DETECTION OF HUMAN PAPILLOMAVIRUS GENOTYPES IN HIV INFECTED WOMEN OF CHILD BEARING AGE.

Thesis Submitted to

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For the award of the degree of Doctor of Philosophy in the Faculty of Medicine

By

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(Registration No: KLEU/Ph.D./2012-13/ DOUN12006)

Under the Guidance of

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APRIL -2022

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Date: ***Mr. Vinay P S***

Place:

LIST OF ABBREVIATIONS

HPV	Human papillomaviruses
HR	High-risk
LR	Low-risk
DNA	Deoxyribonucleic acid
VLPs	Virus-like particles
CIN	Cervical intraepithelial neoplasia
HNSCC	Head and neck squamous cell carcinoma
SCC	Squamous cell carcinoma
OPSCC	Oro-pharyngeal squamous cell carcinoma
HDI	Human Development Index
NCRP	National Cancer Registry Program
IN	Intra-epithelial neoplasia
SIL	Squamous intraepithelial lesions
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
HAART	Highly active antiretroviral therapy
bp	base pairs
ORFs	Open reading frames
cSCC	cutaneous squamous cell carcinomas
EV	Epidermodysplasia verruciformis
NMSC	Non-melanoma skin cancers
pRb	Retino-blastoma protein

PV	Papillomavirus
UV	Ultraviolet
LSCC	Laryngeal squamous cell carcinoma
STI	Sexually transmitted infection
TLR	Toll-like receptor
OPC	Oropharyngeal cancers
Rb	Retinoblastoma
LCR	Long control region
NCCR	Non-coding region
URR	Upstream regulatory region
pA	Polyadenylation
LMICs	Low- and middle-income countries
SCJ	Squamo-columnar junction
LSIL	Low-grade squamous intraepithelial lesions
HSIL	High-grade squamous intraepithelial lesions
LBC	Liquid-based cytology
Pap	Papinicolaou
WHO	World Health Organization
IAPCOI	Indian Academy of Pediatrics Committee on Immunization
VIA	Visual inspection with acetic acid
TMH	Tata Memorial Hospital
AIS	Adenocarcinoma in situ
MHC	Major histocompatibility complex
ER	Endoplasmic reticulum
TCR	T cell receptors

PBMC	Peripheral blood mononuclear cells
CDC	Centre for Disease Control and Prevention
NARI	National Aids Research Institute
LA-HPV	Linear Array® HPV
PGMY-LB	PGMY line blot
RLA	Roche Linear Array
FFPE	Formalin-Fixed Paraffin-Embedded
PCR	Polymerase chain reaction
SD	Standard Deviation
PPV	Positive predictive value
NPV	Negative predictive value
ICC	Invasive cervical cancer
ASCUS	Atypical squamous cells of undetermined significance

ABSTRACT

Globally cervical cancer is the fourth most frequent cancer in women with an estimated 604 000 new cases and 342,000 deaths in 2020, about 90% of these occur in low- and middle-income countries. Women with HIV are six times more likely to develop cervical cancer compared to HIV negative women. Majority of cervical cancer (> 95%) is due to the human papillomavirus (HPV).

HPV is the most common viral infection of the reproductive tract. Most sexually active women and men will be infected at some point in their lives, and some may be repeatedly infected. More than 90% of the infected populations eventually clear the infection. Cervical cancer is by far the most common HPV-related disease. Approximately all cases of cervical cancer can be attributed to HPV infection. Although most HPV infections clear up on their own and most pre-cancerous lesions heal spontaneously, there is a risk for all women that HPV infection may become chronic and pre-cancerous lesions progress to invasive cervical cancer. It is reported that it takes 15 to 20 years for cervical cancer to develop in women with normal immune systems, however It can take only 5 to 10 years in women with weakened immune systems (HIV positive women).

Present study was aimed to

1. To know the HPV types among women attending Department of Gynecology, KLE's Prabhakar Kore Hospital & MRC, Belagavi.
2. To determine the HPV type distribution among women of child bearing age in according to HIV status (HPV-HIV co-infection)
3. To correlate CD4 counts with HPV infection in HIV Positive patients.

4. To compare and explore variety of molecular methods for implementation in screening of cervical cancer.

In the present cross-section study patient's demographic details were collected. HIV status was diagnosed with Tridot assay, CD4 count, PAP test was done with routine procedures. Roche Linear array for HPV genotyping was done to identify the HPV genotyping

In the present study there were 214 women were HIV positive. Of the 197/214 women with the adequate cervical sample, 86 (43.6%) were HPV positive, and 111 (56.3%) were HPV negative cases. A total of 132 (69.1%) women had normal cervical status, 26 (13.6%) had CIN1 lesions, 1 (0.5%) had CIN2 lesions, and 12 (6.3%) had CIN3 lesions. Single HPV infection was detected in 47 (54.6%) women and multiple (≥2) HPV genotypes were detected in 39 (45.3%). The HPV genotypes detected in descending order of frequency were HPV 16, HPV 33, HPV 35, HPV 52, and HPV 58. Ever pregnant (parous) women were 4.47 more likely to have HPV infection. It was concluded that there was greater prevalence of HPV 16 among HIV-positive women from Belagavi, India, was observed. Parity was the independent factor associated with HPV detection.

Totally there 96 HIV-negative women in the age range of 18- 45 years in the present study. Results proved that a significant linear increasing trend in proportion of carcinogenic and non-carcinogenic genotypes over grade was observed (P=0.039; P=0.0024, respectively).HPV 59 was reported to be the most common genotype followed by 16, 53, 62, 72 but without any statistical significance.

Based on the ours study findings it can be concluded that Screening strategies incorporating HPV genotyping and vaccination should be effective in preventing

cervical cancer in HIV–negative women. In small cities there is a gap in the knowledge which might be essential for the policymakers, and also present research finding from Belagavi region might help treating clinicians towards incidence data. Present finding is also having relevance to cancer prevention programs early screening of the HPV can definitely help in early diagnosis and treatment, which in turn might reduce mortality. However our study might not directly associated with HPV vaccine.

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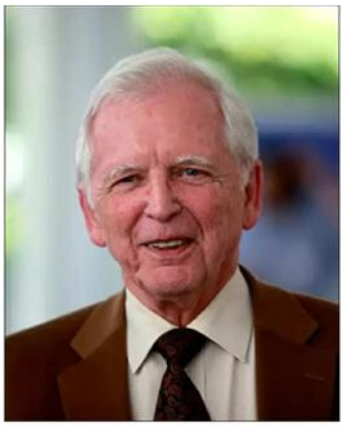
INTRODUCTION

Human papillomaviruses (HPVs) are well-established disease-causing entities which are categorized into almost 80 distinct classes. These viruses give rise to benign warts in several cases and vigorous squamous cell cancers in other cases. Non anogenital warts are accrued by skin-to-skin connection while anogenital warts are generally accrued sexually. Either category of warts bring about much morbidity but do not generally support malignant transformation.[(1)]

Human papillomaviruses (HPVs) are made up of a big family of double-stranded DNA viruses of roughly 8 kilobases that are the causative agents of the anogenital carcinomas and the non-cancerous warts. [(2)] HPVs consists of beyond 100 genotypes. The mucosal strain of virus can be further classified as High-risk (HR) or Low-risk (LR) on the basis of the risk associated with the disease.[(3)]



Harald zur Hausen
Nobel Prize in Physiology or Medicine in 2008

Harald zur Hausen, a virologist from the German Center for Cancer Research, Heidelberg, has been awarded the Nobel Prize in 2008 for his research on the role of human papilloma viruses in causing cervical cancer. He employed restriction

fragment length polymorphisms and DNA hybridization to establish a numerical method of HPV typing and for the classification of HPV types into flat and plantar warts. zur Hausen and his colleagues identified new strains of HPV. They characterized HPV-6 as causing condyloma acuminata (genital warts) and also described a second virus HPV-8 causing epidermodysplasia verruciform. Subsequent to cloning these genomes, the group employed an HPV-6 probe to identify DNA of yet another class of virus (HPV-11) in genital condylomas, laryngeal tumors, and few of the cervical cancer samples. They further made use of the low specificity hybridization to HPV-11 to recognize a novel genome, HPV-16, occurring in 11 out of 18 cervical cancers.[4,5]

HPVs are compact, double stranded DNA viruses that result in mucosal and cutaneous tissue lesions. These viruses contribute to the etiopathogenesis of cancers of the vulva, vagina, cervix and penis. The genetic material of the virus is perpetuated in the episomal configuration through the course of their life cycle and duplicates itself simultaneously as the host cell genome with the assistance viral proteins namely, E1, E2, E4 and E5.[6]

Genital HPV strains have been categorized into subtypes like low-risk strains, which are generally observed in genital warts, and high-risk strains, which are normally associated with the fast-spreading cervical cancer.[7] Among the papillomavirus strains discovered so far, over 100 strains are pathogenic to humans. These HPV strains can be differentiated into cutaneous and mucosal HPV strains on the basis of the tissue they target. Mucosal HPV strains are classified into two classes' namely high-risk (hr) and low-risk (lr). HR-HPV strains especially HPV16 and 18 are carcinogenic and causes the highest percentage of HPV associated cancers sparing no

anatomical regions whereas, low-risk HPV strain give rise to warts. The strains HPV6 and HPV11 give rise to genital warts and occasionally mild dysplasia, but severe dysplasia is uncommon.[8] The low- risk HPV strains including types 6, 11, 34, 40, 42, 44, 53, 54, 55, 57, 61, 70, 71, 72, 81 and 84 are linked to benign lesions like warts. The high-risk HPV strains including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 have the capability to advance into cancerous lesions. [9]

Certain strains of HPVs, like HPV-16, HPV-18, and HPV-31, have been identified to play causative roles in the etiopathogenesis of cervical as well as anal cancer. Infections by these strains, arising through sexual transference, are linked to almost 95% of the cervical cancers. Papillomaviruses target the keratinocytes in the basal level of the stratified squamous epithelial tissue and duplicate inside the nucleus of the targeted keratinocytes in a manner which is dependent on the differentiation of the cells. Expression of the viral genes in the cells which are infected is dependent on the cell differentiation and is firmly monitored at the level of transcription as well as post transcription. [10]

Papillomaviruses primarily infect epithelial cells, and the completion of their life cycles is dependent on the differentiation of the epithelial tissue. The viral gene expression is highly modulated as the affected basal cell moves towards the epithelial surface. The production of E6 and E7 proteins in the bottom epithelial layers guide the cells into S-phase, thus creating conditions beneficial for the viral DNA replication and cell multiplication. The amplification of the viral genome which is required for producing pathogenic virions, is suppressed till the amount of viral replication proteins increases, and is determined by the co-expression of a number of viral proteins.[11]

A bulk of the HPV infections are harmless and eliminated spontaneously however, continuous infection with high-risk HPV (specifically by the strain 16) can result in the cancer of the vagina,vulva, cervix, penis, anus and oropharynx. HPV impedes normal cell-cycle regulations, stimulating the unrestricted cell division and the aggregation of damage to the genetic material. [(12)]

The second most prevalent cancer occurring in women worldwide is cervical cancer. The understanding of the etiopathogenesis of the same is broadening rapidly. Four crucial steps mark the development of cervical cancer: a) the infection of the metaplastic epithelial tissue at the cervical transformation region, b) prolonged or continuous existence of the virus, c) progression of the continuously infected epithelial tissues to cervical precancer, and d) the invasion via the basement membrane of the epithelial tissue.[(13)]

Human papillomavirus infection has been reported to be associated with the incidence of cervical cancer over three decades ago. Recent research have given rise to overwhelming confirmation that certain strains of HPV play causative roles in the development of these cancerous growths.[(14)]

Among several strains of HPV, many (more than 30) infect the genital tract. The correlation of some of the high risk (oncogneic) strains of HPV with the incidence of cervical cancer is well substantiated. Early identification and medicaments of precancerous tissue alterations can avert the advacnement of cervical cancer.[(15)] Invasion of tissue by cancerous HPV strains is a prerequisite for development of cervical cancer which is the second most prevalent cancer affecting females globally. Rates of HPV infections are inflated, especially among adults who are sexually active.[(16)] Numerous HPV strains (more than 100) have been identified so

far, and classified into cutaneous or mucosal strains on the basis of their target tissue types viz. the skin or mucosa of the upper respiratory tracts or the genitals. Biological and epidemiological observations have demonstrated that HPV16 is oncogenic to the greatest extent within the high-risk category. Cancers associated with HPV are closely related to continuance of HPV infection and the aggregation of chromosomal rearrangements.[17] It has been proposed that high-risk HPV strains are associated with a small group of head and neck cancers in which the critical biological process elementary to the HPV-associated tumorigenesis is the blocking of p53 by the HPV E6 oncoprotein.[18]

Cervical cancer is the most common reason for the increasing mortality rate among women who are suffering from cancer in the developing nations and attempts for the prevention of the condition utilizing novel strategies and HPV vaccines require further exploration. The ratio of the incidence to the rate of mortality due to cervical cancer continue to be high, chiefly because of the lack of the benefit of pertinent anti-cancer therapeutics. In several developing nations there is dearth of facilities for the prevention, early detection, therapy and amelioration of tumor associated disorders. [19] The HPV strain that is causative of cervical cancer are sexually accrued, however there is insufficient information regarding the prevention of infection by implementing behavioral modifications, like the use of condoms. On the contrary, preventive vaccines against HPV infections probably have increased efficacy. The efficacy of the immunization against HPV as an approach towards cervical cancer management can be assessed either by observing the fluctuations in the trends in the incidence of cervical cancer or by performing randomized assessments. [20]

Cervical cancers constitute 530,000 fresh patients per annum and is responsible for the bulk of the HPV-associated tumor cases globally. In almost fifty percent of instances, the disease is identified in women less than50 years of age and approximately two-thirds are detected in less developed nations. The majority of the of cervical cancer cases prevail in Latin America, Africa (sub Saharan region) and South-Eastern Asia (with an exceptionally high disease burden in the Indian population). HPV 16 and 18 jointly are accountable for 71% of the cervical cancer worldwide. HPV contribute to several cases of carcinomas worldwide, around 8,500 instances of vulvar cancer, 12,000 of vaginal carcinoma, 35,000 of anal and 13,000 of penile tumors.[(21)]

Apart from the critical impact of infection by HPV in women, it is also accepted that HPV can give rise to significant illness in males as well. A common presentation of infection by HPV in males are genital warts which are highly contagious. Individuals who have sexual intercourse with affected partners subsequently develop warts. Over 90% of the genital warts are the result of infection of non-cancerous HPV strains 6 and 11. Additionally, respiratory papillomatosis, a rare disorder, is also often linked to infections by HPV strains 6 and 11.[(22)]

Cervical cancer, the fourth most prevalent cancer type and the lead source of cancer associated deaths in females globally, is necessarily caused by HPV infections. It additionally causes tumors of the anus, vagina, vulva, penis as well as oropharyngeal cancers. Preventive vaccines sourced from recombinantly expressing virus-like molecules have been generated. The U.S. FDA has approved two first-generation vaccines to prevent HPV infections and subsequent disease development.

These vaccines are effective against the HPV16 and HPV18 strains that give rise to cervical cancer. Additionally, one of the two vaccines is also effective against HPV6 and HPV11 strains which cause genital warts. Another new generation vaccine which has also been approved by U.S. FDA has been demonstrated to protect against target strains like HPV16, HPV18 as well as further 5 HPV strains inclusive of HPV6 and HPV11.[23]

Incessant infection by high-risk strains of HPV necessarily induces cervical cancer. The HPV genome encodes two genres of genes; early and late. The products of the early genes control the viral DNA replication (E1, E2), viral RNA transcription(E2), cytoskeleton reorganization(E4) and cell transformation (E5, E6, E7) while the late gene products (L1, L2) are structural elements of the viral capsid. Therefore, prophylactic immunization targeting HPV is a promising approach for the prevention of cervical cancer. Immunization could be administered as preventive vaccines which produce neutralizing antibodies to obstruct HPV infection or as therapeutic vaccines, used to remove infection by triggering a virus-specific T cell-mediated feedback mechanism. Contemporary approaches for the generation of safe and potent preventive vaccines are supported by the mechanism of triggering of neutralizing antibodies which target the major and minor capsid proteins (L1 and L2) of HPV. [24]

HPV-associated screening strategies and HPV immunization provide vast capability for prevention of cancer especially cervical cancer. Broadening the manifestations for HPV immunization and expansive testing for HPV in the screening schemes has the capacity to speed up the decrease in the incidence of cervical cancer. [25]

Screening of women has reduced the occurrence and mortality rate associated with cervical cancer. Precancerous cervical tissue damage (cervical intraepithelial neoplasia) and cervical tumors are deeply linked to the sexually accrued high-risk HPV strain infection, which bring about greater than 99% of cervical carcinomas. Screening approaches involve cytology (Papanicolaou test) and HPV testing, either individually or combined.[26]

The immune system utilizes innate and adaptive immunity to detect and encounter external elements that gain access to the body; however, these systems are occasionally incompetent against HPV. HPV possess numerous methods to circumvent the surveillance by the immune system. These viruses infect and proliferates in keratinocytes which are far off from immune foci and inherently have shorter life span. HPV suppresses the production of interferons.in spite of the properties of the viruses which aid in immune evasion, the immune system virtually holds of the bulk of HPV infections and is firmly linked to localized cell mediated immune reactions. It is suggested that vaccines against HPV 16 &18 as well as HPV 6, 11, 16 & 18 are safe, generate elevated amounts of antibodies and are successful at impeding HPV infection. [27]

Carcinogenic HPV is a common genital infection that can potentially progress into cervical carcinoma in few females. Even though women generally are rid of infection in a short time (few months), the virus prompt a shift in the direction of immune tolerance that assist in the persistence of infection and thereby enable tumorigenesis. HPV is critical in the evaluation of the probable contribution of prophylactic immunization in curtailing the incidence of cervical carcinoma. [28]

