benign cervical lesions such as condylomata accuminata and dysplasias are termed as 'low-risk' HPV types. These include HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 81 and CP6108. Among these, HPV6 and HPV11 cause approximately 90% of genital warts.

The rates of HPV infection are reported to be very high in sexually active women younger than 25 years of age. In young HPV-negative women, the cumulative incidence for a first HPV infection has been estimated as 32% at 24 months and 43% at 36 months. Globally, 50–80% of sexually active women are infected by HPV at least once during their lifetime. Although the peak of HPV prevalence is reached in the first half

of the third decade (18–25 years) of life, cervical cancer is found much later, usually after 40 years, with a flatter peak of incidence around age 45 years. Interestingly, unlike in the West, HPV infection is most common in Indian women a decade later, at around 26–35 years of age, but a very low frequency (~3%) of HPV infection occurs during adolescence as the majority are not sexually active at this age (9–13 years of age) in India and cancer arises much later, with a peak at approximately 45–55 years of age. Therefore, there is a long gap between infection and invasive cancer, and the phenomenon provides a suitable window period for cancer control.

Natural history of HPV infection demonstrates spontaneous clearance of HPV infection in approximately 90% of cases, and in approximately 10% of cases viral infections, particularly the high-risk types, persist and can lead to development of low-grade CIN lesions. Therefore, persistent infection of HPV for 1 year or more is a prerequisite for the development of CIN lesions and cervical cancer. Studies indicate that infections with high-risk types usually take longer for spontaneous clearance and pose a greater risk for the development of cervical lesions, which can progress to invasive cancer if not treated. Apart from genetic predisposition and chronic localized inflammatory co infections, viral persistence is facilitated by the integration of viral DNA into the host cell genome, which makes it almost impossible for the precancer lesions to revert back to normalcy.

Although HPV is a necessary factor for the causation of cervical cancer, it appears to be insufficient. Various other factors including host cell factors are necessary for progression of HPV-infected precursor lesions to cancer. Long-term use of hormonal contraceptives, multiple pregnancies and high parity lead to folate deficiency and immunosuppression, contributing to cancer causation. Tobacco smoking, co infection with HIV, *Chlamydia trachomatis* or herpes simplex virus type 2 (HSV-2), and certain dietary deficiencies have also been identified as important cofactors. The risk of contracting HPV infection is influenced primarily by sexual activity, in particular the sexual behavior of the partner(s).

Cervical cancer is the principal cause of cancer-related mortality and is a major reproductive health problem in Indian women. Despite the successful development of vaccines against its causative agent (human papillomavirus [HPV]), which could be used as primary prevention tool, the highly beneficial effects of cervical screening by visual inspection with acetic acid, cytology and recently HPV DNA detection, and advances in technologies for detection of incipient lesions and HPV infection, the problem of cervical cancer is still prevalent in the country.

## HPV work undertaken at ICPO

1. Prevalence of HPV in cancer, especially in Cervical Cancer.
2. Cytological screening for HPV lesions
3. Development of HPV detection system in various biological materials.
4. Interaction of host gene with HPV genes and understanding carcinogenesis process.
5. Vaccine development against HPV.

1. Prevalence of HPV in cancer, especially in Cervical Cancer

ICPO started working on HPV way back in 1986. During this time cheaper and easier methods were being developed for population screening. At ICPO we used following techniques before PCR technique became preferred technique:-

- Southern Blot Hybridization using full length HPV DNA probe labelled with $P^{32}$.
- Insitu Hybridization to localize HPV infection in chromosomes, using H3 labelled full length HPV DNA probes.
- Filter In situ Hybridization using cells blotted onto nitrocellulose disc membranes.
- Dot/Slot blots Hybridization. (Radioactive and Non radioactive)
- Polymerase Chain Reaction.
- Realtime PCR
- Using commercial Kits like Hybrid capture II.

**I. HPV Screening:**

- Human Papillomavirus (HPV) Infection among Young Adolescents in India: Impact on Vaccination.
- We showed that urine HPV can be detected from urine samples of young adolescence in and also prevalence of HPV is found to be less among school children in comparision to other countries. **J Med.Virol, 84: 298-305, 2012.**

**II. Host Genetic Factors:**

- **1. Association of p53 and MDM2 gene polymorphism with the advancement of cervical cancer.**
- We showed that Arginine at codon72 of p53 and GG genotype at 309 in second promoter of *MDM2* together exhibit a direct proportionality with increasing grade of cervical cancer along with HPV infection in postmenopausal women. **DNA & Cell Biology, 2013.**
- **2.** Association of p16 (CDKN2A) and RB1 Polymorphisms with Susceptibility to Cervical Cancer in Indian Population.
- We showed that p16 (540C/580T) has emerged as a major risk haplotype whereas p16 (540G/ 580T) as a chief protective haplotype for the development of cervical cancer among Indian women. **Mol.Biol.Report, 2011. DOI 10.1007/s 11033-011-0752-z.**
- **3.** Novel Missense Mutation in *FHIT* Gene: Interpreting the Effect in HPV Mediated Cervical Cancer in Indian Women.

