Review Article

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Infection of human papillomaviruses in cancers of different human organ sites

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Clinico-epidemiological and molecular studies have established the casual link between Human Papillomavirus (HPV) infection and cervical cancer as also association of HPV infection with several other cancers. In India, cervical cancer is a leading cancer among women and almost all cases of cervical cancer show prevalence of High Risk (HR)-HPV infection. HPV has been also detected in a significant proportion of oral, esophageal, anal, vaginal, vulvar, and penile cancer and in a small percentage of lung, laryngeal, and stomach cancer in India. Due to lack of organized HPV screening program, insufficient infrastructure and trained manpower and inadequacy in cancer registries, there are not much data available on the countrywide HPV prevalence and its type distribution in different cancers in India. Forthcoming introduction of recently developed HPV vaccines in India given a new urgency to know the prevalence and distribution of various HPV types in different organ sites for the management and monitoring of vaccination program and its impact on prevalence of other cancers. This review, summarizes studies on the prevalence of HPV infection in cancers of different organ sites in India.

Key words Anogenital cancers - cervical cancer - head and neck cancers - human papillomavirus - India - international standards - organ sites - WHO HPV LabNet

Introduction

Human papillomaviruses (HPV) are small, double stranded DNA viruses that belong to family papillomaviridae[1]. Papillomaviruses were first identified, cloned and sequenced from cervical tumor specimens and were subsequently established as important causative agents for development of cervical cancer, the discovery which was honored by conferring Nobel Prize of Physiology and Medicine for the year 2008 to its inventor Harald zurHausen[2-6]. Subsequent research demonstrated infection of papillomaviruses in cutaneous and mucosal tissues of the oral cavity, upper gastrointestinal tract, anogenital tract and skin of hands and feet[7]. These viral infections were also found in other mammals but without any inter-species transmission. Papillomaviruses exhibit a high degree of specific cellular tropism for squamous epithelial cells and have been associated with various clinical manifestations ranging from benign hyperplastic epithelial proliferative innocuous lesions (warts, papillomas) to invasive cancer. These proliferative hyperplastic lesions can be cutaneous (skin warts) or can involve mucosal squamous epitheliam of genital tract, oral, pharynx, or esophagous.

At least 15 HPV types associated with malignancy of both genital tract and non-genital tract have been categorized as High Risk (HR) types (HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 59, 66, 68, 69, 73 and 82),

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whereas those associated with benign lesions such as genital and skin warts, as Low-Risk (LR) types (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108). More than 100 different HPV types have been described till date, associated with infection of anogenital tract and other organ sites. Though epidemiological and molecular studies have established the casual relation of HPV infection with development of cervical cancer, a sizable number of studies also demonstrated associations of HPV infection with other cancers[8]. The majority of high-risk HPV types are associated with the development of cancer of uterine cervix, vagina, vulva, adenocarcinoma in women and anus, oro-pharynx, and esophagus in both men and women. Persistent infections with HR-HPVs have been identified as an essential although not sufficient factor in the pathogenesis of anogenital and other epithelial carcinomas[9]. HPV infection is also reported in skin cancer, lung cancer and in retinoblastoma[10], but the frequency has been low in India. Though controversial, presence of HPV DNA sequences has also been reported in Breast cancer[11]. Presence of HPV in sites other than reproductive organs clearly indicates passage of the virus via routes other than sexual transmission, which are as yet unknown.

In view of diversity in HPV types and their association with different lesions with respect to their site, it is important to analyze the extent of prevalence of HPV infection in general and to determine HPV type-specific association to cancers of different organ sites in particular. In an International Agency for Research in Cancer (IARC)-initiated study (Globocan 2002), to determine the global cancer incidence demonstrated about 5.17 per cent of all cancers can be attributed to HPV and cancer of anus is next to cancers of uterine cervix that show as high as 90 per cent cases attributed to HPV infection (Table I). Cancers of vulva, vagina, penis and oro-pharynx also show moderate HPV infection. Similar data on HPV prevalence in India where two promising HPV prophylactic vaccines against high-risk HPV type 16 and 18 and low-risk HPV types 6 and 11 are going to be introduced soon is, however, highly fragmentary. In addition, majority of the available information is related with cancer of uterine cervix only and has been collected using variety of detection methods which have variable sensitivity and specificity and are susceptible to intra- and inter-laboratory variations. Since HPV vaccination will also affect the HPV-associated disease pattern other than cervix, in this article, we tried to present studies which analyzed presence of HPV infection in cancers of various organ sites (Table II). Though all these studies[14-16,23,26,27,32,34,37-39,41,49] were performed using various technologies available at that time, and had considerable variations in results and these might have been carried out in a small subset of samples, yet they provide significant preliminary data on HPV prevalence in Indian population.