The recognition of the HPVs as the causative agent of cervical carcinomas provided the opportunity for the establishment of HPV vaccines. After two decades of recognition of HPV strain the first HPV vaccine was introduced in the market. The protection effectiveness of these vaccines against cervical cancers is comparatively high. These vaccines comprise of virus-like particles (VLPs). The VLPs of the HPV strain 6 and 11 provide effective protection against genital warts. [29]

The evidently increased sensitivity and reproducibility of HPV DNA testing for high-grade cervical intraepithelial neoplasia (CIN) has given rise to the extensive appeal to develop it as the primary screening test. However, in order to attain its maximum potential, novel strategies for HPV testing with superior specificity are required, either in the form of triage tests which comprise of HPV classification, methylation (and subsequent repression) of both host and viral genes, and improved cytological techniques or in the form of alternative primary screening methods. [30]

Presently three HPV vaccines have been endorsed by U.S. FDA to avert HPV infection namely Gardasil 9, Gardasil, and Cervarix. Each of these vaccines safeguard against HPV strains 16 and 18 which together is responsible for around 70% of cervical carcinomas. The Gardasil vaccines also shield against HPV strains 6 and 11, causative agents for 90% genital warts. Contemporary research of the substance carrageenan has exhibited encouraging outcomes in its promise to reduce HPV spread. Cervical carcinoma screening recommendations encompass the inclusion of HPV testing to cervical cytology. HPV-DNA investigations can be carried out on cervical samples by signal amplification using polymerase chain reaction. [31]

Given the unmistakable etiological association between high-risk HPV infection and cervical carcinoma, high-risk HPV testing is contemplated as an

alternate for cytological cervical carcinoma screening. Several investigative approaches have been initiated to identify the wide range of hr-HPV strains using a single test. For the purpose of screening, the identification of high-risk HPV is not essentially helpful except when it is instructive of the presence of high-grade cervical intraepithelial neoplasia or cancer. [32]

Cervical cancer is the second most prevalent potentially fatal cancer amidst women globally. HPV high-risk variants play the critical part in the etiopathology of the disorder. The postulation of cervical intraepithelial neoplasia (CIN) was initiated in 1968 as analog to the word dysplasia, which signify anomalous maturation. Cervical carcinoma advances slowly from pre-invasive CIN to invasive carcinoma and hence screening for dysplasia is a critical public health issue globally.[33]

Far reaching randomized controlled studies suggest that HPV based screening yield superior defense against cervical carcinoma than cytology singly through refined detection of premalignant disorder in the preliminary screening stage preceding progression. Females 30 years and above is recommended to be evaluated for HPV infection every 3 to 5 years. [34]

Squamous cell cancers typify the most common malignancy of the head and neck. Frequent risk elements for the head and neck squamous cell carcinoma (HNSCC) are smoking and alcohol ingestion. The link between HPV and squamous cell lesions at several regions of the body together with oral cavity was first identified in 1983 by Syrjanen *et al.*[35] HPV infection was initially accepted as causative of head and neck carcinoma. Oro-pharyngeal squamous cell and oral cancer is a disorder generally associated with tobacco and alcohol exploitation. HPV seems to possess a

causative role in numerous malignancies of the oro-pharynx region, especially tonsils and throat, and also probably a small subclass of cancers of the oral cavity.[36]

The most commonly identified HPV strain during characterization of squamous cell carcinoma (SCC) is HPV16 followed by the variant HPV18. HPV18 is the variant deeply linked to adenocarcinoma of the cervix, the incidence of which is rising in an indirectly proportional manner to that of SCC. The bivalent HPV variants 16 and 18 and the quadrivalent HPV strains 6, 11, 16 and 18 vaccines are being assessed in phase III clinical trials, and show promise to safeguard against 70% of cervical carcinomas.[37]

The frequency of occurrence of oro-pharyngeal squamous cell carcinoma (OPSCC) is increasing in comparison to the reducing frequency of carcinomas in further sub-locations of the head and neck, despite the decreased prevalence of smoking. HPV infection specifically by the variant 16 is identified as a key player at the inception of the HPV positive OPSCC, with distinct behavioral, prognostic, clinical, radiological, anatomical, biological and epidemiological attributes in comparison to HPV negative OPSCC. [38]

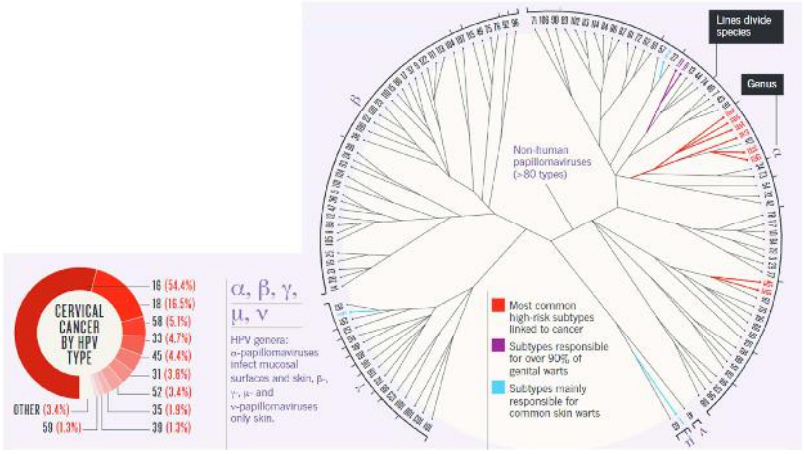


Figure 1. Mapping of HPV strains by genus. (Adapted from) [39]

Several distinct papillomavirus strains infect human beings, however, only a small subset is harmful. Mapping HPV strains by genus (Figure 1) unveil that few species frequently give rise to comparable warts and lesions, with majority of the HPV strains that are carcinogenic arising from the same species.

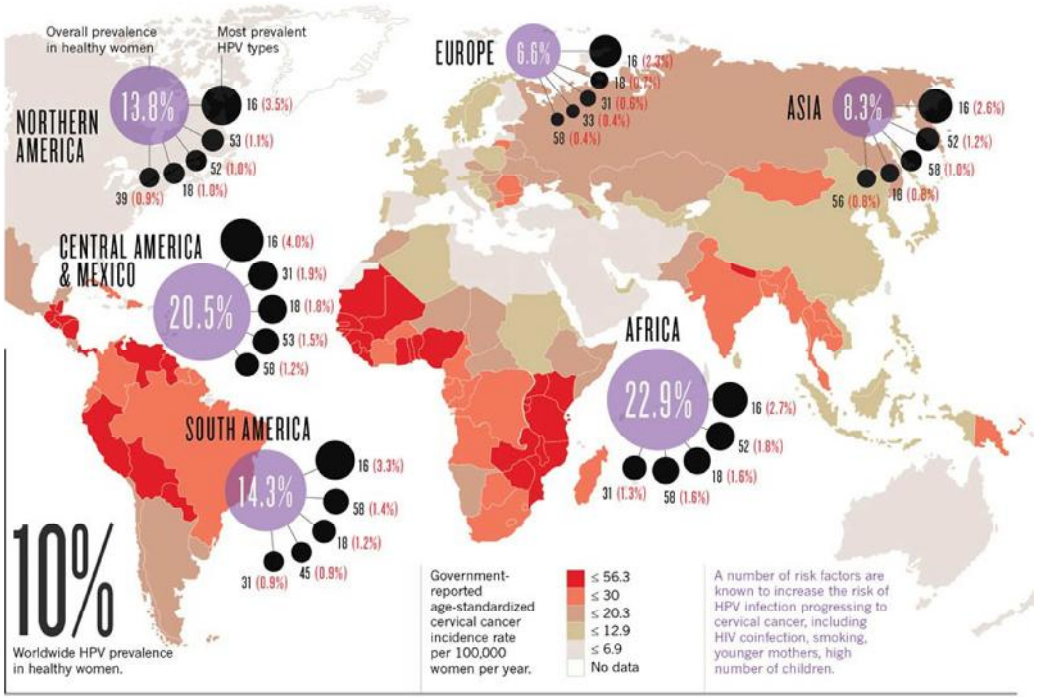


Figure 2. Global prevalence of HPV infections. (Adapted from) [39]

Cervical HPV infection incidence differ globally (Figure 2), as does the frequency of the infected females who proceed to develop cervical cancer. [39]

The global frequency of infection by human papillomavirus (HPV) in females without cervical anomalies is 11–12% with increased rates in Eastern Europe (21%), sub-Saharan Africa (24%) and Latin America (16%). The most common variants are HPV16 (3.2%) and HPV18 (1.4%). The frequency of incidence increases in females with cervical abnormalities in proportion with the intensity of the lesion extending to almost 90% in females exhibiting grade 3 cervical intraepithelial neoplasia and

invasive carcinoma. HPV infection has been recognized as a conclusive human carcinogen for six classes of cancer: penis, vulva, vagina, cervix, anus and oropharynx (inclusive of the base of the tongue and tonsils). Cervical carcinoma is the third most frequent female cancers and show a deep relationship with the stage of development, the rates of incidence being at least four-times elevated in nations described as within the low ranking of the Human Development Index (HDI) in comparison to those in the higher ranking. The all-inclusive global burden of HPV infection is most favorably evaluated by combining observations from studies utilizing well established, quality-controlled techniques to identify HPV in females with normal cervical cytology. Considerable regional disparity exist with the prevalence being the highest in Eastern Europe (21%), sub-Saharan Africa (24%) and Latin America (16%). Exceptionally high incidence is observed in Eastern Africa and the Caribbean, where rates go beyond 30% (Figure 3).

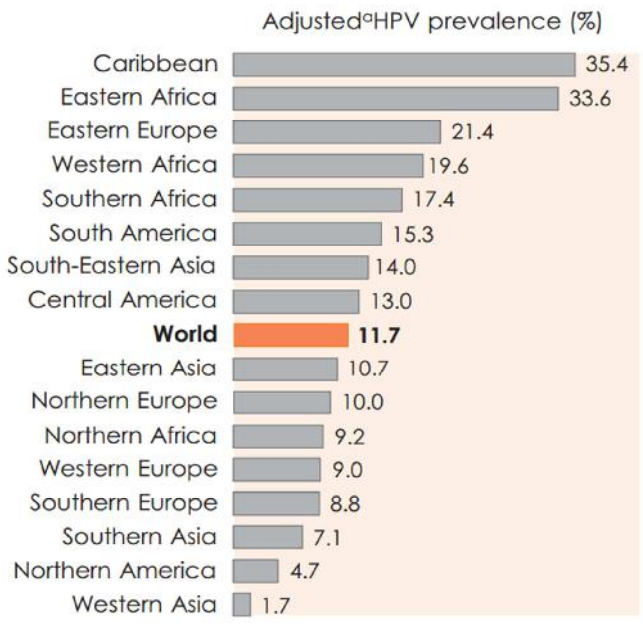


Figure 3. HPV prevalence among women with normal cytology: meta-analysis based on results from 1,016,719 women. [(40)]

In India, cervical carcinoma give rise to around 6–29% of most of the cancers in females. The National Cancer Registry Program (NCRP) has been in established since 1982, and a permanent centre (National Centre for Disease Informatics and Research) under the guidance of Indian Council of Medical Research maintains the pivotal repository of data from the participating cancer registries based in hospitals and other medical colleges/institutions spread around India. The rate of incidence of cervical carcinoma normalized according the age demonstrated wide variation among the repositories, the highest recorded incidence was 23.07/100,000 in Mizoram State, the second highest was recorded at 22.54/100,000 in Pasighat and the lowest was recorded at 4.91/100,000 in Dibrugarh district. [41]

There is a decline in trend of incidence of cervical carcinoma in India in as per the registries formed on the basis of population; however, it still remains a principal public health issue for females in India. The frequency of occurrence of cervical carcinoma is peaked at the age interval of 55–59 years, and a sizeable proportion of cases are reported in the later stages of the disorder.Specific variants of carcinogenic HPV-16 and 18 have been detected in cervical cancer subjects. Immunization against strains 16 and 18 can also be taken up taking into consideration all the associated individuals, inclusive of the parents of pubescent girls. Prevention and treatment of cervical carcinoma and thereby bringing in a reduction on the disease burden are feasible by focusing the management strategies into the high prevalence areas. In India, 122,844 females are reported to be confirmed to be affected with cervical carcinoma yearly and 67,477 of the cases succumb to the disease. With a population of 432.2 million females of ages 15 years and above, India has a high number of individuals at risk for developing carcinomas. Cervical carcinoma is the second most frequent cancers in females between the ages of 15 and 44 years. According to the

recent reports, India at 22 years, has the highest age standardized incidence of cervical carcinoma in South Asia region, in comparison to 19.2 years in Bangladesh, 13years in Sri Lanka, and 2.8 years in Iran. [42]

Cancer is a complex multi-factorial disorder, with various contributing factors, both intrinsic and environmental factors, in its etiopathology. Human papillomavirus (HPV) has been linked to the etiogenesis of many malignancies. India acutely suffers from three HPV-related malignancies, namely, cervical, oral and oropharyngeal carcinomas. HPV has been recognized as a contributing factor for malignancies and is responsible for over 600,000 cases globally per annum. [43,44] HPV has been linked to cancer incidence since its discovery in 1970s by Zur Hausen in common genital warts and cervical carcinoma. [43,45]

The rate of survival for cervical cancers have been enhanced during the last four decades on account of the huge impact of the screening strategies like Pap smear. The capability to investigate and manage females with preinvasive condition, viz. cervical dysplasia, is the critical element resulting in the decrease of the incidence of invasive cervical carcinomas. The feasibility of assessing females for the etiopathogenic agent HPV has come up as a prospective screening mechanism. Recent investigations have been directed on novel strategies for Pap smear screening like thin layer technology, the pertinent intervals for assessments and the suitable approaches of including of HPV evaluation into the screening approaches. Automated repeated screening strategies employed may help reduce the frequency of false-negative Pap smears.[46]

HIV-positive males and females are at higher risk for developing anogenital as well as oral HPV infection. The probability for HPV-related life threatening intra-

epithelial neoplasia (IN) and carcinoma are also higher.The frequency of incidence of oral, cervical and anal HPV infection among HIV-positive subjects in comparison to HIV-negative subjects heightens with the steady decrease in CD4+ levels, similar to the incidence of high-grade IN.Accumulation data indicate a direct effect of HIV infection in the etiopathogenesis of HPV-related neoplasia, however, HIV-linked debilitation of HPV determined immune feedback may facilitate the continuance of high-grade IN and ample time for aggregation of genetic modifications that are pivotal in the evolution into cancer.[47]

Infection by HPV is critical in the etiopathogenesis of anogenital carcinoma as well as its predecessors. HIV-infected subjects present an elevated abundance of HPV DNA. Numerous studies have additionally provided evidence that HIV-infected subjects have a higher frequency of squamous intraepithelial lesions (SIL) of the anus, vulva and cervix. The prevalence of invasive cervical carcinoma is higher in HIV-positive females while that of anal carcinoma in HIV-positive females and males.[48]

Human immunodeficiency virus is responsible for causing acquired immunodeficiency syndrome (AIDS). HIV infection may impact the etiopathogenesis of HPV-related cervical disorders either directly via molecular interconnection with viral genes or indirectly via modulating the immune responses of HIV-infected subjects.With enhanced antiretroviral therapy and prevention of HIV-related opportunistic infection and increased survival of females infected by HIV, infection by HPV and its frequent debilitating outcome, cervical carcinoma, probably would commandeer higher importance in the management females infected by HIV globally.

A refined comprehension of the contribution of HIV in facilitating the clinical presentation of HPV invasion is critical for the regulation of this disorder. [49]

HPV have been recognized as being high-risk or low-risk, on the basis of their association with the progression of malignancy. Women affected by HIV are at higher risk for being infected by HPV and its persistence, thereby raising the risk of anomalies in the cervical cells as well as the invasive cervical carcinoma. HIV infection results in a decrease in the frequency as well as function of the CD4+ T cells. This further leads to an increased rate of HPV, thereby reducing the opportunity for their impetuous elimination.[50,51]

HIV-associated immunodeficiency affects the genital HPV in women in a complex manner. This include higher infection risks by multiple variants, persistence, repeated activation and the risk to progress into pre-invasive and invasive conditions. Restructuring of immunity with the help of anti-viral medication enhances cellular immunity, however, the risk of HPV-associated carcinoma continues to be greater than contextual incidences and manifests at earlier ages. Advance introduction of antiretroviral therapy (ART) permits enhanced retention of immune processes via already operating antibodies and T-cell copies and thereby boost the long-term consequences. Interaction between HIV and HPV should be considered while making public health policies by concentrating on extensive prepubescent HPV-immunization initiation, prevention of secondary cervical carcinoma and early screening schemes for HIV-infected females and advance introduction of ART.[52]

Interactions of HPV and HIV facilitating the infection by the each other have been demonstrated at the cellular level. Infection of HPV assists the acquisition of HIV in males and females, additionally, HIV-infected subjects possess a higher risk for of HPV-influences dysplasia and carcinoma resultant of a continuous immune repression. Both HIV and HPV promote the diabolical circle which is responsible for

the escalation of the pandemics in certain regions on the globe. Highly active antiretroviral therapy (HAART) is perhaps advantageous in decreasing HPV infection and related tissue damage subsequent to numerous years of most favorable regulation of HIV proliferation and pronounced immune restructuring. [53]

Since the specificity and sensitivity of Pap smear is poor, cervical carcinoma screening approaches are switching from cytology-based screening to high-risk HPV testing. Although PCR testing is a sensitive and non-invasive technique for determining the occurrence of high-risk variants, however limited data are present on circulating genotypes especially among HIV-negative women. In the light of the above knowledge the current study was designed to gauge the prevalence of HPV genotypes in HIV negative women in Belagavi, Karnataka and investigated the types of HPV most frequently found in these patients.