- We showed a novel missense mutation in *FHIT* gene at nucleotide position 655, at codon 98, His to Arg substitution in substrate-binding domain may generate catalytically inactive protein with loss of tumor suppressor activity in HPV associated cervical cancer. **Mol Cell. Biochem** 335: 53-58, 2010.
- **4.** Homocysteine levels are associated with Cervical Cancer independent of Methylene Tetrahydrofolate Reductase gene (MTHFR) polymorphisms in Indian population.
- We showed that homocysteine and consequently cysteine levels may be associated with HPV associated cervical cancer but no association was found between SNPs in MTHFR and risk of development of cervical cancer. **Biomarkers** 15(1): 61-68, 2010.
- **5.** Association between HLA Class II alleles with HPV mediated Cervical Cancer in Indian women.
- We showed that HLA DR-DQ polymorphisms/haplotype may be associated with cervical cancer or HPV infection in North Indians. **Human Immunology, 70, 222-229, 2009.**
- **6.** Genetic Variant of *CCND1*: Association with HPV Mediated Cervical Cancer in Indian Population.
- We showed that G870A of CyclinD1 may play as risk factor where G1722C variant may play protective role in HPV associated cervical cancer in Indian. **Biomarker**, 14 (4): 219-225, 2009.
- **7.** Effect of aberrant promoter methylation of FHIT and RASSF1A genes on susceptibility to cervical cancer in a North Indian population.
- We showed that differential promoter methylation of FHIT & RASSF1A genes correlate well when comparing various clinical stages & histological grading of cervical carcinoma in Indians. **Biomarker**, 13 (6): 597-606, 2008.
- **8.** TNFα –308G/A polymorphism as a risk factor for HPV associated Cervical Cancer in Indian population.
- We showed TNFα promoter polymorphism –308G/A may confer risk to HPV infection & subsequent development of cervical cancer in Indians. **Cellular Oncology**, 29 (3): 249-256, 2007.
- **9.** Allelic Variations in CYP2D6 Gene and Susceptibility to Cervical Cancer.
- We showed CIN I/II smokers with CYP2D6 EM genotype are susceptible to wards progression of CIN III. **Drug Metabolism Letters, 1 (4): 276-280, 2007.**

**III. Vaccine programme**

**1. Development of DNA based India specific HPV16 Vaccine:**

A total of 16 major variations were observed among which 13 variations were biallelic and one was trialleic. Out of these major variations, 4 variations causes change in the predicted epitopes in comparison of variant protein sequence to the reference sequence; which were in the vicinity of the major variation were noted. We predicted five new epitiope in our variant sequence and some get vanished in our variants sequence. On the other hand majority of the variations caused the increase or decrease in the binding affinity of the epitope as their IC50 values increased or decreased. Out of these 4 major variations, two of them V3 and V8 may cause the reduction of immunogenicity as they are present in close vicinity of epitope and ultimately cause disappearance of predicted binder peptide for MHC alleles. Therefore, these variations may be important for the carcinogenic potential of the HPV 16 and development of the vaccines which we are in the process of validation in experimental animal model.

2. Study on different micro RNA (miRNA) in HPV associated Cervical cancer:

miRNA profiling of different grades of cervical cancer tissue biopsies is ongoing in bead array system in collaboration to IGIB. We found several miRNAs are down regulated and only few are up regulated. Now we are in the process of validation of these miRNAs.

## HPV work undertaken at NARI

1. Estimation of prevalence of Pap smear abnormalities and human papillomavirus infection among HIV-infected women (2003 – 2004).

   We reported a high prevalence of Pap smear abnormalities and HPV infection with types 16 and 18 among HIV-infected women. **Journal of Medical Virology 2005, 76 (4), 470–475.**

2. Prevalence estimates and evaluation of risk factors of CIN among HIV-infected women in Pune, India using colposcopy and histopathology as diagnostic tools (2006 – 2008).

   We reported a high prevalence of CIN 2+ lesions in HIV-positive women and the presence of cervical high-risk HPV-DNA and women currently receiving ART as independent predictors of increasing severity of CIN. **PLoS One. 2010; 5(1): e8634.**

3. Comparison of visual inspection with acetic acid and cervical cytology to detect high-grade cervical neoplasia among HIV-infected women in India (2007 – 2010).

   Overall, VIA performed better than cytology in this study with biologically rigorous endpoints, suggesting that VIA as a practical and useful alternative or adjunctive screening test for HIV-infected women. **Int J Cancer. 2011; 130 (1), 234-40.**

4. HPV Genotype Distribution in Cervical Intraepithelial Neoplasia among HIV-Infected Women (2008-2010).

- We found that HPV genotypes were present in 52.5% and 'carcinogenic' HPV genotypes were present in 35.3% in HIV-infected women. Increased presence of HPV genotypes other than 16/18 like 56, 35, 31, 33, 52 were reported. **PLoS ONE 2012, 7(6): e38731.**
- We reported the HPV incidence rate and clearance rate as 11.1 and 18.3 per 100 person-years. HPV 52 was the most incident genotype, while HPV 16 showed highest clearance. **BMC Infectious Diseases 2012, doi: 10.1186/1471-2334-12-S1-O2.**

## Currently the studies ongoing include:

1. Determination of intratype genomic variants of HPV amongst HIV-positive women (2011-2013).
2. Anal HPV infection in HIV-positive women (2012-2014).
3. Improving Cervical Cancer Prevention among HIV-Infected Women Using Novel HPV Based Biomarker Assays like (i) immunocytostaining by $p16^{INK4a}$/Ki-67 (biomarkers correlated with the oncogenic transformation of cervical cells following persistent carcinogenic HPV infection) and (ii) testing for HPV E6/E7 mRNA (expressed during progression of a transient to a transforming HPV infection) (2012-2014).
4. ACTG 5282: A Randomized, Phase II Trial to Compare an HPV Test-and-Treat Strategy to a Cytology-based Strategy for Prevention of CIN 2+ in HIV-infected Women (2012-2016).

## Future studies planned:

1. HPV infection in MSM population
2. Comparison of genomic variants in HIV-positive and HIV-negative women in different cervical disease stages.
3. Studies on novel biomarkers and microRNA profiles in different cervical disease states.

## Facilities available at NARI for HPV testing

1. Abbott HP HPV
2. Roche Linear Array
3. Sequencing