Cancers of anogenital tract

Cancer of uterine cervix: Squamous cell carcinoma of the uterine cervix, a malignant cancer of the women develops through a defined series of pre-neoplastic lesions with increasing cellular dysplasia referred to as cervical intraepithelial neoplasia (CIN) that are graded CIN1, CIN2, or CIN3 depending upon the severity of the lesion[50]. It is the second most common cancer among women worldwide. There were estimated 493,000 new cases and 274,000 deaths due to cervical cancer in 2002 with more than 83 per cent cases occurring in developing countries[12]. In India, it is estimated that 132,000 new cases of cervical cancer and over 75,000 deaths are reported annually. Proportionately a quarter of the global burden accounting to 27 per cent of total cervical cancer cases are reported from India while it houses only 16-17 per cent of world's women population. Cancer of the cervix constitutes about 15-51 per cent of all female cancers and rates of age-standardized incidence range from 17.2 to 55 per 100,000 women in different regions of India[51].

Infection of HPV 16/18 is estimated to account for more than 80 per cent of invasive cervical cancer

Table I. Global incidence of HPV attributable cancer in 2002: GLOBOCAN 2002[12,13]

Gender-specific cancers	Human organ site	HPV attribution (%)	Total cancers	Attributable to HPV infection	Per cent of all cancers	HPV 16/18 association (%)	Per cent of all cancers
Females	Uterine cervix	100	492,800	492,800	4.54	344,900 (70)	3.18
	Vulva, vagina	40	40,000	16,000	0.15	12,800 (80)	0.12
Males	Penis	40	26,300	10,500	0.10	6600 (63)	0.06
In both	Anus	90	30,400	27,300	0.25	25,100 (92)	0.23
	Mouth	3	274,300	8200	0.08	7800 (95)	0.07
	Oro-pharynx	12	52,100	6200	0.06	5500 (89)	0.05
	All-sites		10,862,500	561,000	5.17	402,900 (72)	3.71

Table II. Attribution of HPV infection in cancers of different organ sites in India

Organ site	HPV prevalence (%)	Prevalence of HPV 16/18 (%)	Detection methods	HPV types	References
A. Anogenital cancers					
Uterine Cervix	50-98*	60-90	SBH, ISH, PCR-TS, RLB, PCR-ELSIA,HCII	HPV16,18,31,33,35,39,45,51, 52,56,58,59,66,71,73,6,11	14-28
Anus	0-22	22	PCR L1, PCR-TS, IHC	HPV16, 18	29, 30
Penis	30	30	PCR L1, PCR-TS	HPV16, 18	30
Ovary	0	0	PCR L1, PCR-TS	HPV16, 18	30
B. Head and neck cancers					
Oral cavity	15-74	6-47	SBH, PCR L1, PCR-TS, PCR-Sequencing	HPV16,18,6,11	31-38
Esophagus	26.7	18.8	PCR L1, PCR-TS	HPV16,18	39-41
Larynx	30-70	30 (HPV 16),	PCR L1, PCR-TS	HPV16,18	42
Naso-pharynx	38.8	ND	IHC		43
C. Other cancers					
Lung	5	5	PCR L1, PCR-TS	HPV16,18	44
Urinary bladder	10-20	10-20	ISH, PCR	HPV16	45
Stomach	8.3	8.3 (HPV 16)	PCR L1, PCR-TS	HPV16,18	30
Eye	0-17	ND	IHC	HPV16,18	30, 46
Breast	0	0	SBH, RQ-PCR,	HPV16,18	47, 48