Need for the study

1. The idea that HPV testing could play a crucial role in cervical cancer screening programs becomes more universal. Several applications for HPV DNA detection have been proposed, therefore Considering the rising importance of HPV testing, the performance of HPV tests should be carefully assessed and validated.
2. It is important to identify the viral type involved which is of interest in the prevention and treatment of infection.
3. Hence the present study is designed to investigate the presence of various HPV genotypes in cervicovaginal samples and its association with HIV positive women.

OBJECTIVES

1. To know the HPV types among women attending Department of Gynecology, KLE's Prabhakar Kore Hospital & MRC, Belagavi.
2. To determine the HPV type distribution among women of child bearing age in according to HIV status (HPV-HIV co-infection)
3. To correlate CD4 counts with HPV infection in HIV Positive patients.
4. To compare and explore variety of molecular methods for implementation in screening of cervical cancer.

REVIEW OF LITERATURE

Human Papillomaviruses (HPV)

Introduction

Papillomaviruses are compact non-enfolded viruses having icosahedral capsids with a diameter of 55nm enclosing double-stranded DNA of roughly 8000 base pairs (bp) as genetic material. They are broadly spread all around the animal kingdom, especially infecting the squamous epithelia and generating warts (anomalous tissue proliferation).[54] (Figure 6). These viruses target epithelial tissue, and is dependent on cell differentiation for completing the life cycle.[55] The whole genome of HPV exhibits a well conserved generic arrangement. All presumptive open reading frames (ORFs) are limited to a single DNA strand. The alternate strand, seemingly the non-coding strand accommodates ORFs which are preserved irrespective of location and configuration. The distinct frames are categorized into "early" (E) or "late" (L) genes unlike other DNA viruses, where the genes are activated in accordance with a particular schedule in the trajectory towards effective infection. [36]

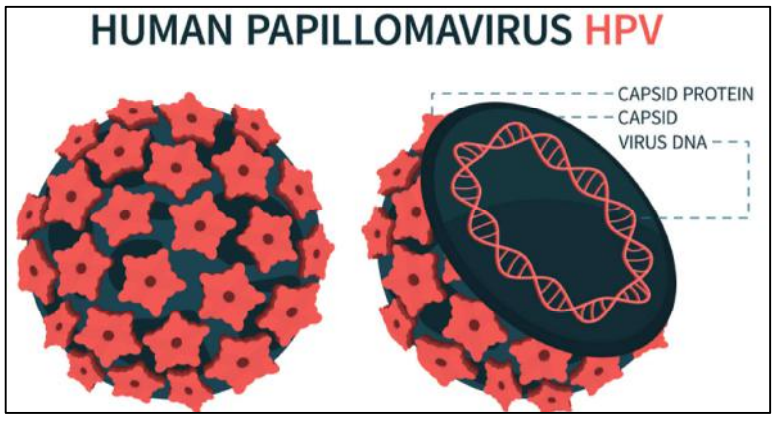


Figure 4. Internal and external structure of human papillomavirus, HPV

(Source: https://www.thegreatcoursesdaily.com/hpv-and-cervical-cancer-causes-and-cures)

Papillomaviruses possess a general non-encased icosahedral arrangement which is 50-60nm in diameter. Their genetic material consists of double-stranded episomes of roughly 8000 base pairs, which accommodate eight or nine open reading frames.(56)

HPV have been identified in a considerable number of anal, esophageal, oral, penile, vaginal, vulvar and head-and-neck malignancies in India.(57) HPVs constitute the most frequent infectious organisms that are accrued sexually worldwide, the main risk aspects are behaviors related to sexual activity. Even though nearly all infections do not show any symptoms and are eliminated within a span of 2 years, infection by genital HPV can result in clinical manifestation encompassing cervical neoplasia, anogenital warts, cervical carcinoma and other anogenital malignancies.(58)

Infection by HPV plays a causative role in the etiogenesis of cervical carcinoma, and is related to several other genital malignancies, encompassing vaginal, vulvar, and anal carcinoma. The main preventive measures with HPV immunization is safe and efficient, and a lately validated HPV vaccine imparts extensive defense against numerous tumorigenic HPV variants. Screening programs for HPV are swiftly progressing, indicating the critical role played by HPV infection in the etiology of cervical malignancies.(59)

Types

Genital HPV variants have been subclassified into low-risk strains, which are detected largely associated with genital warts, and high-risk strains, which are customarily linked to invasive cervical carcinomas.[7] Of all the studied papillomavirus strains so far, over 100 variants are contagious for human beings. These HPV strains can be differentiated into cutaneous and mucosal HPV classes, on the basis of the type of epithelial tissue they can target. Mucosal HPV variants are segregated into low-risk (LR) and high-risk (HR) HPV strains. Hr strains are tumorigenic especially, HPV 16 and 18 which are responsible for the highest percentage of HPV associated malignancies at all anatomical sites whereas, low-risk HPV strains produce warts. HPV6 and HPV11 produce genital warts or mild dysplasia, but seldom serious dysplasia.[8] Low- risk HPV strains comprises of variants 6, 11, 34, 40, 42, 44, 53, 54, 55, 57, 61, 70, 71, 72, 81 and 84. These are linked to non-malignant lesions like warts. High-risk HPV strains comprise of variants 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. These have the capability to develop into tumorigenic abrasions.[9]

HPV strains 16 and 18 have been detected in two distinct human cervical malignancies. The DNA of the viruses were cloned at the molecular level and utilized as probes to screen substantial number of genital carcinomas by Southern blot technique. HPV 16 or 18 variant sequences, were identified in high frequency in cervical cancers, nevertheless in scarce amounts in flat condylomas or condylomata acuminata. The bulk of the latter tissue damage, comprised of HPV 6 or 11 DNA sequences, which as opposed were identified seldom in malignancies in situ or invasively proliferating cancers.[60]

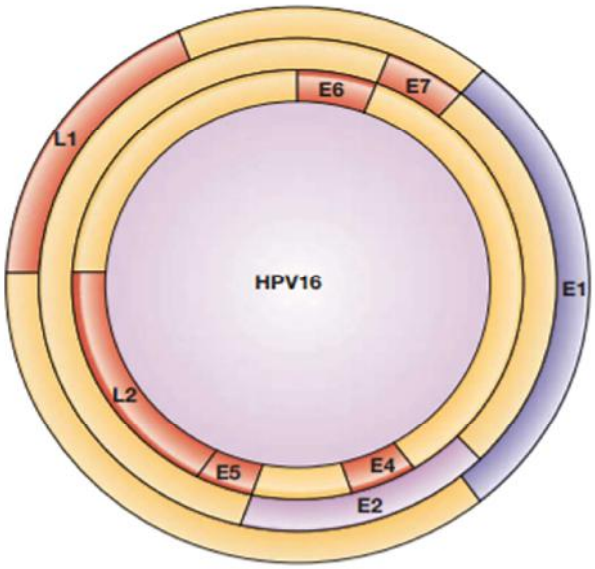


Figure 5. Schematic diagram of the human papillomavirus 16 (HPV16) genome showing the arrangement of the major non-structural and capsid genes.

The three rings draw parallels to the three ORFs using which the coding strand are translated. No gene products have been identified as originating from the antisense strand. The early viral protein (E4) is coded for by a mRNA copy that encompass the first amino acids of the E1 gene. The segments between late 1 (L1) and E6 is critical transcriptional controlling region− mRNAs coding for most non-structural (E6, E7, E1, E2, E4 and E5) and capsid (L1 and L2) genes arise in this segment. Most of the papillomavirus genetic materials parallels HPV16 in the broad arrangement (Figure 5). [(61)]

The HPV family includes roughly 200 variants that are capable of infecting the cutaneous and mucosal epithelial tissue. They are subcategorized into species and genera in the phylogenetic tree of HPVs on the basis of the sequence of DNA of the late gene (L1). The genus *Alphapapillomavirus* encompasses the mucosal high-risk (HR) HPV variant that is apparently associated with the progression of anal and

cervical carcinomas and a subgroup of additional genital tract carcinomas, like penile, vaginal and vulvar cancers in conjunction with a subgroup of head and neck carcinomas. Analogous to the high-risk HPV strains, cutaneous beta HPV strains have also been implied in tumorigenesis, even though the disease mechanism is relatively distinct. Biological and epidemiological investigations favor the prototype of collegial cooperation of cutaneous beta HPV strains and UV radiations in the establishment of cutaneous squamous cell carcinomas (cSCC).

Infection by beta HPV seems to have a critical role in the preliminary stages of skin tumorigenesis, however it is not necessary for the survival of the cancer cells once they have turned malignant. The initial cutaneous beta HPV variants HPV 5 and 8 were derived from the cutaneous tissue of individuals with epidermodysplasia verruciformis (EV), an autosomal recessive hereditary disorder outlined by the establishment of wart-like tissue malignancies in several regions of the body that often evolve into cSCC in areas exposed to UV. Beta HPV variants are subclassified into 5 distinct species, beta-1, beta-2, beta-3, beta-4, beta-5 where beta1 and beta 2 HPVs are the most prevalent strains in the skin. Other variants are seldom identified in skin. [(62)]

The International Human Papillomavirus (HPV) Reference Center supports quality and order in HPV research and diagnostic. The center allots HPV strain numbers to newly discovered HPV strains, retains a reference cloned archive, and supply international proficiency panels for HPV genotyping. The currently identified HPV strains till HPV225 can be classified into 5 distinct genera: alpha (65 strains), beta (54 strains), gamma (98 strains), mu (3 strains), nu (1 strain). Since 2014, 23 new variants have been discovered, 82.6 % of these come under the genus gamma.[(63)]

The HPV strains identified in humans belong to five genera, the alpha and the Beta/Gamma constituting the biggest groups HPV variants from the alpha genus are frequently categorized as no-risk cutaneous, low-risk mucosal or high-risk. The high-risk variants are established as "human carcinogens" based on the epidemiological data. The rest of the high-risk variants are "probable" or "possible" carcinogens (Figure 6). [64]

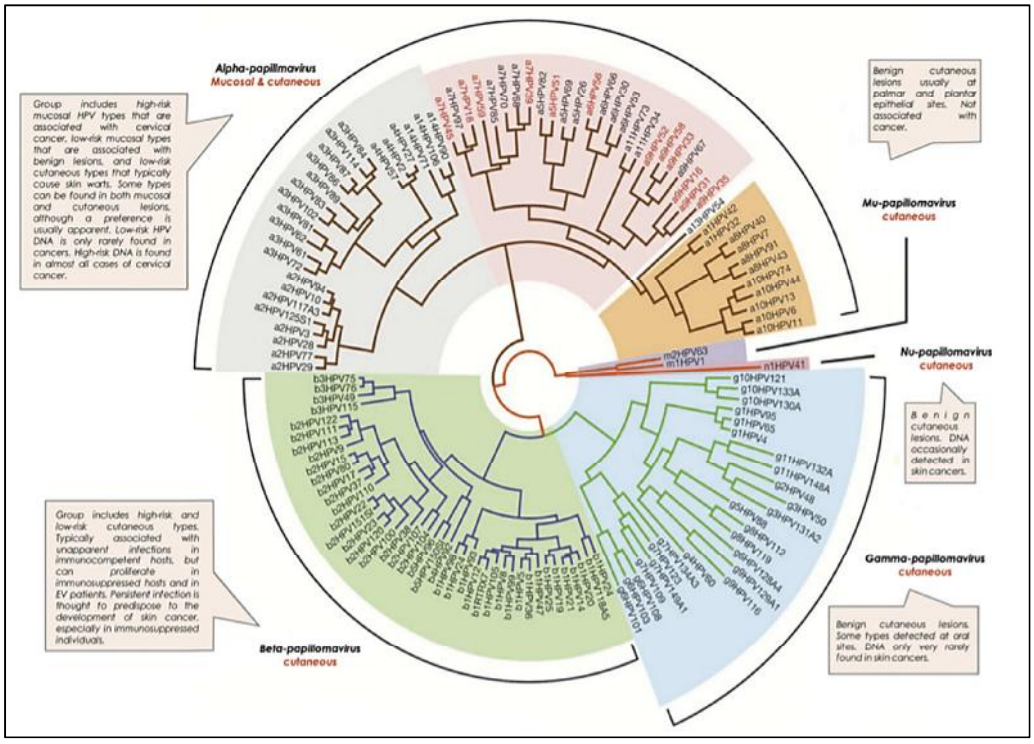


Figure 6. Evolutionary Relationship between Human Papillomaviruses.

HPVs consists of five evolutionary classes with distinct epithelial tropisms and disease correlations. The alpha papillomaviruses encompass the low-risk mucosal variants (a number of which are inside the orange shaded section) that are causative of the genital warts, and the high-risk mucosal variants (accommodated within the section highlighted in pink) that cause cervical neoplasias and carcinoma. Even though the cutaneous HPV variants (most of which are accommodated in the grey

(Alpha), green (Beta) and blue (Gamma) shaded sections) are not frequently linked to malignancies, some of the beta strains have been involved in the progression of non-melanoma skin cancers (NMSC) in immune compromised subjects as well as in individuals suffering from epidermodysplasia verruciformis (EV).

Table 1. Protein Function and Gene Expression in High and Low-Risk HPV Disease.

	High-Risk Alpha	Low-Risk Alpha
E6	encodes E6* products	no E6* products
	binding and degradation of... •p53 •specific PDZ-domain proteins (e.g. Dlg, MAGI-1, Scribble)	weaker binding (no degradation) of... •p53 •no binding of PDZ-domain proteins
	interact with the E6AP ubiquitin ligase inhibition of p53 transactivation and acetylation	
	inhibition of apoptosis	unknown
	bypass of growth arrest following DNA damage	normal growth arrest following DNA damage
	inhibition of keratinocyte differentiation	unknown
	inhibition of interferon response	weaker inhibition of interferon response
	activation of signaling pathways... •Akt •Wnt •Notch •mTORC1	unknown
	telomerase activation	no activation
	c-myc activation	no activation
E7	binding and degradation of... •pRb •p107 •p130 binding (no degradation) of... •E2F1 •Cullin2 •HDAC	weaker binding (no degradation) of... •pRb •p107 •E2F1 binding of... •p130
	binding of regulatory proteins including E2F6, p600, HAT, PP2A induction of cell cycle entry and DNA synthesis role in genome amplification	
	induction of genome instability	no stimulation of instability
	suppression of STAT-1 function	no suppression
	immortalization and transformation functions	no such functions
	activation of signaling pathways... •Akt	unknown

The proteins E6 and E7 of the high-risk and low-risk HPV variants have distinct roles, which indicate their divergent biologies. The capacity of the high-risk HPV strains to regulate cell proliferation in neoplasia is contemplated as indicative of the capability of their E7 protein to attach and disintegrate members of the Retino-blastoma protein (pRb) family, as well as the capability of E6 to successively degenerate p53 and to weaken the effect of PDZ-domain proteins that modulate cell contact and signaling cascades. In the life cycle of the viruses the E6 and E7 proteins have critical function in guiding S-phase reactivation in the outer epithelial layers to facilitate viral DNA proliferation. This also necessitate the E1 and E2 proteins, which rise in abundance subsequent to the "late" promoter upregulation (viz. p670 in HPV 16) in cells which persists to express E6 and E7 from the early promoter (viz. p97 in HPV 16).[65] In the situation of low-risk HPV variants, genetic amplification necessitates cell cycle reactivation in the middle to upper epithelial layers on the contrary to occurring in tissue that have continued to being in cycle after being removed from the basal layer. In case of both high-risk and low-risk HPVs, amplification of the genome continues as the infected cell progresses from S-phase to G2- like phase prior to perpetrating into complete differentiation. [56,66,67]

The low-risk and high HPV variants have remarkable variations in the promoter location and regulation, in addition to the mRNA splicing motifs. These distinctions influence the expression of the E6 and E7 genes.[11,55] Distinct motifs of viral protein synthesis (in addition to the differences in the protein functions) have critical role in influencing the disease manifestation subsequent to the infection.[64]

Papillomavirus (PV) derivatives are generally catalogued as “types”. Several PV variants have been identified in human beings, the single extensively investigated host. Approximately 100 human HPV variants have been reported on the basis of the information from the genetic material isolated so far, however, a greater number of variants are assumed to prevail on the basis of the already identified subgenomic amplicons. Several of these HPV strains have revealed to be ubiquitous and spread worldwide.[68]

The beta genus consists of greater than 50 beta HPV strains that are reckoned to be implicated in conjunction with ultraviolet (UV) radiation in the progression of non-melanoma skin cancer (NMSC), the most frequent type of human carcinoma. HPV5 and HPV8, members of the genus beta, were initially detected in individuals with the genetic condition epidermodysplasia verruciformis (EV) that favors greater risk for predisposition to infection by beta HPV and NMSC progression. It is evidenced that recipients of organ transplant who have compromised immune system have an increased risk of NMSC. Beta HPV strains gave rise to the theory that they may be instrumental in the tumorigenesis of skin cancers in non-EV subjects. [69]

HPVs are classified on the basis of the homology of the nucleotide sequence of the L1 gene, the most conserved segment of the viral genome and codes for vital capsid protein. On comparison of the DNA sequences from various isolates, HPV strains from distinct genera within the family *Papillomaviridae* share similarity of less than 60%. Within a genus, distinct species share percentage similarities between 60% and 70%. [63]

Table 2. Classification of papillomavirus

Group	Prototypes	Site of infection	Acute consequences	Chronic consequences	Other features
Cutaneous	HPV1, HPV2, BPV1	Skin	Warts	None	Synchronous regression, lasting immunity
Mucosal	HPV6, HPV11, COPV, ROPV	Genital mucosa	Warts	None	Slow resolution in immunosuppressed individuals
Mucosal high risk	HPV16, HPV18, HPV31, H. 33, HPV45, BPV4	Anogenital mucosa (other mucosal surfaces)	Flat lesion (CIN 1)	~2% persist, ~1% progress to invasive cancer	Slow resolution in immunosuppressed individuals, variable malignant potential
Cutaneous high risk	HPV5, HPV8, CRPV	Skin	Flat lesion or none Warts	Promotes SCC (?)	SCC more common in immunosuppressed individuals

BPV, bovine papillomavirus; CIN, cervical intraepithelial neoplasia; COPV, canine oral papillomavirus; CRPV, cottontail rabbit papillomavirus; DTH, delayed-type hypersensitivity; HPV, human papillomavirus; ROPV, rabbit oral papillomavirus; SCC, squamous skin cancer.