ELISA, enzyme linked immunosorbent assay; HCII, hybrid capture II; ISH, *in situ* hybridization; PCR, polymerase chain reaction, PCR L1-HPV L1 Consensus PCR; PCR-TS- HPV type specific PCR; RQ-PCR, real time quantitative-PCR; SBH, Southern blot hybridization; RLB, reverse line blot hybridization; (PGMY), PCR-sequencing, ND-not determined

including CIN3 and for 50 per cent of CIN2 lesions[52]. In India, 85-90 per cent cervical cancer cases are squamous cell carcinoma and the HPV 16 is the most prevalent type among them compared to other parts of the world where the proportion of HPV16 is much lower and ranges only upto 70 per cent when both HPV16 and 18 are considered[1, 53,54]. In India, HPV type 16 alone in cervical cancer is 70-90 per cent while occurrence of HPV type 18 varies from 3 to 20 per cent. Other high-risk HPV types such as HPV 45, 33, 35, 52, 58, 59, and 73 have also been reported are rare and constitute only a minor group[25-28]. In a national HPV mapping study, prevalence of HPV type 16 was found to be highest in Chennai (88%), whereas it was very low in Jammu & Kashmir (14.2%)[55]. The possible reason for low prevalence of HPV infection in Jammu & Kashmir is possibly due to the practice of circumcision in Muslim population[56]. The age-wise prevalence of HPV and HR-HPV16/18 infections among healthy female subjects from different geographical regions of India has also been reported[26-28,49,57,58] . Interestingly, the peak of HPV infection, particularly HPV 16, appears to reach at later stage in third decade of sexual life at 26-35 years in Indian women in contrast to 18-25 years reported in western countries[59,60].

Adenocarcinoma of uterine cervix: The adenocarcinoma of cervix originates adjacent to the squamous epithelial neoplastic lesions. Though majority cases of cervical cancer are squamous cell carcinomas (SCCs), adenocarcinoma of cervix also contributes to the overall burden of cancer of uterine cervix. The higher proportion of adenocarcinoma cases has been observed in those areas where incidence of cervical cancer is low and in some western countries it accounts for more than 25 per cent of cervical cancer cases[61]. Unlike squamous cell carcinoma where HPV 16 contributes upto 70 per cent of cases, in adenocarcinoma, infection of HPV 18 is reported to be more prevalent worldover (>86%)[62,63]. In contrast, only 10-15 per cent of cervical cancer cases in India are adenocarcinoma and HPV16 is the most prevalent type, though it is less frequent than what is found in the squamous cell carcinoma (42% vs 70-90%). As compared to preferential occurrence of HPV 18 in adenocarcinoma reported worldwide, only 17 per cent cases showed infection with HPV18 in India that too was present as co-infection with HPV16[18, 64].

Cancer of vagina: Vaginal cancer is a less frequent cancer in women and the age standardized rate is 0.3-0.7 per 100,000 worldwide[13]. The number of studies on the role of HPV infection and occurrence of vaginal cancer is limited and have analyzed only in fixed tissues for a few types of HPV[65]. Epidemiological studies indicate that the cancer of vagina resembles the cancer of uterine cervix and HPV DNA sequences are detected in majority of vaginal tumors and their precursor lesions. Like in cervical cancer HPV16 is the most prevalent

type found in vaginal cancer[66,67] and the frequency of HPV varies between 82 to 100 per cent of VAIN-3 and 64 to 91 per cent in vaginal cancer[65].

In India, the occurrence of vaginal cancer is low and vary between different geographical regions. According to cancer registries data among different places the age standardized rate of vaginal cancer varies from 1.4 per 100,000 in Pune to 0.3 in Bhopal[68]. The detection of HPV infection in vaginal cancer has not been reported in India and hence the information on the prevalence of HPV infection in cancer of vagina is not available.

Cancer of vulva: The worldwide ASRs of vulvar cancer lie between 0.5 and 1.5 per 100,000. The geographical pattern of vulvar cancer is not similar to cervical cancer and high rates are observed in several European populations (Scotland, Denmark, Spain, Italy), while it showed a low prevalence in sub-Saharan Africa, Southeast Asia, and Latin America[13]. Distinct subtypes like the warty and basoloid types have been recognized but majority of tumors are squamous cell carcinoma. Etiologically vulvar carcinomas are heterogeneous and thus the presence of HPV infection in invasive vulvar cancer cases varies[69-71]. Vulvar cancer with basaloid histopathology in young women is often associated with HPV[69]. HR-HPV types 16, 31 and 33 are the frequently detected types in vulvar cancer and its precursor lesions, VIN[72]. On the other hand, vulvar cancer with verrucus subtype and some cases of the precancerous lesions of vulvar intraepithelial neoplasia (VIN), are not associated with HPV infection[73]. In India, no report is available on the prevalence and type distribution of HPV infection in vulvar cancer.

Cancer of anus: Cancers of the anus are those arising in the anal canal and tumors of the external skin (anal margin) are classified along with skin cancers. The canal is lined in its upper part by colorectal-type of mucosa, and in its lower third by squamous epithelium, with specialized transitional zone epithelium in between. Therefore, cancers are predominantly SCCs, adenocarcinomas, or basaloid and cloacogenic carcinomas. In most populations SCC is twice more common in females than in males[61]. Incidence is specifically high among homosexual males and the risk is increased further by infection with HIV. Risk is also reported to increase by cigarette smoking, anal intercourse, and the number of lifetime sexual partners[74]. According to Cancer Registries of India, the observed incidence of anal cancer is higher in females than that of males in India[61]. The role of HPV infection in etiology of anal cancer has not been explored extensively in India. A study by Gupta *et al*[29] showed presence of HPV 16/18 in 22 per cent cases out of 36 anal cancer cases studied by immunohistochemical methods. On the other hand, analysis by consensus and type specific PCR revealed complete absence of HPV infection in 20 anal cancers studied[30]. The observed difference in the prevalence of HPV infection in anal cancer may be attributable partly to techniques used for HPV detection and partly because of low prevalence of anal sex in India.

Cancer of penis: Globally penile cancer is rare cancer and accounts for less than 0.5 per cent of all cancers in men[13]. The concordance of cervical and penile cancer in married couples and geographical distribution of these cancers suggest that it shares a common etiology[75]. Serological studies have confirmed the role of HPV16 and HPV18 in etiology of penile cancer and HPV DNA is detected in 40-50 per cent of penile cancers[76]. Cancer Registries have demonstrated that observed incidence rates of penile cancer are higher in India in comparison to western countries.

Although the incidence of penile cancer is higher in India, the role of HPV infection in the etiology of this cancer has not been very well studied. There is no comprehensive study that demonstrate prevalence and type distribution of HPV in penile carcinoma in India. In a small sample size (n=30) HPV 16 was detected in 30 per cent of penile cancers[30]. In contrast, Gupta *et al*[77] using penile smears demonstrated HPV 16 infection in 67 per cent (20/30) of male partners of women with invasive cervical cancer. It reiterates that HPV16 is the most frequent type found in penile cancer in India. As India harbors more than 1/4th of total global cervical cancer cases, the prevalence of penile cancer in male partners of women with cervical precancer and cancer could be higher. Therefore, an organized and regular screening of penile cancer is very important particularly in view of upcoming HPV vaccine trials in India. Man being the main host reservoir of HPV infection, it is important to give HPV vaccine to males thus creating herd immunity against the HR-HPV types responsible for the disease.

Ovarian cancer: Ovarian cancer is most commonly formed in the epithelial lining of the ovary resulting in epithelial ovarian cancer or in the egg cells resulting in a germ cell tumor. Ovarian cancer is the fifth leading cause of death from cancer in women and the leading

cause of death from gynecological cancer[78]. A woman has a lifetime risk of ovarian cancer of around 1.5 per cent. Primary squamous cell carcinoma of the ovary is rare, most cases represent malignant transformation of ovarian teratomas or are associated with preexisting Brenner tumor or ovarian endometriosis. The actual cause of ovarian cancer is unknown. There is an increased risk of ovarian cancer in older women and in those who have a first or second degree relative with the disease[79,80]. The role of HPV infection in etiology of ovarian cancer remains unclear and the results differ worldwide. Some studies have demonstrated that HR-HPV types 16 and 18 play an important role in the development of ovarian cancer[81] whereas some authors suggest that HPV is highly unlikely to play any causal role in the pathogenesis of epithelial ovarian neoplasia[82]. In India, the role of HPV infection in pathogenesis of ovarian carcinoma has not been explored. In a small study on the prevalence of HPV infection in 20 ovarian cancer biopsies using consensus and type specific PCR for high risk HPV types 16 and 18 demonstrated complete absence of HPV infection in ovarian cancer in India[30].

Head and neck cancers

Oral cancer: Oral Squamous Cell Carcinoma (OSCC) is the sixth most common cancer globally and accounts for approximately 5 per cent of all malignant tumors worldwide[78]. In India and South East Asia OSCC is the most common malignancy amounting up to 50 per cent of all malignant tumors[83]. Although most of the OSCC is attributed to tobacco and alcohol consumption, a significant proportion of oral cancers have been demonstrated to contain anogenital HPV infection[84]. The high risk HPV type 16 tends to be the most predominant type detected in oral cancer[9,84].