The classification of papillomaviruses is tabulated in Table 2. Papillomaviruses are classified into cutaneous, mucosal, mucosal high-risk and cutaneous high-risk. [61]

Investigations into HPV has mostly been dominated by the research into a subgroup of alpha papillomaviruses that in conjunction is responsible for approximately 5% of human carcinomas globally, with focal point being HPV16 and 18. Attempts have always been to distinctly identify these viral types, typically known as hr HPV, apart from the other 200 common HPV strains that generally produce only noncancerous epithelial tissue damages. Persistant laryngeal papillomas, which seldom occur in children and adults, necessitates methodical surgical de-bulking to permit breathing. Typical infections are incurable, and in spite of being produced by HPV11, a low-risk variant are linked to 1-3% risk of developing into cancer if not cleared up.[70]

Infection

HPVs are ubiquitous, well acclimatized to their host and intelligently isolated from the immune system responses. Infections by HPV may be productive, subclinical or latent in both mucosa and skin. Papilloma viruses favored infecting

differentiating squamous epithelium and virtually any region of skin can be targeted in human beings. The causative relationship of HPV to cervical carcinoma, and more and more with the increasing numbers of squamous cell carcinoma at alternate sites in both males and females, is gaining recognition. HPVs are categorises into three main classes: mucocutaneous, cutaneous and those related to the autosomal recessive condition, *Epidermodysplasia verruciformis (EV).* [(71)]

More than 120 distinct variants of HPV have been identified so far; more than 40 of these variants invade the epithelial layers lining the mucosal areas like the anogenital tract. In most of the subjects, infection by HPV are not permanent and are non-symptomatic with almost all new infections clearing up within the span of 2 years. Infection by HPV has been strongly evidenced to be the primary causative agent of cervical carcinomas. It is unclear why HPV infections get eliminated in few subjects while in others they give rise to cervical intra epithelial neoplasias. Numerous factors including individual sensitivity, state of the immune system and nutrition, exogeneous and endogenous hormones, tobacco smoking, number of offspring, comorbidity with other sexually accrued agents like HIV, *Chlamydia trachomatis* and herpes simplex virus type 2 as well as viral attributes viz. HPV strain, concomitant infection with other variants, viral load, HPV type and viral integration have been implicated. [(72)]

The whole papilloma virus comprises of a protein coat (capsid) encircling a circular, double-stranded DNA assembled as coding and non-coding sections. Eight early (E1-E8) ORFs and two late (L1, L2) ORFs have been isolated from the coding segment of most of the papilloma viruses. The early ORFs code for the proteins which communicate with the genome of the host to synthesize new viral DNA whereas late

ORFs are turned on subsequent to viral DNA replication and code for the capsid proteins of the viruses. Most of the papilloma viruses are necessarily intranuclear beings with particular tropism towards keratinocytes. Three probable events can occur after the entry of papilloma virus into the cells: (1) viral DNA remain as intra nuclear, extrachromosomal episomes, which replicates along with the host cell in a synchronous manner setting up a latent infection; (2) conversion of the latent infection into a productive one with the congregation of the whole infective virions; (3) integration of viral DNA into the cellular genome of the host, a mechanism observed in HPV infections related with malignant transformation. [(73)]

HPVs are compact, double-stranded DNA viruses that bring about lesions in mucosal and cutaneous tissue and are accountable for cancers of the penis, vulva, vagina and cervix. HPVs are categorized into 5 genera adding up to roughly 150 species that have been sequenced. Its genome consisted of an early (E) segment coding for the viral regulatory proteins, a late (L) segment coding for the viral structural proteins and a noncoding segment that is necessary for the viral life cycle. In order for the infection to happen the virus is required to access the basal epidermal lining where subsequent to endocytosis and disassembly of the viral capsid, the L2 protein facilitates viral genome transference into the nuclei of proliferating keratinocytes. The viral genome remains in the episomal configuration in the normal life cycle and duplicates in a synchronous manner with the host genome under the influence of the viral proteins E1, E2, E4 and E5.[(6)]

HPVs are compact circular DNA viruses that produce warts. Infection by high-risk anogenital HPVs like HPV 16 is linked to human malignancies especially cervical carcinoma. The life cycle of HPVs is deeply tied to the stages of

differentiation of the host epithelial tissue and has two different phases: the non-productive phase and the productive phase. During the nonproductive phase, which occurs in the crudely differentiated basal epithelial section of a wart, the virus continues to maintain a low-copy-number of the nuclear plasmid. In the productive phase which happens as the host cell goes through terminal differentiation, viral DNA is multiplied; the capsid genes L1 and L2, are activated and expressed; and progeny virions are generated. This phase of the viral life cycle depends on the capability of the virus to dedifferentiate cells to assist DNA synthesis.

Papilloma viruses code for multiple oncogenic proteins like E5, E6 and E7. The HPV 16 genome comprises of eight viral genes coding for six nonstructural and two structural proteins. Three of the nonstructural proteins, E5, E6 and E7 are well accepted as oncogenic due to their capability to alter cell in vitro and the E6 and E7 proteins produce tumors in vivo. The nonstructural proteins E1 and E2 are implicated in DNA amplification and the transcription of the viral genome. E4 is speculated to influence the duplication of the viral DNA indirectly during the productive phase. The major and minor capsid proteins being L1 and L2 respectively. [(74)]

HPV initiates its life cycle by invading the basal cells of the epithelial tissue within cells that are replicating; the genomes of viruses are duplicated, retained and transmitted on to the progeny cells. The stratified epithelium hosts the HPVs. It is a complex tissue made up of stratified layers of non-dividing cells in several phases of terminal differentiation, the topmost layer remains the most differentiated. The cells occurring at the lowest layer of this tissue alone, the basal cells, multiply. Even though the lifecycle of the HPV starts with the invasion of the basal cell, it becomes complete only after the infected cell approaches the upper strata of the epithelium. As

a consequence, the HPV DNA originally occurs in the nucleus of an actively dividing cell, but subsequently in that of a non-dividing (differentiating) one. This series of chronological of stages of cell proliferation that has been regulated by the virus has paved the way utilizing which HPV duplicates its DNA over the various locations throughout its life cycle. Soon after infection by the papilloma virus, copy number of the viral DNA is increased to a particular level (50 to 400 per cell). Subsequent to host cell differentiation, the viral DNA goes through one more amplification cycle leading to an elevated copy number of HPV DNA up to hundreds to thousands per cell. This is succeeded by packing of the viral DNA into virus particles. [(75)]

Papilloma viruses (PVs) initiate their productive life cycle in layered mucosa or epithelium, where the undifferentiated multiplying keratinocytes are the preliminary targets for the productive infection by the virus. These groups of cells are complex, comprising of numerous distinct cell variants most of which include tiered sheets of keratinocytes in multiple stages of differentiation. The rest of the cells, up to 20%, include Merkel cells, melanocytes and Langerhans cells. There are two classes of proliferating keratinocytes in the epidermis steadily cycling undifferentiated stem cells, and the cells having ability of transient multiplication in basal cell compartment. These undifferentiated multiplying keratinocytes are the preliminary target for productive infections by the papilloma virus and initiation of latent infection. Few infected cells lose contact of the basal membrane and ascend into the suprabasal section of multiplying cells, where they initiate latently infected multiplying cell groups. Ensuing sequences in the life cycle of the virus are essentially dependent on the differentiation of the epithelium of the host. The triphasic multiplication replica has been suggested for PVs. A fruitful infection of a keratinocyte activates the primary amplification of copy number of PV DNA. This is succeeded by the steady

maintenance stage of the HPV genome per cell. Eventually, vegetative duplication of the viral DNA is carried out. The HPV duplication cycle requires three weeks as this is the time period necessary for the keratinocyte to sustain the complete differentiation life cycle. [76]

Papillomaviruses target epithelial cells, and is dependent on the epithelial differentiation for fulfillment of their life cycle. The production of viral proteins is tightly regulated as the affected basal cell move to the epithelial surface. Production of E6 and E7 proteins in the lower epithelial tier guides cells into S-phase, which set up an environment that is beneficial for viral genome duplication and cell division. Genome duplication, which is a requisite for the assembling of infectious virions, is stopped until the stage where viral proliferation proteins rise, and is dependent on the concomitant expression of numerous viral proteins. [55]

Papillomaviruses are a family of compact non-enveloped DNA tumorigenic viruses whose infection generally gives rise to benign epithelial lesions (warts). Few variants of HPVs, like HPV-16, 18 and 31, have been identified as responsible for cervical as well as anal carcinoma and the infections by them, which are accrued via sexual activity, are linked to more than 95% of cervical malignancies. Papillomaviruses target keratinocytes occupying the basal layer of tiered squamous epithelia and multiply in the nuclear area of infected cells in a manner which is differentiation dependent. Viral protein production in the affected cells relies on the cell differentiation and is closely controlled at the transcriptional and post-transcriptional stages. A critical characteristic of all papillomavirus transcripts is that they are generally synthesized in bicistronic or polycistronic configuration comprising

of two or more ORFs and are poly-adenylated at each or both early or late poly (A) site.

The past decade has seen remarkable development in delineating that the complex viral gene expression is modulated at the transcriptional level (through DNA methylation) and specifically at post- transcriptional level (via RNA splicing, polyadenylation) and finally at the translation level. Current understanding of the mRNA configuration and RNA processing has supplied some indications about controlling the viral oncogene expression.[(10)]

In the cells which are infected, the HPV genome occurs as extra chromosomal component (episome) of roughly 8 kilobase pairs that codes six to eight ORFs. Owing to their restricted coding capability, HPVs aids viral duplication by controlling the host cell DNA duplication and repair mechanism. The HPV lifecycle is closely linked to epithelial differentiation of the host keratinocytes, in which the productive stages of the viral life cycle is limited to the terminally differentiating suprabasal tissue of the epithelium. HPV invades the actively multiplying, undifferentiated basal keratinocytes of the tiered squamous epithelium that are speculated to be exposed due to a micro-lesion. Two promoters of the viral genes, early and late, modulate the gene expression and are triggered at different phases in the life cycle. The HPV life cycle is made up of three phases of replication.

The HPV target the multiplying basal cells of the tiered epithelium revealed through a microscopical wound. Upon gaining entry, viral genomes are set up in the nucleus of affected cells as episomes, early viral proteins (E1, E2, E6, E7) are produced and the virus rapidly duplicates to 50-100 copies per cell. HPV episomes are sustained at low copy number in an actively proliferating basal keratinocytes by

duplicating in synchrony with the cellular DNA. As the infected cell duplicates one cell is retained in the basal layer, while the other cell ascends to the next layer and triggers epithelial differentiation. Differentiation initiates the productive stage of the viral life cycle, leading to viral genome amplification to thousands of copies per cell, the late gene expression and assembly of virions and subsequent release. The early promoter stays active permitting continued expression of the genes E6 and E7 in differentiating cells. While differentiation conventionally led to an exit from the cell cycle, the E6 and E7 proteins removes the cell cycle regulations to make the differentiating cells return to the cell cycle supplying HPV access to the cellular substrates necessary for productive viral replication. Expression of L1 and L2 in the topmost layers of the epithelium leads to the assembly and release of virions. [(77)]

The life cycle of high-risk HPVs is related to the epithelial differentiation with virion production limited to highly differentiated suprabasal cells. Two main viral promoters guide high-risk HPV gene expression and their functions rely on differentiation. The early promoter regulates expression in undifferentiated cells and triggers initiation of viral proteins at regions upstream of the E6 ORF. These polycistrons code for E6, E7, E1, E2, E4 and E5 and stop at polyadenylation sites situated downstream of the E5 ORF. The viral proteins E1 and E2 have also been evidenced to bind upstream of the early promoter segment and have varying roles in episome replication and amplification. E1 binds to the origin of replication and has a consequential helicase activity. E2 acts to load E1 unto the viral origin and functions as a regulator of early expression through selective binding to four sites within the upstream regulatory region. [(78)]

Papilloma viruses display a strong epitheliotropism and specifically infect the epithelial cells of the skin and mucus layers for persistent infection to happen, the virus molecules must penetrate deeper epithelial strata via abrasion or other microtrauma and infect basal cells that have the ability for cell division (stem cells or transient amplifying cells). The synthesis of infectious virions (productive infection) happens only in the homologous species and only in highly-differentiated epithelial layers. In the deeper strata, the early viral proteins are synthesized. As the differentiation increases amplification of the genome happens; expression of the capsid proteins L1 and L2 happens in the upper keratinized layers. These encircle the viral genome compressed by cellular histones to give rise to mature infectious viral molecules which are finally released as the keratinocytes and sloughed off. The viral E4 proteins are capable of destroying the intra cellular filament network of the keratinocytes. This system allows the secretion of the viruses from sloughed off keratinocytes without prior cytolysis and associated inflammation and thereby activate an effective antiviral immune response.

High-risk HPV can result in oncogenic activity in rare cases. As the level of dysplasia raises, viral DNA is also most frequently combined into the genome of the host cell. The resultant over expression of the oncoproteins E6 and E7 give rise to immortalization of the cell and instability of the genome. With the aggregation of genetic alterations, malignant transformation can happen and invasive cancers can develop. [(79)]

Laryngeal carcinoma is the most prevalent head and neck malignant cancer and its major pathological type is laryngeal squamous cell carcinoma (LSCC). In recent years, the frequency of LSCC has been on the rise, seriously threatening the

life and health of humans. Numerous studies have indicated that infection with high-risk HPV (specifically HPV 16 and 18) and the expression of the early coding genes E6 and E7 may be critical risk factors for the incidence of laryngeal carcinoma.

HPV infection majorly influences the incidence and progression of laryngeal cancer by merging into the host cell genome, triggering the expression of proto-oncogenes and inhibiting the expression of tumor suppressor genes. Its DNA go into the nucleus in a free state, and subsequently integrates into the host cells genome immediately after HPV infection occur through the disrupted laryngeal mucosal epithelial cells to the host cells. The merging of the HPV DNA can result in genetic mutation and gene expression anomalies of the host cells, which give rise to cellular metabolic disorder and abnormal proliferation. The expression of high-risk mRNA has a critical role in the initiation and progression of laryngeal carcinoma. Infection by HPV triggers laryngeal carcinoma mostly due to the elevated expression of high-risk HPV E6 and E7 proteins. When HPV infects laryngeal cells, it can result in elevated expression of HPV E6 and E7 proteins thereby influencing the stability and functioning of the proto-oncogenes and tumor suppressor genes directly or indirectly. (80)

Risk factors for HPV infection and cancer

High-risk HPV are recognized as causative agents for the malignancies of the anus, cervix, vulva, penis, vagina and oropharynx. Behavioral risks like number of sexual partners, age at the time of first sexual intercourse, and partner's sexual behavior are linked to an increased risk of infection by HPV, continuing of the infection and the occurrence of neoplastic precursor lesions. HPV infection of the genital tract is considered to be the most frequent sexually accrued virus. The

frequency of this infection is dependent on the age and is greater in females between the ages of 15 and 25 years. Continuous HPV infection is necessary for cervical carcinoma and its predecessor lesions. The elevated risk of HPV infection in younger females have been linked to the absence of adaptive immune reactions and or the comparatively substantial area of cervical epithelium developing into squamous metaplasia.[81,82]

Risk factors for HPV infection comprises of early onset of sexual activity as well as multiple sexual partners. Factors posing a risk for viral persistence and development to high grade cervical pre-cancers and invasive cervical carcinoma encompasses exogenous agents like tobacco smoking, high parity and comorbid infection with additional sexually transmitted infections; viral factors viz. HPV genotype, specifically HPV 16 and 18, viral load and HPV types; and host cofactors like factors associated with immune response.[83]

HPV is evidenced to be the most prevalent sexually transmitted infection (STI). Since it is contemplated to be an STI, several risk factors for cervical HPV infection relate to the sexual behavior. Happening to be under the age of 16 years at the time of first sexual intercourse and higher number of sexual contracts, long-term use of oral contraceptives, smoking and HIV infection raises the risk of HPV infection. In comparison to cervical HPV infection, anal HPV infection is linked to the sexual behavior in females, the number of sexual partners during the lifetime and presence of HPV infection at the genitals were observed to raise the risk of anal HPV infection.[8]

Cancer of the cervix is one of the chief malignancies in the developing countries like India. Considering this, health care schemes will have to be

reconceived. Majority of the data are obtained from the developed nations, which depend mainly on cytology screening on routine basis. This nevertheless may not be possible in developing nations due to of different constraints. Thus, alternate strategies are necessary based on risk reduction methods. The critical life styles linked to cervical carcinoma and which are compliant to fundamental prevention approaches via health education, legislative strategies, behavioral interventions and modification of the health care seeking behavior were recognized. These factors mostly concern with early sexual debut, multiple sexual partners, menstrual hygiene and unprotected sex. Influence of male partners has also been described in the mechanism involved in cervical carcinogenesis. [84]