The prevalence of HPV in oral cancer has been well documented in several studies from different geographical regions of India. However, the prevalence of HPV differs in different geographical regions within Indian subcontinent. The infection of HPV 16 is reported in 27 per cent of oral cancer from north India[38] whereas from western part of the country it ranges from 25 to 31 per cent[34,37]. Multiple HPV infection was observed in about 14 per cent of cases[37]. The reports of HPV prevalence in oral cancer from southern India seems to be highly variable. The overall frequency of HPV infection has been reported to be 74 per cent while 41 per cent showed multiple HPV infection[32]. HPV type-specific infection for HPV types 6, 11, 16 and 18 was found to be 13, 20, 42 and 47 per cent, respectively[32].

Cancer of esophagous: Esophageal Squamous Cell Carcinoma (ESCC) is highly divergent and demonstrates wide regional variation in incidence and causal associations in different geographical regions of the world[61]. Infection with HPV has been implicated as one of the possible etiological factors but occurrence of HPV infection is conflicting, varying from complete absence to detection of up to 60 to 70 per cent of HPV positivity mainly of HPV type 16 and 18 in the cancer biopsies[85].

In India, the incidence of esophageal carcinoma also differs from region to region and the association of HPV infection has been demonstrated in a number of studies. Agarwal *et al*[39] reported a significantly higher number (63%) of esophageal cancer cases immunopositive for HPV16 E6 oncoprotein. In an interesting study carried out in esophageal cancer patients from three different high incidence geographic regions of India with different food habits, smoking, tobacco chewing and ethnicity showed significant difference in the frequency of HPV infection[41]. Of a total of 101 biopsy specimens of carcinoma esophagus analyzed, the frequency of HPV was found to be the highest (44%) in Dibrugarh followed by 33 per cent in Kashmir, but, no high-risk HPV could be detected in New Delhi patients (0%) which showed significantly a high frequency of p53 mutations as compared to that of the other two regions[38].

Laryngeal carcinoma: Laryngeal cancer is the most common cancer of the upper aerodigestive tract. Age-standardized incidence rates ranged from 2.5 to 17.1 per 100,000 person-years at risk in men while 0.1 to 1.3 per 100,000 person-years at risk in women. In European and Asian countries, most squamous cell carcinomas of the larynx result from an exposure to carcinogens, such as tobacco and alcohol, which cause diffuse mucosal changes. HPV has been found in a good proportion of laryngeal cancers[86]. A meta-analysis of 60 HNSCC specimens has demonstrated prevalence of HPV DNA in 24 per cent (ranges 0-100%) of laryngeal squamous cell carcinoma[86]. HPV 16 was accounted for the 69.2 per cent whereas HPV 18 was found in 17 per cent of laryngeal carcinoma cases. In India, the prevalence of HPV infection was found in 34 per cent (15/44) of invasive laryngeal squamous cell carcinoma[42].

Nasopharyngeal carcinoma (NPC): Nasopharyngeal cancer is an uncontrolled growth of cells that begins in the nasopharynx, the passageway at the back of the nose. Nasopharyngeal carcinoma (NPC) is a common

cancer in Southeast Asia and is frequently associated with Epstein-Barr virus (EBV) infection. Recent studies have indicated HPV, particularly HPV31, is also found in a significant number of NPC cases. In India, prevalence of HPV infection was found in 38.8 per cent (14/36) cases of NPC by immunohistochemical localization of HPV antigens, whereas 10 per cent (1/10) cases of adenoid lesions also showed presence of HPV[43].

Other cancers

Cancer of lung: The malignant proliferation of lower respiratory tract epithelial cells results into development of lung cancer. Lung cancer is one of the most common form of malignancy worldwide and is the leading cause of cancer related deaths around the world including India. Smoking is considered to be one of the principal causes of lung cancer; however, genetic factors such as mutation or over-expression of oncogenes *e.g*., c-myc, erbB2, polymorphism in P450 (CYP1A1) and glutathione transferase M1 genes, functional inactivation of tumor suppressor genes eg, Rb, p16, p53 genes including p53 codon 72 polymorphism, and infection of specific types of human papillomaviruses, have been implicated with the development of lung cancer[87,88].