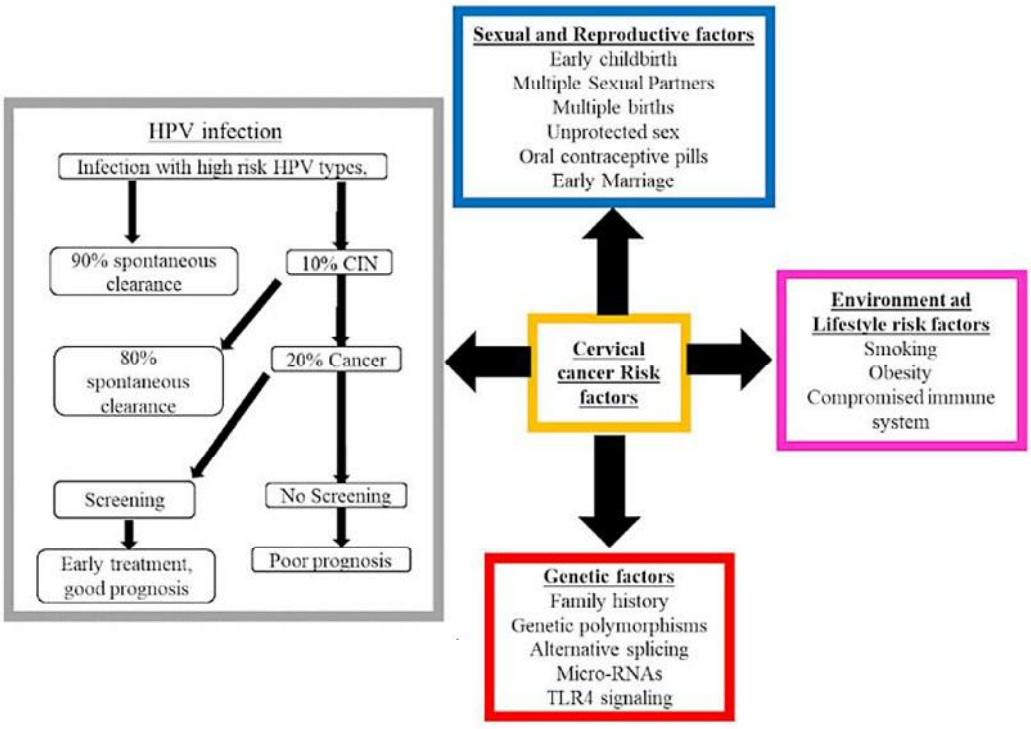


Figure 7. Risk factors associated with the development of cervical cancer. HPV, human papilloma virus; CIN, cervical intraepithelial neoplasia; TLR, toll-like receptor

Infection by HPV is the most critical risk factor linked to the progression of cervical cancer. The risk of HPV infection is related to a number of reproductive and sexual factors, which additionally raise the risk for the development of cervical carcinoma.[85]

HPV related HIV infection

HPV infection of the lower genital tract is regarded as the critical factor in the establishment of neoplasia. HIV infection seems to modify the natural history of HPV-related oncogenesis, but its influence on gynecology has been described recently. Anal HPV infection and anal squamous intra-epithelial tissue damage have been established to be highly frequent among HIV-positive homosexual males and women as well. HPV infection and related lesions are also seen in body regions other than anogenital area, specifically the skin and oral cavity.[86]

HPV infections have crucial part in the etiopathogenesis of the anogenital carcinoma and its predecessors. HIV-infected subjects show a high amount of HPV DNA and further studies exhibit that HIV-infected individuals have an elevated prevalence of squamous intraepithelial lesions (SIL) of the anus, vulva and cervix.The frequency of occurrence of cervical HPV is thrice higher in HIV-positive females than in HIV-negative females. Condyloma acuminate are HPV-associated benign epithelial tumors detected on cutaneous layers chiefly the resultant of the non-oncogenic HPV variants 6 and 11. Among HIV-positive females, the frequency of HPV 6 and 11 is remarkably high in comparison to the HIV-negative females.[48,87]

HIV-positive males and females are at higher risk of anogenital and oral HPV infection. The risks for HPV-linked high-grade intra-epithelial neoplasia (IN) and

carcinoma are also elevated. The frequency of occurrence of oral, anal and cervical HPV infection in HIV-positive subjects is higher when compared to the HIV-negative subjects with progressively lower CD4+ levels, as does the incidence of high-grade IN. [47]

Anogenital HPV infections continue to be highly frequent and persistent in HIV affected subjects. HIV is linked to a varied diversity of HPV strains and a high occurrence of anogenital cytological anomalies. The prevalence of anogenital HPV-associated cancers continues to be on the rise in the highly active antiretroviral therapy age, increasing concerns regarding HPV infections as a potential health burden among HIV-infected subjects. Interfering measures aimed at stopping HPV infections with immunization require to be contemplated in HIV-infected subjects.[88]

HPV is a frequent sexually accrued virus and a critical etiologic factor in head and neck malignancies. HIV-infected subjects are at higher risk of developing oropharyngeal cancers (OPC) when compared to the general population. HPV-positive OPC's are also more and more a crucial cause of morbidity and mortality for HIV-infected subjects in the era of efficient combination antiretroviral therapy. Oral HPV infection is rarer than anal infection, and more frequent among HIV-infected individuals than the general population. Hence, prevention of OPC is critical in HIV-infected subjects.[89]

Mechanism of HPV infection

HPV is the main etiological factor in the progression of cervical carcinoma. Dysregulation of viral gene expression and instability of the host genome play a pivotal role in virus-mediated tumorigenesis. Vital events like viral genomic integration and epigenetic alterations may result in the dysregulation of viral as well

as host gene expression. HPV integration is initiated with a double strand break brought about by either oxidative stress or by HPV proteins and the ensuing steps are guided by the response towards DNA damage. Oxidative stress and inflammation could be regarded as cofactors in triggering viral integration and dysregulation of cellular and viral oncogenes through the development of cervical malignancies.[90]

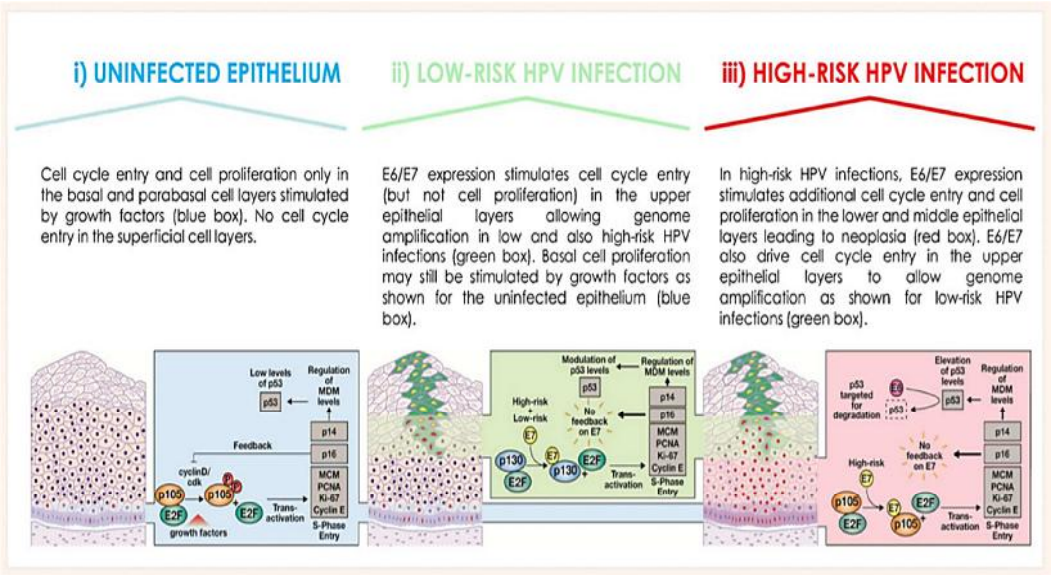


Figure 8. Regulation of Cell Cycle Entry and Proliferation in Infected and Uninfected Epithelium

The functioning of the viral proteins influences the disease phenotype. This is evident when the effect of the high- and low-risk E6 and E7 proteins are taken into consideration in the context of disease. Distinct patterns of viral protein synthesis as well as protein functions have a chief role in deciding disease manifestation ensuing infection (Figure 8).

Uninfected epithelium. In an uninfected epithelium (left), entry into cell cycle (red nuclei) and cell proliferation in the basal/parabasal cell strata is regulated by growth factors that trigger the response of G1 cyclins encompassing CyclinD/Cdk.

CyclinD/Cdk phosphorylates pRb (Retinoblastoma protein) and dislodges it from E2F, which permits the transactivation of genes required for progressing into S-phase. According to this controlled stimulation of entrance into the cell cycle, p16ink4a creates a negative feedback loop that reduces cyclinD/cdk activity, thereby blocking the over-expression of itself and other E2F-activated genes (MCM, Ki-67, PCNA). Due to this, levels of p14arf stays low, which permits MDM to sustain its normal function of degrading p53. The molecular mechanism and their control are depicted on the right of the diagrammatic representation of the epithelium.

Low-risk HPV infection. In tissue damage caused by low-risk HPV variants (centre), it is speculated that basal cell multiplication is mainly controlled by the presence of growth factors, as is observed in uninfected epithelium (left). The principal role of the HPV E6 and E7 proteins in these lesions is to guide cell cycle entry over the basal layer to facilitate HPV genome amplification (red nuclei in mid epithelial layers). It is speculated to be dependent on the capability of E7 to adhere to the Rb (Retinoblastoma) family protein p130, and to remove it and the related E2F4 and the five transcriptional repressors from target promoters necessary for S-phase gene expression (i.e., without the need for p130 phosphorylation). The transcriptional activators E2F1,2 and 3 can then engage these vacant sites and trigger the expression of the host genes required for DNA replication and progression through cell cycle (e.g., PCNA, MCM, CyclinA, CyclinE). Cells producing HPV E4 protein are depicted in dark green, with L1 expression depicted in yellow. Cells in the proliferation cycle are represented with red nuclei. The molecular cascade involved is depicted to the right of the diagrammatic representation of the epithelium.

High-risk HPV infection. In high-risk HPV infections (right), an auxiliary role of the high-risk E7 protein results in the displacement of E2F 4 and 5 from Rb and p130 without the necessitating Rb phosphorylation. The lack of effective blockage of cell cycle progression by p16ink4a can result in it accumulation in the cell and thereby an increase in MCM, Ki-67 and PCNA levels all over the infected epithelial layers. The analogous increase in p14arf levels ameliorates the normal function of MDM in degrading p53, which ultimately results in an increase in p53 abundance. P53-regualted cell cycle arrest is however impeded in the proliferative cell strata by the high-risk E6 proteins, which is associated with E6AP and controls the ubiquination and proteosomal degradation of p53. Recent investigations have indicated that few biomarkers of high-risk HPV infection (such as p16ink4a) may be also be switched on by E7 utilising epigenetic programming.[64,91]

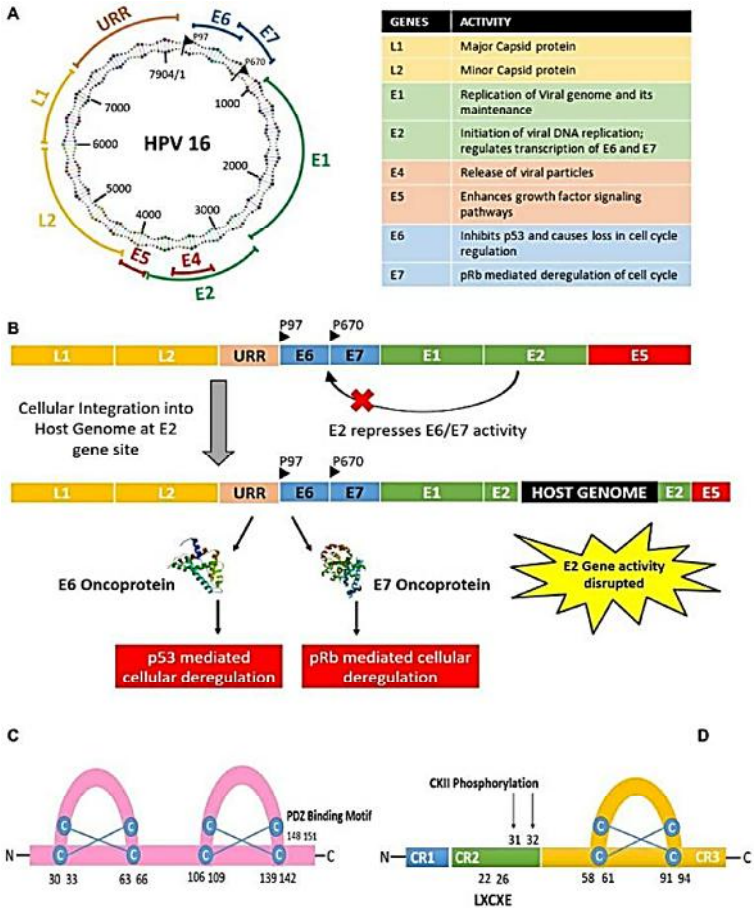


Figure 9. (A) Structure and organization of HPV16 genome. (B) Integration of HPV genome into the host genome via disruption of the E2 gene leading to the expression of the oncogenes E6 and E7. (C) Structure of E6 oncoprotein. (D) Structure of E7 oncoprotein.

The HPV genome configuration and the oncogenes are being discussed here with reference to high-risk HPV16 variant (Figure 9). The HPV16 genome is a 7.9 kb long nucleotide chain, divided into three segments: the early gene-coding section (E), the late gene-coding section (L), and the long control region (LCR), also called as non-coding region (NCCR) or upstream regulatory region (URR). These gene segments contain two polyadenylation (pA) sites: early pA (AE) and late pA (AL). The 5′ end starts with the early gene coding section, which has six ORFs, namely E1, E2, E4, E5, E6, and E7. E1 and E2 are demonstrated to regulate the replication of the

viral genome and the transcription of early proteins, whereas E5–E7 are the ones which trigger oncogenesis. E5 is identified to help in keratinocyte differentiation and in avoiding immune response during the later stages, whereas E6 and E7 are considered to be in charge of multiple cellular checkpoints to set up the cancer hallmarks. The late gene coding segment has two sections: L1 and L2. L1 encodes a major viral capsid protein, while L2 encodes a minor viral capsid conformation. Even though the 850 bp stretch of LCR does not include any protein coding region, it encompasses the origin of replication and several transcription factor binding sites for the RNA polymerase II mediated transcription.

HPV infection is initiated in the basal layer of the tiered squamous epithelium, wherein preliminarily E1 and E2 take charge of the viral DNA duplication at a low copy number. Subsequently, as the basal cells differentiate to form the epithelial suprabasal layer, viral genome duplication shifts into high copy number mode. After this, the virions are released as a result of epithelia desquamation, resulting in infection of the neighboring cells. HPV genome can be either integrated to the host genome or stay independently in an episomal configuration. Around 83% of the HPV-positive cervical carcinoma cases show indications of HPV genome integration with the host cell genome.[92] In situation of viral genome integration with the host genome, this frequently results in the disruption of E2 gene site. The E2 gene causes repression of E6 and E7 genes, thereby making it possible for E6 and E7 to get activated upon viral genome integration into the host genome.

HPV E6 and E7 viral oncoproteins are critical in pushing the cells toward tumorigenesis. In their mechanism of duplicating the viral genome, they induce all the hallmarks of a cancer cell, like uncontrolled cell division, invasion, angiogenesis,

metastasis, and unrestricted telomerase activity in addition to the avoidance of apoptosis and growth suppressor activity. Numerous in vitro and xenograft investigation have demonstrated that cancer cells undergo senescence or apoptosis when there is absence of E6 and E7 protein activity thus proving that both proteins are absolutely required for persistence of HPV-mediated carcinogenesis.[93]

Cervical cancer

Cervical carcinoma is the second frequent type of cancer in females globally, next to breast cancer. Most females with cervical carcinoma encounter a long nonsymptomatic period prior to the disorder becoming clinically conspicuous. Early detection of anomalous cytologic alteration through routine screening may avert progression from pre-invasive to invasive condition.[94]

Cervical carcinoma is the second most frequent malignancies in females around the globe, and comprehension of its cause and etiopathogenesis is broadening swiftly. There are four main steps in cervical carcinoma progression: infection of metaplastic epithelium at the cervical transformation zone, viral persistence, progression of persistently infected epithelium to cervical precancer and invasion via the basement membrane of the epithelium.[13]

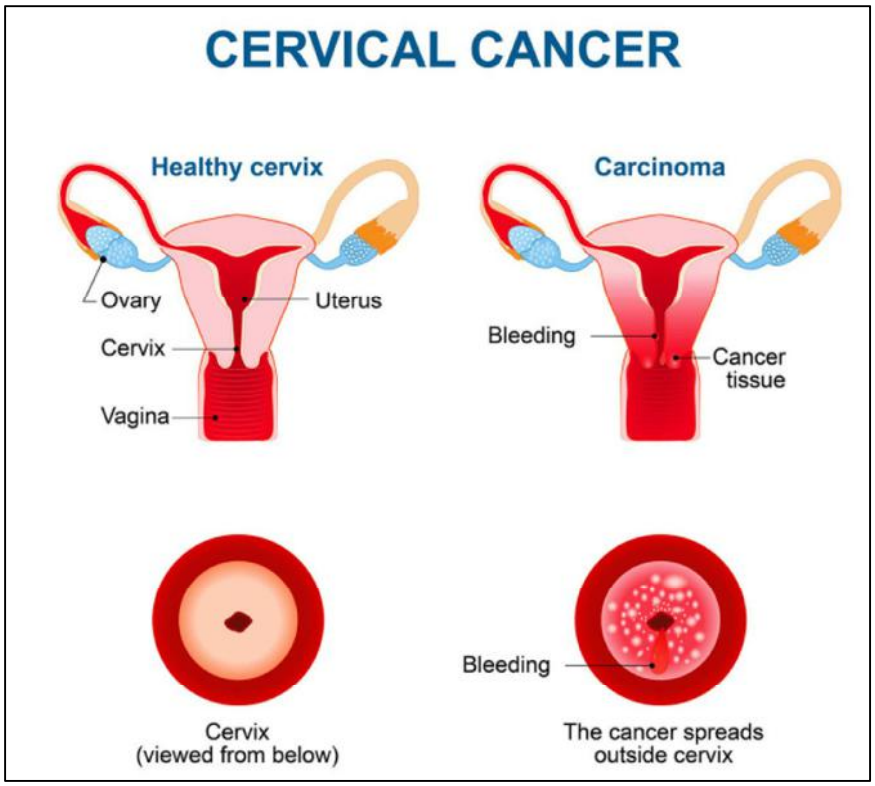


Figure 10. Cervical cancer (Source: https://www.netmeds.com/health-library/post/cervical-cancer-causes-symptoms-and-treatment)

Out of the several strains of HPV, more than 30 target the genital tract. The relation between particular oncogenic (high-risk) variants of HPV and cervical carcinoma is well evidenced. Even though HPV is necessary for the transformation of cervical epithelial tissue, it is not adequate, and a number of cofactors and molecular phenomenon affects the progression of cervical carcinoma. Early discovery and treatment of pre-cancerous tissue damage can stop the progression to cervical carcinoma. In developing nations, cervical carcinomas is frequently the most prevalent cancer in females and constitute up to 25% of all cancers found in women. (15,95)

Cervical carcinoma is a malignant tumor that arise in the cervix and is categorized into two histological variants, adenocarcinoma and squamous cell carcinoma (SCC); SCC is more prevalent and is responsible for 70% of all the cases. In 2018 there were ~569,000 new instances of cervical carcinoma diagnosed globally and ~311,000 demises were ascribed to cervical carcinoma. Among these, between 84 and 90% happen in low- and middle-income countries (LMICs) like South Africa, India, China and Brazil. The prevalent reason of cervical carcinoma is continuous infection produced by the sexually transferred human papilloma virus. Other factors that promote the incidence of cervical carcinoma include traditional practices and beliefs, geography, the screening levels, healthcare access, socioeconomic status, public awareness, smoking, use of oral contraceptives and co-infection with HIV.[(85)]

Cervical carcinoma is one of the main sources of cancer death among females. A bulk of the cases of cervical carcinoma result from HPV infection, with HPV DNA detected in roughly 95% of malignant cervical lesions. The cervix is lined by tiered squamous epithelium that protect the exocervix and mucus-secreting columnar epithelium an attribute of the endocervical canal. The transition between these two populations of cells is known the squamocolumnar junction, and it is this region that is considered to be at the greatest risk of viral neoplastic transformation. [(96)]

More than half a million females are confirmed to have cervical cancer annually and the disease is causative of over 3 lakh deaths globally. High-risk sub variants of the HPV are the source of the disease in a bulk of cases. The disease is preventable to a great degree. Medicaments depend on the extent of the disease at the time of diagnosis and the locally accessible resources, and might include chemo radiation or radical hysterectomy or a combination of both. Progress in radiotherapy

technique, like intensity regulated radiotherapy have led to a decrease in treatment associated toxicity for females with locally progressing disease. [97]

Cervical carcinoma is one of the most frequent neoplastic disorders affecting females, with a combined global incidence of almost half a million fresh cases yearly, next to breast cancer. In the course of the past 15-20 years biological and epidemiological investigation carried out have supplied abundant proof for an etiopathologic role for the infection with specific types of sexually transmitted HPV as the main reason of cervical carcinoma. Other risk factors for cervical cancer like smoking, use of oral contraceptives, parity, comorbid infections and host susceptibility traits should be delineated under the circumstances of mediation of acquisition of HPV infection. Effectively all cervical cancer specimens encompass HPV DNA which indicate that HPV infection is a prerequisite for causing cervical neoplasia.[98]

HPV has been recognized as a chief factor that results in cervical carcinoma, even though HPV infection singly is not capable of causing the disease. In fact, HPV-guided malignancy is a small probability phenomenon since most infections are temporary and is cleared instinctively by the host immune system. With persistent infection by HPV, a long time is required for progressing into cervical carcinoma. Hence, this long-time window gives golden chance for clinical intercession, and the basis here is to delineate the tumorigenic pattern and the relevant targets during the HPV-host interaction.[99]

Cervical cancer, chiefly caused by HPV infection, is the predominant cancer in Indian females and the second frequent cancer in females globally. Although there are numerous approaches of prevention of cervical carcinoma, prevention by

immunization is arising as the most efficacious approach, with the access to two vaccines. Globally, high-risk variants HPV-16 and 18 cause over 70% of all cervical carcinoma cases (the most frequent one being HPV-16 conferring 50–60% and HPV-18 conferring 10–12%). Comparably, in Indian females, the most prevalent variants are HPV-16 and 18.[13,100]

The rapidly growing AIDS epidemic is currently overlapping the high rates of cervical carcinoma incidence and mortality in India. HIV-infected females represent one of the largest risk groups for the development, progression, and recurrence of HPV- associated cervical precursor tissue damage and cervical carcinoma. Prevention of cervical carcinoma must hence include contemplating regarding alternate screening approaches including low-cost methods like the "visual inspection with acetic acid" test (for which training can be provided to nurse midwives, gives on-the-spot results and can relate diagnosis to treatment in a single clinical visit); in addition to the modern advanced technology like the liquid-based cytology (to enhance the detection rates of conventional cytology), automated cytology (to avoid procedural and reading errors in conventional cytology, and to overcome the lack of trained cytotechnologists), and HPV testing (for triage of women with abnormal Pap smears)(28) utilizing self-administered swabs (for ensuring privacy and thereby improving uptake of screening). A probable HPV vaccine that might protect from "high-risk" HPV subvariants could be the ultimate intervention tool and may be on the horizon in the future years. The combining of cervical cancer prevention in the clinical management of HIV-infected females in India and other developing nations is thus a convincing priority.

Reinforcing cancer screening, treatment, and care services requires to be prioritized by increasing the funding, mobilization, and public awareness. Reducing the influence of the double jeopardy of HIV and cervical cancer will necessitate a reassessment of current approaches, establishing guidelines for enhancing preventive clinical care, and realigning the efforts to combat both disorders affecting millions of females in India. [101]

Causes

Multiple factors have been indicated to raise the likelihood of the establishment of persistent infection and ensuing malignant transformation, inclusive of use of long-term oral contraceptives, high parity cigarette smoking and coinfection with type 2 herpes simplex virus or the HIV (96). HPV infection is the chief risk factor for the cervical carcinoma nevertheless there are few other factors which raises the risk like the number of sexual partners, infection by sexually transmitted diseases, age at first sexual intercourse, use of hormonal contraceptives, age, parity, smoking, food and diet.[102]

There are two chief histologic variants of cervical cancer, squamous cell carcinoma (about 75%) which generally begins at the transformation region of the ectocervix and adenocarcinoma (about 25%) which appear in the glandular columnar layer of the endocervix. The HPV is critical to the progression of cervical neoplasia and can be identified in 99.7% of cervical carcinomas.[103,104] It is generally caused by chronic infection with high-risk variants of HPV (chiefly the sub variants 16 and 18). The HPV sub variants linked to squamous cancer are distinct from those linked with adenocarcinoma.[104,105]

HPV cervical cancer

Cervical carcinoma is the most frequent malignancy affecting females in developing nations. It has been estimated to be causative for almost 2,60,000 deaths annually and approximately 80% of them occur in developing countries. Persistent infection by specific oncogenic HPV strains is strongly evidenced as the required to cause most of the premalignant and malignant epithelial lesions of the cervix. There are more than 100 identified HPV variants of which roughly 15 cause carcinomas of the cervix and other regions. HPV 16 and 18, the two most frequent oncogenic strains roughly cause 70% of all cervical carcinomas globally. HPV is extremely transmissible and it is contemplated as the most prevalent sexually transmitted infection in most populations. Even though most females infected with the virus turn out to be negative within 2 years, women with persistent high-risk HPV infections are at greater risk for contracting cervical cancer. Since the recognition of HPV as the requisite cause of cervical carcinoma, HPV-based approaches have become pivotal for novel primary and secondary cervical carcinoma prevention schemes by the introduction of HPV testing and screening and of HPV immunization in prepubescent girls and young females.[106]

In recent investigation of the global burden of malignancies among females, cervical carcinomas ranked second to breast cancer. The generation of cervical cancer is dependent essentially on the infection of the uterine cervix with HPV that requires to persist for many years and decades. The establishment of high-grade precursors and cervical carcinoma appears to rely on the infection of a discreate cell population situated at the squamo-columnar junction (SCJ) at the border between ecto and endocervix. HPV 16 is crucial HR-HPV variant which is linked to roughly 50% of

cervical carcinomas globally. HPV 18 ranks second. HR-HPV varies from other HPV strains by oncogenic attributes of two proteins E6 and E7 that may impede with cell regulation and differentiation.

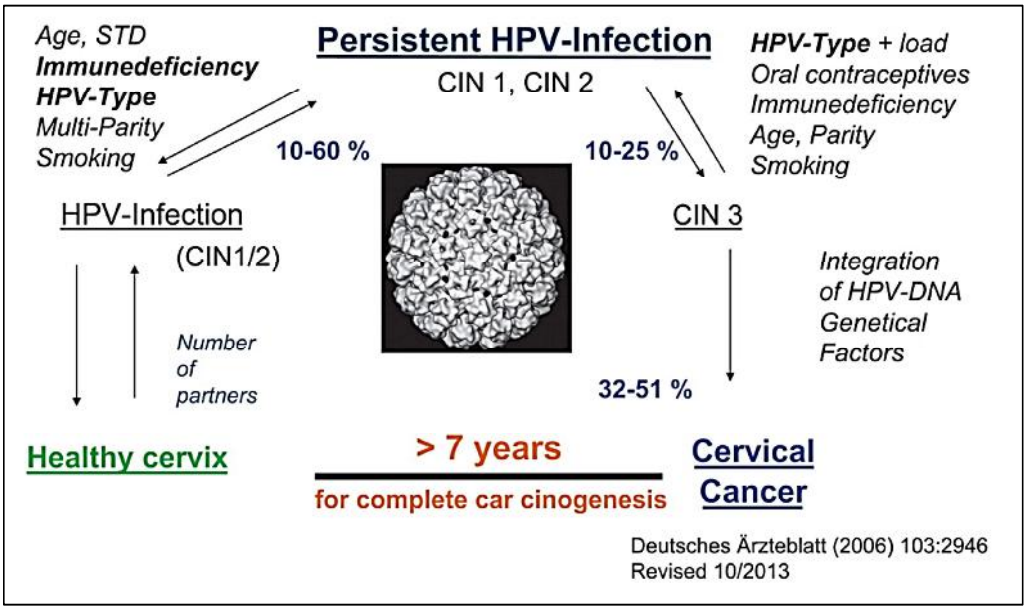


Figure 11. A model of the genesis of cervical cancer.

Figure 11 summarizes the four critical steps and identified co-factors for the genesis from the initial permissive HPV infection to persistent transforming infection with the development of true precursors (CIN3) that will progress to cancer in more than 30 % of cases.[107]

Epidemiologic research assisted by molecular technology have imparted adequate evidence for the casual function of some HPV infections in the establishment of cervical cancer. The discovery is consistent globally, HPV has been suggested as the first recognized as the necessary cause of cervical carcinoma. Such acknowledgement translates into the notion that cervical carcinoma does not develop in the absence of persistent presence of HPV DNA.[108]

HIV infection is linked to a higher risk of HPV positivity and cervical intraepithelial neoplasia (CIN) which may develop into cancer. Therefore, it is critical to screen all HIV positive females for this cancer which is feasible to cure during the early stages. HIV positivity and cervical neoplasia are both frequent health issues in India, the latter being the most prevalent genital tract carcinomas in females in India, with an appreciable prevalence of precursor intraepithelial lesions in the reproductive age group. This is thus one of the few malignancies which can be subjected to preliminary prevention by screening tests. Cervical screening tests should be accessible to the general population, and specifically to HIV positive females, who are at a higher risk of developing cervical cancer. It is well acknowledged that females with AIDS exhibit a high incidence for cervical carcinoma. HPV 16 has been established to be the most frequent strain and appears to initiate progression earlier and more consistently than the other sub variants. This is further magnified by the immunosuppression in HIV positive females leading to chronic persistent HPV infection specifically with numerous strains simultaneously, which has more probability to develop into invasive cancer. [109]

Mechanisms of HPV carcinogenesis

Figure 12 represents some of the main components of the transition from HPV infection to cervical cancer. While transient infections are generally subclinical, progression is deeply linked to persistent presence of viral DNA. This mechanism frequently falls in line with viral disruption in the early (E) E1/E2 segments of the viral genome and integration into the cellular DNA. E2 disruption let out the viral promoters of E6 and E7 and raises expression of these transforming genes. The E6 and E7 viral proteins are able to selectively degrade the proteins of the p53 gene and

of the retinoblastoma (pRB) gene, resulting in the inactivation of two critical cellular negative regulatory proteins.[108]

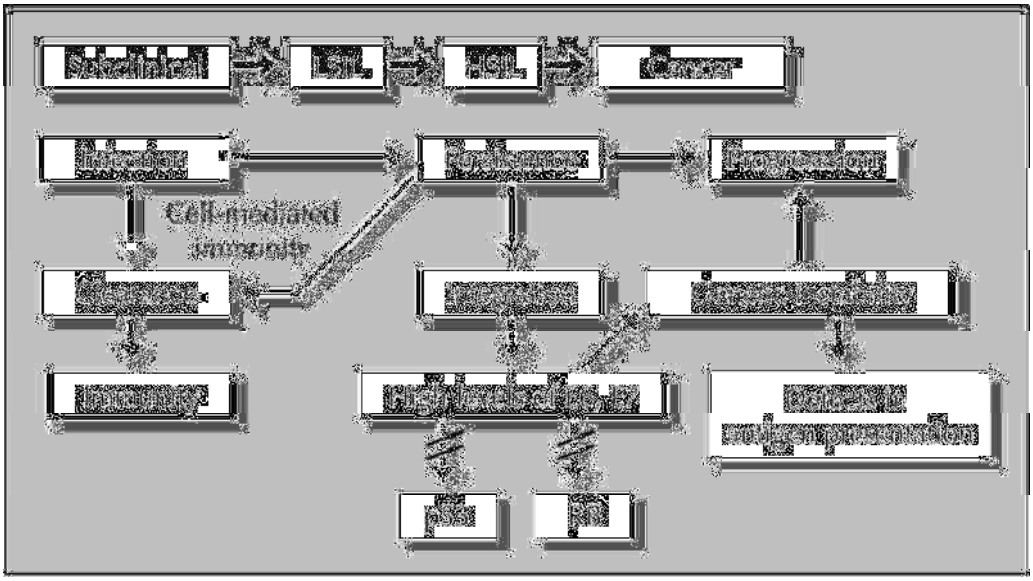


Figure 12. Mechanisms of HPV carcinogenesis

LSIL – Low-grade squamous intraepithelial lesions, HSIL – High-grade squamous intraepithelial lesions

Screening

Cervical carcinoma is the most prevalent malignancy amongst women in developing nations, chiefly due to absence of precursor screening. The lack of screening is resultant of inherent disadvantages of the Pap smear: low sensitivity, high cost, the requirement for laboratory with exemplary human competence and a composite screening program management practice. The precondition for screening in a developing nation consist of screening approach that is inexpensive, and can be constructively put to practice once during the life time between ages 30-35 years, give an immediate result and thus permit an on-site treatment of positive cases. None of

the present screening techniques is in accordance with these necessities. Further research is required with different coalition of tests, which enhance sensitivity. On-site HPV detection, singly or in combination with supplemental tests, is promising. Another encouraging occurrence is vaccination against HPV infection, either as a prophylactic measure or for triggering immunity in infected women.[110]

In the past decade colossal steps have been taken in the direction of reducing the frequency and mortality of cervical carcinoma by executing various prophylactic and screening programs. The causative factor associated with cervical carcinoma progression and its instigator is HPV. Preventive and screening approaches for cervical carcinoma is of utmost importance since the ability to detect and remedy the illness at its pre mature phase generally interrupts the progress into neoplasia. Almost all HPV infections are eliminated spontaneously. Screening for the disease is the best preventive measure for cervical carcinoma.

The eventual aim of cervical carcinoma screening is to detect high-grade cancer instigator lesions and early non-symptomatic invasive cervical carcinoma, while evading the detection and needless medication of transient HPV infection and resultant nonmalignant lesions. The methodical screening with the Papanicolaou cytological investigation (pap-smear) to identify pre-invasive cervical anomalies and early-stage carcinoma has significantly decreased the incidence and mortality linked to cervical malignancies in the United States and other industrialized countries. [111,112]

There are two classes of Pap smears, traditional and liquid-based cytology. In the traditional technique cells are acquire from the neck of the cervix and subsequently the cells are smeared on a glass slide. In the liquid-based cytology

approach the cells are procured from the neck of the cervix, in lieu of being smeared on a glass slide, they are put in a small glass vial that holds preserving fluid. [112]

The American Cancer Society in 2011, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology refined the screening guidelines for the early identification of cervical cancer and its precursors. The proposed screening approaches were cytology and co testing (cytology in combination with HR-HPV testing). [113]

Survival rates for malignancies of the uterine cervix have been boosted over the past four decades hugely due to the influence of screening strategies such as Pap smear. The capability to screen and medicate females for pre-invasive disorder, cervical dysplasia, is the crucial factor resulting in the decrease in the incidence of invasive cervical carcinoma. The capability to test female for the causative factor, the HPV, has come out as a prospective screening tool of late.