The reports on the association of HPV infection in lung cancer are not only rare but also controversial. Several authors have reported that HPV has no role to play in lung carcinogenesis, whereas others have observed a low frequency (4-18%) of specifically HPV18 infection in lung cancer. In contrast, a moderate to a very high frequency of HPV infection (30-79%) has been reported by others[89,90]. In India, a very low frequency (5%, 2/40) of only HPV18 infection has been observed but no other HPV types including most prevalent HPV16 were found in lung cancer[44].

Cancer of urinary bladder: Over 90 per cent of bladder cancers are transitional cell carcinomas, or cancers of the epithelial cell lining of the bladder, ureters and urethra. Though the exact causes are unknown, both genetic and environmental factors are responsible[91]. The role of HPV infections in the development of bladder cancers is unlikely as the most of the cases are transitional cell carcinomas in the developed world. Although some studies have demonstrated HPV sequences in less than 10 per cent of cases, a majority of studies rule out any role of HPV infection in development of bladder cancer[92-94]. The most significant risk factor associated with bladder cancer is smoking; and the carcinogens are absorbed through the lungs into the bloodstream, where they are filtered out by the kidneys and enter the urinary tract. An environmental risk is presented by a class of organic chemicals called arylamines. People who are in the leather, rubber, printing, and textile industries or work with large quantities of paint are often exposed to these chemicals chronically and are susceptible to developing bladder cancer.

In India, carcinoma of urinary bladder is more prevalent in men than women. Cancer Registries of India show an age standardized rate of uninary bladder varies from 2.3 to 5.8 per cent per 100,000 for men and 0.4 to 1.5 per cent in women. In a study carried out by Gopalkrishna *et al*[45] showed presence of HPV16 in 20 per cent (2/10) cases by PCR. This study showed a small but significant probability that HR HPV infection found in urinary bladder may be involved in development of malignancy at this organ site.

Stomach cancer: Stomach or gastric cancer can develop in any part of the stomach and may spread throughout the stomach and to other organs; particularly the esophagus and the small intestine. Stomach consists of four different layers of cells. The outermost layer is made up of epithelial cells which make gland like structures on stomach wall. Globally, a large number of studies have indicated that most cases of stomach ulcers, gastritis, and stomach cancer are caused by *Helicobacter pylori* infection. The association of HPV infection with stomach cancer/gastric cancer has been controversial. Some of the studies have ruled out presence of HPV in stomach cancer[95,96], whereas other investigators indicated role of HPV 16 in gastric cancer[97].

In India, the number of new stomach cancer cases in 2001 was estimated to be approximately 35675 (n = 23785 in men; 11890 in women)[98]. The AAR of gastric cancer in urban registries (3.0-13.2) is on the lower side among those reported worldwide (4.1-95.5). It is a disease mainly of males. Gastric cancer is highly prevalent among South Indians and it occurs a decade earlier as compared with the North Indians[99]. The etiology of gastric cancer is multifactorial and infection with *Helicobacter pylori* has been accepted worldwide as the main etiological factor for the development of stomach cancer. *H. pylori* causes carcinogenesis by inducing chronic atrophic gastritis, and prevalence of its infection is high (49.4 to 83.3%) in India[100,101], but gastric cancer rates are relatively low. There is no study that has focused on the role of HPV in stomach cancer in India. Recently, a very low percentage HPV16

(8.3%; 2/24) infection in stomach cancer cases has been detected[30]. However, the mechanism of pathogenesis and the route of infection of HPV in stomach cancer is not clear. The exclusive occurrence of HR HPV type 16 in stomach cancer from India needs further studies.

Cancer of eye: HPV DNA sequences have been reported in benign conditions of the conjuctiva and in dysplastic and invasive lesions of the eye also. HPV16 and 18 E6 gene expression was found in intraepithelial neoplasia of the conjuctiva[102]. However, some authors suggest that there is very limited evidence for the pathogenesity of HPV in squamous cell carcinoma of eye conjuctiva[76]. In a recent study of retinoblastoma specimens no HPV infection could be detected[30]. In a meta-analysis of 65 cases of ocular neoplasia comprising 35 papillomas and 30 ocular surface squamous neoplasias (OSSN) including 30 normal controls, were studied by immunohistochemistry[46]. Interestingly, the results demonstrated 17 per cent HPV positivity for conjunctival papillomas but all cases of ocular surface squamous neoplasias (OSSN) were negative for HPV. Since majority of retinoblastoma is sporadic, it is suggested that this is possibily due to inactivation of Rb gene the product of which binds to HRHPV E7 protein and the HPV could be transmitted in utero during vaginal delivery or otherwise[103]. Therefore it is important to look for prevalence of HPV infection in retinoblastoma cases in a larger study.