Contemporary research has been concentrated on novel approaches for Pap smear screening like thin layer technology, the pertinent intervals for screening, and the relevant methods for including HPV testing into the screening program. Literature reviews evaluating the efficiency of novel technologies indicate that there is inadequate data to confirm better outcome; nevertheless, results till date demonstrate that thin layer Pap smear methods may enhance sensitivity to the identification of cervical tissue anomalies. One of the crucial reporting methods for cervical carcinoma screening include the novel Bethesda System for the reporting of Pap smear put forward by the American Society of Colposcopy and Cervical Pathology. These suggestions are inclusive of HPV testing based on a multicenter investigation

recording its efficiency in the triage of females with a standard squamous cell on Pap smear. [46]

Quality assured and organized cytology-based screening schemes have significantly decreased cervical carcinoma incidence in several developed nations. Nevertheless, there are substantial barriers for arranging cytology-based screening approaches, specifically in developing nations. This has prompted the investigation for new and alternative strategies to cytology for prevention of cervical cancer. The probability of associating screening to therapy in a single visit approach seems to be safe, achievable and effectual. [114]

The refined sensitivity and reproducibility of HPV DNA testing for high-grade cervical intraepithelial neoplasia (CIN) has resulted in widespread calls to launch it as the primary screening test. The major concern has been its reduced specificity, since it cannot differentiate between transient and persistent infections, and only the latter are linked to an increased risk of high-grade CIN and malignancies. Therefore, even those in support of HPV testing normally suggest it for females above the age of 30 years (or in few cases 35 years). Strategies that may be useful, specifically the triage tests, involve HPV typing, methylation (and consequent silencing) of host and viral genes, and novel cytologic techniques, like $p16^{INK4a}$ staining, which attempts possible identification of proliferating cells. [30]

Cytology-based cervical screening had unequivocal success in decreasing the incidence and mortality of cervical malignancies in the last century. The identification of the role of HPV infection as a prerequisite for cervical cancer led to the establishment of HPV testing. There has been a gradual shift from reflex HPV testing for mild cytological anomalies, to co-testing with cytology and HPV, and recently to

primary HPV screening, on the basis of the evidence from well-designed large-scale randomized controlled experiments and meta-analyses. Benefits of primary HPV screening involve increased sensitivity to identify pre-neoplastic lesions, enhanced re-assurance with a negative test, and safe extension of screening intervals. Nonetheless, clinicians and policy makers should make sure the accessibility to clinically validated HPV tests and triage protocols to screen positive cases before implementing the primary HPV screening. It is likely to decrease the probable harm from excess-treatment as well as additional burden on the health care organization. [115]

Novel technologies for cervical carcinoma screening look forward to provide an accurate, efficient and cost-effective way of recognizing females at risk for cervical carcinoma. Contemporary screening utilizes HPV DNA testing in combination with cytology and requires multiple visits at a huge cost to the patient and society. New approaches for screening involve HPV investigations (identification of either the presence of HPV or integration of the virus into the host cell), cell multiplication and detection of epigenetic modifications, either in the host or virus. [116]

Globally cervical carcinoma is the leading causative agent of cancer associated morbidity and mortality for over five decades, cervical cytology has been the gold standard for cervical carcinoma screening. The Pap smear is broadly accepted as the model screening method. Since its initiation, many researchers have investigated the Pap Smear and observed that it is not without its limitations including reduced sensitivity for identification of cervical intra epithelial neoplasia 2/3. In addition, the evidence backing that infection with the HPV is necessary for the development of cervical carcinoma has resulted in the establishment of HPV testing as an auxiliary to

cytology screening. Not too long-ago researchers have compared HPV testing and cytology in the preliminary screening of cervical cancer.[117]

Evidence -based research have demonstrated that novel techniques for cervical carcinoma screening have an increased diagnostic yield than traditional cervical cytology (Pap test). Automated screening machines that utilize liquid-based, thin-layer cytology and HPV DNA testing can possibly become the standard for the regular preliminary screening for cervical malignancies and its precursors in the 21st Century. The higher initial expense of the novel techniques will most likely be the absorbed by establishing longer intervals for safe preliminary screening, in both low-risk and high-risk population. [118]

With the aide of both screening and early identification, cervical cancer is preventable. Insufficient screening in low- and middle-income countries (LMICs) lead to increased rates of late-stage disorder upon clinical manifestation. The disease burden of cervical malignancies in LMICs is on the rise; options for prevention and therapy are inadequate in quantity and quality to have substantial impact on this tendency. On the contrary, for roughly more than fifty years, nations with extra resources have decreased deaths due to cervical malignancies by broadening the adoption of screening by Pap test. LMICs grapple with the execution of Pap testing owing to insufficient infrastructure for this cytology-based technique necessitating well-trained technologists and pathologists.[119]

Table 3. New Tests for cervical cancer screening

Test	*Goals*	*Advantages*	*Disadvantages*
Liquid-based/thin-layer preparations (e.g., ThinPrep, AutoCyte Prep)	Improve the quality of the Pap smear Decrease unsatisfactory Pap smears Increase detection of cancer precursors	High-quality smear for review Improved transfer of cells from collection device Residual material may be used for HPV testing	Cost Increased detection of low-grade lesions in initial studies* Retraining of cytotechnologists
Computer-assisted screening (AutoCyte Screen)	Improve Pap smear interpretation Increase laboratory productivity Increase detection of cancer precursors	Increase cytotechnologist productivity May decrease false-negative reports	Cost From studies on PAPNET, increased detection of low-grade lesion*
HPV testing (e.g., Hybrid Capture II)	Potential use in triage of patients with ASCUS or noncorrelating colposcopy	Detect presence of high-risk HPV types	Cost ASCUS/LSIL Triage Study Group indicated lack of utility for LSIL Studies on ASCUS are ongoing

Pap = Papanicolaou; HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion.

**—There is controversy about whether this significantly benefits patients.*

Table 3 presents a summary of each tests including its goals, advantages and disadvantages.

The liquid-based /thin layer preparation arrangement is conceived to resolve the issues which occur during Pap smear slide preparation such as slides may be impeded by poor sampling, uneven sample distribution or improper fixation of the slide. Critical findings may be masked by uneven sample distribution, cellular clumping and debris. Benefits of the liquid-based method involve improved transfer of cells from the collection device and uniformity of the cell population in each sample. One of the agents that can impact the false-negative rate of Pap smears is whether anomalies that remain on the slide are detected and interpreted accurately by the cytotechnologist. Two approaches of computer-assisted screening are currently available: AutoPap and AutoCyte screen. With AutoPap, the device analyses the

specimen on the slide and, on the basis of an algorithm includes a variety of visual attributes like shape and optical density of the cells. The device generally does not show the cytotechnologist which of the cells are likely to be anomalous.

The Auto Cyte screen device displays cell images to a human reviewer who can then determine if a manual review is necessary. After the human reviewer enters the opinion, the device reveals its analysis based on ranking as to whether manual review is warranted. Once the human reviewer and computer reach an agreement if review is not needed, a diagnosis of "within normal limits" is given. Manual review is warranted for any case so designated by either the cytologist or the computer ranking.

Hybrid Capture II is the latest refined version of HPV tests and has been described as having enhanced sensitivity. It can identify thirteen high-risk variants of HPV. [(120)]

HPV is an etiologic agent for cervical carcinoma and is the most frequent sexually transmitted disease in females. PCR amplification of HPV genomes is the most sensitive technique for the identification of cervicovaginal HPV. PCR has been evidenced to be the most sensitive approach for detecting HPV infection in clinical samples. A number of distinct primer combinations amplifying DNA segments from various sections of the HPV genome have been established and utilized for the identification of HPV. Primers amplifying DNA fragments in the conserved L1 sections have become the most broadly utilized in clinical and epidemiological investigations. While contemplating the optimum PCR primer system for utilizing in clinical and epidemiological investigation should also take into consideration the source of the clinical specimen, the length of the PCR product, the spectrum of HPV variants amplified, the capability to amplify several HPV strains, and the accessibility to strain-specific probes for the detection of distinct HPV genotypes and variants. [(121)]

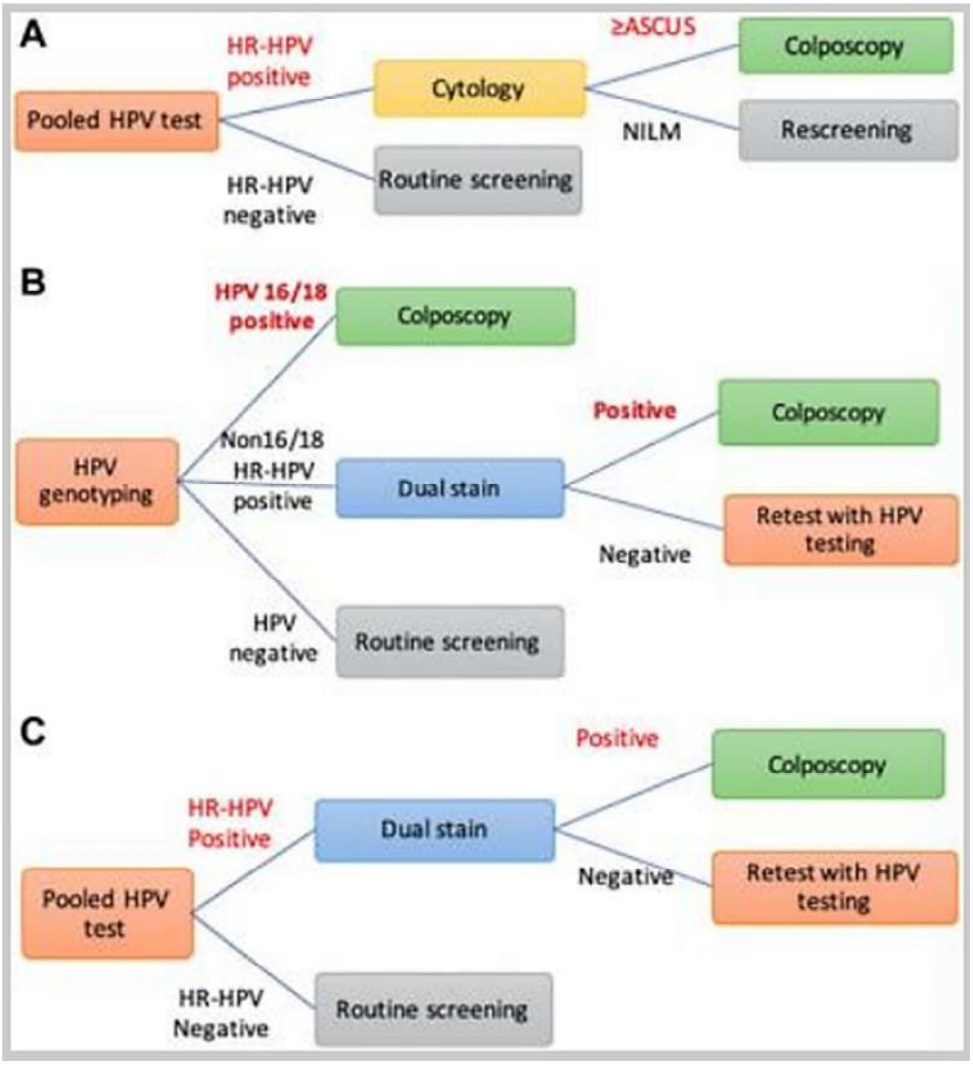


Figure 13. Screening model. A, Pooled HPV test with reflex LBC triage. B, HPV genotyping test with reflex dual stain. HPV indicates human papilloma virus.

High-risk HPV evaluation with reflex liquid-based cytology (LBC) triage: Pooled high-risk HPV testing is a primary tool, succeeded by LBC for females with the high-risk HPV positive result. A cytology of ASCUS or worse results in immediate colposcopy. A repeat HPV testing at 12 months will be conducted for HR-HPV-positive females with normal cytology. If the result of high-risk HPV is negative, the subject will return for routine screening in 5 years (Figure 13 A).

Human papilloma virus with 16/18 genotyping and reflex dual stain: Screening with HPV genotyping then referring to colposcopy if the result is positive for HPV 16 or 18. The dual staining is conducted in cases of other 12 high-risk HPV positive and those with positive result undergo colposcopy. For those who negative dual staining, HPV testing will be done at 12 months interval. Females with a negative result of HPV genotyping return to screening in 5 years (Figure 13 B).

High-risk HPV testing and reflex dual stain: using high-risk HPV testing alone every 5 years followed by dual stain if the result is positive for high-risk HPV. The subjects with positive results of both high-risk HPV testing and dual stain will be referred for colposcopy. Repeat the HPV test in 12 months for a woman with HR-HPV-positive and -negative dual staining (Figure 13 C).

Prevention

A subclass of HPVs promotes anogenital carcinoma, including cervical cancer, prevention and treatment strategies that reflect the causal role of HPV are being developed. Vaccines based on HPV virus-like molecules induce genotype-specific virus-neutralizing antibody and stop infection with HPV. [61,122]

Cervical cancer is a preventable disorder yet remains the frequent cause of cancer mortality among females in poor nations. Contemporary research into alternate methods for the secondary prevention of cervical carcinoma offers novel possibilities for more affordable and implementable programs, specifically ‘screen and treat’ programs that have been tested in randomized trials. HPV-based screening combined with immediate therapy using cryotherapy remarkably decreases both cervical carcinoma precursors and cervical malignancies.

In addition to novel approaches to secondary prevention of cervical carcinoma, the off late availability of two extremely effective vaccines against HPV infection has critical implications for future prevention. [19]

Cervical cancer impacts females in their reproductive ages. Screening is critical for secondary prevention strategy. The long process of tumorigenic transformation from HPV infection to invasive cancer gives ample chances to identify the disorder at a stage when treatment is highly effective. Appropriate screening tests are cytology, visual inspection after acetic acid application and HPV detection tests. Ensuring suitable therapy and follow-up of screen-positive subjects is critical to the success of the screening program. [123]

Abundant evidence conclude that almost all cases of cervical carcinoma are attributable to persistent infection by a variant of HPV specifically HPV-16 and HPV-18.Even though cervical carcinoma screening, primarily with Papinicolaou (Pap) smear, has decreased the incidence of this malignancy. The detection of HPV as the causative agent has created progresses that have critical impact on methods to decrease the incidence of this disease. The first progress is the establishment of a preventive vaccine. Ensuing second generation vaccines may be able to protect against oncogenic infections by a wider array of HPV variants. The second is the introduction of HPV testing into screening program. In women aged more than 30 years, HPV testing can detect high-grade cervical intraepithelial neoplasia prior to Pap smears with acceptable rates of specificity. The mode in which immunization and screening strategies are combined should be contemplated carefully so that they are effective in decreasing the overall incidence of cervical carcinoma. [124]

Two very efficient prevention methods for cervical cancer exist – immunization against HPV and cervical screening with primary HPV testing succeeded by treatment of precancerous lesions. In 2018, the World Health Organization called for action towards attaining the global elimination of cervical carcinoma, and a strategic plan including elimination goals and targets for the scale-up of HPV immunization, cervical screening and precancer and cancer treatment, especially in low- and middle-income countries, will be presented to the 2020 World Health Assembly. The first published estimates indicate that attaining rapid scale-up of both immunization and twice lifetime cervical screening in all nations would avert up to 13.4 million cervical malignancy cases over the next half century, with the most (but not all) nations achieving incidence of <4 per 100,000 women by 2100.[(125)]

Over the past four decades mortality due to carcinoma of the cervix has decreases as a result of improved treatment and the introduction of national screening strategies. Awareness and health-seeking practices have been evidenced to be poor in many developing nations, necessitating the need for proper awareness and immunization program. The HPV vaccination is of public health importance. The Indian Academy of Pediatrics Committee on Immunization (IAPCOI) recommends immunization with HPV vaccine to all women who can afford the vaccine. Vaccination can be administered to females as young as 9 years to those aged 13–26 years who have not previously been vaccinated. There is bivalent, Quadrivalent and Nonavalent HPV vaccines available based on protection against number of HPV variants. HPV vaccination and regular cervical screening is the most efficacious way to prevent cervical carcinoma.