Cancer of breast: Breast cancer is the second most common cancer in the world, after the cancer of the lung, affecting one in eight women during their lifetime. It is also the leading cancer among women worldwide[9]. In India, breast cancer is the second most common cancer in women and is showing rising trend in urban India (NCRP 2005). Various epidemiologic risk factors both exogenous as well as endogenous, including mutations in breast cancer genes BRCA1 and BRCA2[104] have been associated with the development of breast cancer. Moreover, several oncogeneic viruses such as Epstein-Barr Virus (EBV), murine mammary tumor virus and HPV have been implicated in the development of human breast cancer[11]. Reports on the infection of HPV in Breast cancer are not only limited but also controversial. While several studies[47,105,106] have shown that there is complete absence of HPV infection in breast cancer; a moderate frequency of 25-45 per cent HPV infection was reported by many other studies worldwide[105,107]. Recent report on prevalence of HPV infection in breast cancer shows 65-85 per cent breast cancers positive for HPV DNA[11]. In view of controversial reports, recently a highly sensitive real time PCR methodology was employed to screen large number of breast cancer specimens but no HPV could be detected[48].

Benign/Non tumorous lesions

The ano-genital warts, skin warts and recurrent respiratory papillomatosis are associated with the infection of low risk HPVs particularly HPV types 6 and HPV11[7,108]. Recent studies have established that more than 90 per cent of genital warts are caused by either HPV6 or HPV11 but studies also show that 20–50 per cent of lesions may contain co-infections with HR HPV types[109,110]. Genital warts are a highly prevalent sexually transmitted disease which affects around 1-2 per cent of the sexually active population[111]. As compared to USA, UK, and some other countries where robust data are available on the prevalence and incidence of genital warts, in India no formal reporting system for genital warts is available. Data on the genital warts are available only from small epidemiological studies carried out randomly using anti-HPV sera-based immunostaining[112,113], demonstrating presence of HPV antigens in 46-83 per cent of genital warts. The presence of viral antigen by immunologic test is indicative of productive viral infection but not suitable to detect latent infections. Thus the positivity represented by these studies could be an underestimation.

Recurrent respiratory papillomatosis (RRP), also known as laryngeal papillomatosis, is a rare condition characterized by the recurrent growth of benign papillomas in the respiratory tract. The papillomas occur anywhere in the respiratory tract but most commonly in the larynx[108]. HPV types 6 and 11 are the causative agents of almost 100 per cent of juvenile onset RRP and adult onset RRP. HPV-11 is often associated with more severe form[114] which has a greater risk of malignant conversion[115]. Though sample sizes have been very small, sporadic studies from India have shown considerable proportions of laryngeal papillomas infected with HPV. The HPV infection was found to vary from 80 -100 per cent and comprised both LR as well as HR-HPV types such as HPV11 and HPV16 respectively[30,42].

Detection methods and their impact on HPV prevalence

The prevalence of HPV infection and distribution of HPV types at different human organ sites especially in cervical and oral precancer and cancer show a

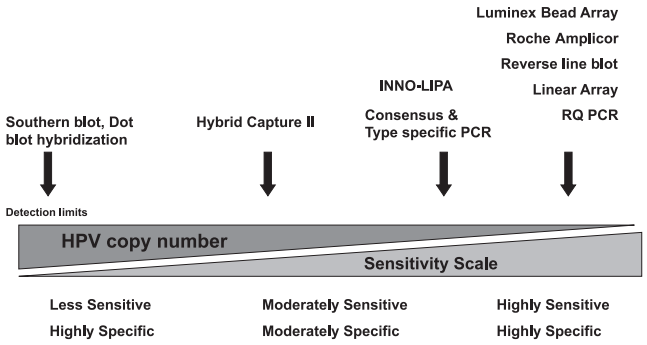

Fig. Relative sensitivities of HPV nucleic acid detection techniques.

considerable variation, whereas in Indian perspective the data on HPV prevalence in other organ sites is limited. The variations can be partly attributed to geographical and ethnic diversity in Indian population and partly influenced by the detection techniques employed[14-16,23,26,27,32,34,37-39,41,49]. In India, the prevalence of HPV infection has been determined by using various techniques which may also the one of the potential reason for observed differences (Table II). Furthermore, the new emerging technologies for HPV DNA testing such as linear array, reverse line blot, real time PCR, Luminex bead-based assays and microarray chips are more sensitive and have higher specificity in addition to detection of multiple HPV infections. However, these techniques are expensive, labor intensive, require dedicated infrastructure and trained manpower. In comparison, immunodetection, Southern blot and In situ hybridization, or HCII methods have low specificity and sensitivity (Fig.) and are gradually getting replaced with advanced technology but the data generated using these techniques demands revalidation.

Prevailing data using various HPV tests not only show inter-test variations but also susceptible to inter- and intra-laboratory variations which is valid not only for India but for data available globally. This is primarily due to lack of harmonization of HPV detection methodologies, differences in sensitivities and specificities of the tests, and lack of appropriate international standards for HPV detection. This usually results in inappropriate judgment of prevailing HPV infection and is a major obstacle in surveillance of HPV-associated diseases.

In addition, studies where multiple HPV infections have been investigated[26-28] have used some or the other hybridization technique which are highly prone to cross reactivity and always bear a mark of interrogation which can only be resolved by conducting confirmatory type-specific PCRs and sequencing. These confirmatory tests have not been done in majority of the cases in India hence the data indicating high degree of multiple infections remains questionable. With newly developed more sensitive and specific tests, it will be easier to delineate and determine the prevalence and type distribution of HPV infection more precisely.

Development of international standards (IS)

In view of tremendous variations in HPV prevalence data, World Health Organization (WHO) has developed a structured global network of various laboratories working in the area of HPV diagnostics (HPV LabNet) to improve quality of laboratory services for effective surveillance and monitoring of HPV vaccination impact. HPV LabNet has three levels, (*i*) Global reference; (*ii*) Regional reference; and (*iii*) National/local laboratories based on the expertise and capabilities of respective laboratories[116]. The objectives of the network are to provide scientific and technical advice, to develop nodal points for quality assurance, training and communications in HPV diagnostic services. These reference laboratories are participating in WHO's international collaborative HPV study coordinated by National Institute of Biological Standards and Control, UK for establishment of international standards (IS) in harmonizing HPV16 and HPV18 DNA Nucleic Acid Amplification Technology-based assays which will be subsequently transferred to local laboratories. These efforts will be immensely important in monitoring the impact of HPV vaccination programs worldwide and will be of great help for fruitful evaluation of data at uniform platform. To extend these efforts, HPV laboratories working in India are also getting networked under the umbrella of Indian HPV Laboratory Consortium. The primary objective of the Consortium is to harmonize various HPV detection assays and to minimize inter-laboratory variations by bringing all the laboratories involved in HPV research and diagnostics to a single platform and collectively establishing a strong and effective Quality Assurance and Quality Control program.

Impact of HPV vaccine on cervical and other cancers

Despite variations in sensitivities, and specificities of various HPV detection technologies used to determine the prevalence of HPV in cancers of different organ sites, it is well accepted that a

significant proportions of cancers other than cancer of cervix harbor HPV DNA sequences[8]. As in a majority of cases these infections are of high risk type, especially HR HPV type 16, it is quite likely that HPV oncogenes must be playing an important role in the development of cancers at these sites too. With the availability of two successful vaccines Gardasil (quadrivalent), Cervarix (bivalent) against HPV 16, 18, it is expected that a significant number of patients bearing such HPV-associated cancers will also be benefited by the introduction of these vaccines. Therefore, programs designed to study HPV vaccine response and post-vaccination surveillance should also look into this aspect as secondary end points or response indicators of HPV vaccines. Moreover, because of almost exclusive occurrence of HPV16 in cervical cancer and precancer lesions, it is expected that current HPV vaccines which are against HPV16 and 18 will be much more effective compared to other regions of the world where other HR-HPVs are also involved in cervical cancers. In addition to above, it is also interesting to note that high prevalence of HPV16 in penile smears of women harboring HPV16 positive cervical lesions[117] unequivocally advocate immunization of male population not only to protect them from HPV-associated penile cancer but also to augment the herd immunity against HPV in general.

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