The HPV immunization is of public health importance. Compliance with cervical Pap smear screening is less in India. The vaccines available currently are safe and efficacious. HPV immunization is now well accepted in several nations and has been incorporated in the immunization program. The Indian Academy of Pediatrics Committee on Immunization (IAPCOI) recommends offering HPV vaccine to all females who can afford the vaccine. Since protection is provided only when the vaccine is administered before infection with HPV, the vaccine should be given before to sexual debut. The vaccine should ideally be introduced to patients as a cervical cancer-preventing vaccine and not as a vaccine against a sexually transmitted infection.[126]

The yearly global burden of cervical carcinoma, a preventable disease, is more than 530,000 new cases and 275,000 deaths, with the most of them happening in low- and middle-income countries (LMICs), where screening for cervical cancer and early medicament is rare. Broadly utilized in high-income nations, Pap smear (cytology-based) screening is costly and demanding for implementation in LMICs, where lesser priced cost-effective alternate approaches such as visual inspection with acetic acid (VIA) and rapid human papillomavirus (HPV)-based screening tests offer the potential for scaling up prophylactic services. Combining HPV screening with VIA in "screen-and-treat-or-refer"' strategy provides the dual advantage of HPV screening to augment detection and applying VIA to sort for advanced lesions/carcinoma, and pelvic exam to address other gynecologic complaints. The chief issue in LMICs is comorbidity of HIV and HPV infection, which additionally increases the risk for cervical malignancies and marks a population with considerable need for the prevention of cervical cancer. [127]

In developed nations, due to the high rate of dysplasia, a thorough gynecologic investigation including a pap smear has been suggested at the initial assessment and at subsequent appointments. Considering the dual burden of HIV infection and cervical malignancies in India, locally possible screening recommendations need to be established on the basis of data on prevalence of cervical anomalies and HPV infection among HIV positive females. This will aid in the detection of the maximum possible HIV positive women in early stages of CIN and decreasing morbidity due to CIN and cancer in this category of subjects.[109]

Comprehension that HPV is required for causing most of cervical malignancies has led to two critical progresses in the direction of prevention of cervical cancer, HPV immunization as a highly efficient preliminary prevention program and HPV DNA testing as novel strategy for screening and/or management of screen positive females. Many cervical carcinoma prevention strategies are currently going through a critical transition stage, with immunized females reaching the age of screening and new molecular investigation being launched. The greatest advantage of an integrated HPV immunization and screening strategy can be attained in a systematic setting with immunization and screening records that permit tailoring screening in accordance with the immunization state of the specific female or to the immunization coverage of the group.[128]

Cervical malignancy is the fourth most prevalent cancer among females globally and the second most frequent cancer in Indian women. India singly bears 23% of the worldwide cervical carcinoma burden. The population-based cervical carcinoma screening in India is mostly nonexistent in most regions owing to competing healthcare priorities, inadequate financial resources and limited trained

providers. Therefore, most of the cases are brought to notice at advanced stages of the disease, thus resulting in higher mortality and decreased survival. Several screening choices like cytology, visual-based screening and testing for high-risk HPV are obtainable. Numerous cross-sectional investigations have focused on the comparative efficiency of distinct screening tests. Three critical randomized controlled clinical trials from India have demonstrated the efficiency of screening once during life time with HPV DNA, one-time screening using VIA by trained nurses and four-time screening with VIA by trained primary health workers, decreasing the mortality due to cervical carcinoma. Prevention of cervical malignancies with two-dose HPV immunization and early identification of precancerous cervical lesions of the eligible population via screening and relevant treatment in a single-visit 'screen-and-treat' strategy appear to be encouraging for low-middle-income countries including India. (129)

Mortality Rate

Every year, more than half million females are diagnosed with cervical malignancies and the disease leads to over 300 000 deaths globally. High-risk variants of the HPV are the source of the disease in most cases. The condition is mostly preventable. Roughly 90% of cervical malignancies occur in low- income and middle-income nations that do not have organized screening and HPV immunization programs. In high-income nations, incidence of cervical cancer and mortality have more than halved over the past three decades since the initiation of formal screening programs. Treatment is dependent on the extent of the disease at the time of diagnosis and locally available resources, and might include chemoradiation or radical hysterectomy singly, or a combination of both. Conservative, fertility-preserving

surgical methods have become conventional of care for women with low risk, early-stage condition. Progress in radio therapy technology, like intensity-modulated radiotherapy, have led to reduced treatment- associated toxicity for females with locally- advanced condition. For females with metastatic or recurrent condition, the overall prognosis remains below par. [97]

The understanding that persistent HPV infection is the chief cause of cervical carcinoma has led to the establishment of prophylactic drugs to avert HPV infection and HPV assays that identify nucleic acids of the virus. Cervical carcinoma remains the main public health threat affecting middle-aged females, specifically in less-resourced nations. The worldwide scale-up of HPV immunization and HPV-based screening−inclusive of self-sampling−has prospects to make cervical carcinoma a rare disorder in the coming decades. [130]

Annually 260,000 women succumb to cervical carcinoma, and roughly 85% of these deaths happen in developing countries, where it is the leading reason for deaths due to cancer in females. inconsistencies of health and poverty have a large influence in the high mortality rate. While routine Papinicolaou and HPV testing has significantly decreased deaths due to cervical carcinoma. Research on HPV DNA testing and the low-technology procedure of “screen and treat” are encouraging. Additionally, decreasing the cost and raising the accessibility of HPV vaccines in developing nations bring about hope and promise to the next generation. [45]

Cervical malignancies remain the fourth most prevalent cancer, affecting females globally with substantial geographic variations in cervical carcinoma incidence and mortality rates. Recent evidence models for cervix carcinoma screening

are being modified in high-resource settings from cytology-based screening to accepting of molecular screening and contesting to attain program effectiveness. [131]

Cervical carcinoma is the prime cause of death due to in India, attributing to 17% of all cancer deaths in women of ages 30 to 69 years. As per the current incidence rates, the yearly burden of fresh cases in India is estimated to rise to 225,000 by 2025, however, there are some large-scale, regulated cervical malignancies prevention programs in the nation. Research and preventive programs in India have reflected the feasibility and acceptability of cervical cancer prevention strategies and that screening approaches needing limited auxiliary human resources and laboratory facilities can decrease the morbidity and mortality. Nevertheless, further evidence generated by implementing basic research is required to make certain that efforts to prevent cervical cancer have the expected impact while being cost-effective. With a quarter of the global disease burden of cervical cancer being from India, there is no better time than the present to translate the research evidence from bench to the bedside. Implementation science can aid in ensuring that investments in cervical carcinoma prevention and control have the greatest impact. [132]

Cervical carcinoma remains the most frequent cancer affecting Indian women. India alone account for 25.41% and 26.48% of the world-wide burden of cervical malignancy cases and mortality, respectively. Ironically, dissimilar to most other cancers, cervical carcinoma can be prevented via screening by detecting and treating the precancerous lesions, at any point during the course of its long natural history, thereby preventing the possible progression to cervical carcinoma. Numerous screening techniques, both conventional and novel technologies, are available to screen females for cervical precancers and cancers. None of the screening tests are

perfect and therefore the choice of screening test will be dependent on the scenario where it is to be used. Likewise, several methods are there for treatment of cervical precancers and the selection of the method will depend on the cost, morbidity, necessity of reliable biopsy specimens, availability of resources, etc.

The most common cancer type seen among Indian women is cervical cancer and was estimated to have been the cause for 134 420 fresh cases and 72 825 deaths in the year 2008. India chip in for around 25.4% and 26.5% of the world burden of cervical carcinoma cases and mortality, respectively. The age-standardized rate of incidence and age-standardized rate of mortality for cervical cancers are 27.0 and 15.2, respectively, in Indian women.

Screening can decrease both the frequency and mortality of cervical carcinoma. The mortality due to uterine cervix cancer has decreased drastically in the developed nations from the advent and the broad application of cytology-based screening with Pap smear test, established by George Papanicolaou in the 1950s. In India, till date, there is no regulated cervical cancer screening program. Therefore, a large proportion of these cases get noticed in advance stages during diagnosis, when cure is impossible. Screening for cervical carcinoma is crucial as the women do not often experience symptoms until the disease has progressed. [(133)]

The frequency and mortality rates of cervical carcinoma around the globe are $13.1/10^5$ and $6.9/10^5$, respectively. In India, it is approximated that there are 96,922 fresh cervical carcinoma cases and 60,078 deaths. The incidence and mortality rates are $14.7/10^5$ and $9.2/10^5$, respectively, even though the frequency differs within Indian population. Most of the cervical cancer subjects gets reported at a late-stage of disease in health-care facility owing to lack of awareness. Tata Memorial Hospital (TMH),

Mumbai, India, a premier cancer institute is a tertiary cancer center in India. On a yearly basis, out of 45,000 fresh cancers reported, roughly 800–1000 fresh cervix cancers are diagnosed, of which around 75% receive complete treatment at TMH. The huge amount of data acquired gives an opportunity to investigate and estimate outcomes in terms of survival rates from clinical trials. In India, it is approximated that there are 96,922 fresh cervical carcinoma cases (9.2%) having an age-standardized rate of incidence of $14.7/10^5$ (more than the rates reported in several nations across the globe) and 60,078 deaths due to cervical cancer (8.4%) with a rate of mortality of $9.2/10^5$ based on data from World Health Organization, International agency for research on cancer. The rates incidence of cancer cervix within India exhibit variation. The incidence rates for cervical cancer in major Indian cancer registries are, 15.3 in Bengaluru (2012), 16.1 in Barshi (2012–2014), 15.9 in Chennai (2012–2013), and 19.0 in Mumbai (2012) (National Cancer Registry Programme. Three-year report of population-based cancer registries 2012-2014).

Treatment

The benefit of simultaneous chemoradiotherapy above radio therapy alone in subjects with cervical carcinoma has been recorded in a series of prospective randomized trials. Individual trials have indicated that epirubicin and a combination of mitomycin plus 5-fluorouracil are efficacious when administered together with radiotherapy. Efficient management necessitates specifically close monitoring of hematologic parameters, electrolyte levels, fluid balance, diet and social support. Cautious coordination of care givers is critical for benefiting from adjoining concurrent chemotherapy to radiotherapy. This should every time be considered against the risk of significant side effects, specifically in subjects who have profound

coexisting medical issues that would have prevented or discouraged enrollment in the clincal trials.[134]

Drastic progresses have been possible in brachytherapy for cervical carcinoma. Radiation therapy designing has progressed from two-dimensional to three-dimensional, including computed tomography and /or magnetic resonance imaging into the treatment plan. This permits an explanation and coverage of the malignancies, as well as enhanced evasion of the surrounding organs.

Brachytherapy includes the application of a radioactive source in close vicinity of the tumor. In practice this permits directing a high dose into the tumor relatively sparing the surrounding normal structure. Brachytherapy for cervical carcinoma can be carried out using an intracavitary, interstitial, or combination strategy. Intracavitary brachytherapy includes placing the source of radioactivity using an applicator, via the vaginal cavity, and thereby treat the upper vagina, cervix and uterus. For interstitial brachytherapy, catheters (small tubes) are kept in and around the residual disease, with a transperineal/ vaginal approach.[135]

Most of the cases of cervical cancer are preventable by regular screening and therapy for precancerous lesions. The therapy of cervical carcinoma is stage-specific. While early-stage condition can be eliminated with radiotherapy or surgery, the most efficient therapy for locally advanced stage subject is concurrent chemotherapy and pelvic irradiation. Among several chemotherapeutic drugs like, Carboplatin, Cisplatin, Paclitaxel, Ifosfamide and Topotecan. Cisplatin is deemed the most efficient chemotherapeutic drug for advanced cervical carcinoma. Inorganic, lipidic and polymeric nanocarriers are potential candidates for the establishment of systemic delivery systems in cervical carcinoma chemotherapy. Their appealing properties

include low toxicity, biocompatibility, lower clearance rates, the ability to target particular tissues, and controlled release of chemotherapeutic agents. Nevertheless, the toxicology of Nano-carriers in humans still requires to be fully investigated. Localized delivery of chemotherapeutic drugs to the cervix provide a several advantages like efficiency and lesser side effects owing to the direct delivery to the site of tumor, which evades the systemic circulation of the chemotherapeutic drugs. (136)

Cervical carcinoma is the second most frequent cause for death due to cancer in females worldwide and the establishment of novel diagnostic, prognostic, and treatment program deserve special attention. Even though chemo-radiotherapy and surgery can heal 80%–95% of females with early-stage carcinoma, the recurrent and metastatic condition continues to be a chief source for cancer death. Several attempts have been made to develop novel drugs and design gene therapies to treat cervical carcinoma. Recent research on therapeutic strategies has put forward several choices, involving the role of HPV E6 and E7 oncogenes, which are conserved and expressed in most cervical carcinomas and their respective gene expression products are significant for the initiation and continuance of the malignancy. Other endeavors have been concentrated on antitumor immunotherapy programs. It is understood that through the development of cervical carcinoma, a series of anomalous events are initiated, encompassing disruption of cellular cycle regulation, disruption of antitumor immune activity, modification of gene expression, and dysregulation of microRNA expression. (137)

HPVs are the main etiologic agents of cervical carcinoma. Therefore, cervical carcinoma and other HPV-related malignancies can be averted or treated by HPV vaccines. Transference of papillomavirus can be averted by the production of antibodies against capsid proteins L1 and L2 that counteract viral infection. Nevertheless, since the capsid proteins are not synthesized at identifiable levels by the infected basal keratinocytes or in HPV-transformed cells, therapeutic vaccines mostly target nonstructural early viral antigens. Two HPV oncogenic proteins, E6 and E7, are crucial to the initiation and maintenance of cellular transformation and are co expressed in the most of the HPV-containing carcinomas. Therefore, therapeutic vaccines targeting E6 and E7 may furnish the best choice for controlling HPV-linked malignancies. Several potential therapeutic HPV vaccines are presently being tested by which E6 and/or E7 are delivered to live vectors, in the form of peptides or protein, in nucleic acid configuration, as constituents of chimeric virus-like particles, or in cell-based vaccines. Promising results from experimental vaccination research in animal models have given rise to several prophylactic and therapeutic vaccine clinical trials. In case these therapeutic and preventive HPV vaccines are successful in subjects, as in the animal models, then tumorigenic HPV infection and other related malignancies may be regulated by vaccination.[138]

Cervical carcinoma is a chief gynecological malady which includes unregulated cell division and tissue invasiveness of the uterine cervix. With the access to novel technologies scientists have increased their endeavors to develop new biomarkers for early diagnosis, and assessment of therapeutic schemes. This strategy will aid in the establishment of early diagnosis and in elevating treatment efficiency with reduced recurrence. The inquiry of specific proteins, enzymes and metabolites

will find more useful biomarkers for precise detection and management of gynecological malignancies specifically cervical carcinoma. [139]

Vaccination

Vaccination conventionally represents the most cost-effective strategy to combat infectious disease. Two approaches to immunization are considered: preventive (prophylactic) and therapeutic immunization. [140]

Viral vaccines that are currently licensed for use in human beings are prophylactic against the future confrontation with infectious virus. It is stipulated that all prophylactic vaccines function via the synthesis of virus-neutralizing antibodies, and markedly decrease the number of cells that are infected after encountering the virus, and thereby prevent the clinical disorder linked with infection. Vaccines might also be designed to eliminate cells that are previously infected with virus. These vaccines, recognized as therapeutic vaccines, may be designed to initiate the antigen-specific T-cell-mediated effector processes that are utilized by the host immune system to regulate and eradicate viral infections. [61]

HPV strain 16 in particular, has been linked with more than 99% of cervical carcinomas. The two HPV oncogenic proteins, E6 and E7, plays a critical role in inducing and maintaining cellular transformation. Hence, immunotherapy focusing on these proteins can be used to control HPV-linked cervical lesions. As T-cell mediated immunity is critical for eliminating the already established HPV infections and the associated lesions, therapeutic HPV vaccine aims at generating efficacious E6 and E7-specific T-cell mediated immune responses. DNA vaccines have been designed as an

encouraging approach for antigen-specific T-cell mediated immunotherapy to confront infection and cancer. [141]

Cervical carcinoma carries on causing significant morbidity and mortality globally, making prophylactic cervical carcinoma vaccines critical for cervical carcinoma prevention. The increasing availability of these vaccines globally has the likelihood to greatly reduce the frequency and disease burden in the future. Prophylactic schemes involve focusing on more oncogenic virus strains utilizing the minor capsid antigen L2 and/or by raising the number of variants used to derive virus-like particle vaccines. Therapeutic approaches involve the establishment of vaccines against HPV early proteins (targets for cellular immunity) for the resolution of precancerous lesions and cervical carcinoma. [142]

HPV is causatively linked to cervical carcinoma, the fourth most frequent malignant disease among females globally. The establishment of first-generation prophylactic HPV vaccines in numerous national vaccination programs has significantly reduced the global prevalence of HPV cervical infections. The licensed bivalent and quadrivalent L1(the major HPV capsid protein) virus-like particle (VLP)-based vaccines (2vHPV and 4vHPV) is not without limitations, like a virus-strain restricted protection, the inflated cost of manufacture, and a lack of therapeutic activity on the already developed lesions. The second-generation prophylactic HPV vaccines, consisting of alternate viral elements (capsomere or minor capsid HPV L2 protein) or produced by more cost-effective approaches of production, are currently under intense clinical assessment. [143]

A novel 9-valent HPV vaccine (9vHPV) was sanctioned by the FDA in December 2014 for females of ages between 9–26 and males of ages between 9–15. Apart from the four HPV variants (6, 11, 16, and 18) seen in the quadrivalent vaccine (viz., the variants that cause 70 % of cervical carcinoma and 90 % of genital warts), it consists of five additional oncogenic HPV variants (31, 33, 45, 52, and 58), which is causative of an additional 15 % of cervical carcinoma. HPV infection is frequent and is linked with several genital malignancies. Immunization is effective for the preliminary prevention of HPV infection, and the recently licensed 9vHPV vaccine will provide protection against multiple hrHPV variants when administered early, prior to the onset of sexual activity. [(59)]

With crucial progress in delineating the infectious etiopathology of cervical carcinoma, preventive medicine has procured highly promising novel tools. HPV vaccines, in combination with a growing arsenal of HPV-based screening tests, have the possibility to radically alter public health but need diligent, large-scale execution to attain the final goal: the elimination of cervical carcinoma. Presently, there are 3 commercially available HPV vaccines: the bivalent (targeting high-risk types HPV16 and 18), quadrivalent (targeting HPV16, 18, and low-risk types 6 and 11) and nonavalent (targeting HPV6/11/16/18 and a further 5 high-risk types), which have all demonstrated excellent efficiency against cervical carcinoma precursor lesions and, in the case of the latter 2, against external genital warts. [(144)]

Two vaccines approved globally are available in India; a quadrivalent vaccine (Gardasil™ marketed by Merck) and a bivalent vaccine (Cervarix™ marketed by Glaxo Smith Kline).[(100,145)] Both vaccines are synthesized by recombinant DNA technology that generates non-infectious VLPs consisting of the HPV L1 protein.

Clinical trials with both vaccines have used efficiency against CIN-2/3 and adenocarcinoma in situ (AIS) produced by HPV variants present in the concerned vaccine as primary end points.

Cervical carcinoma is one of the frequent cancers posing threat to women's health, and the persistent infection of high-risk HPV is closely associated with the pathogenesis of cervical carcinoma and multiple other cancers. The tumorigenesis is a complex procedure from precancerous lesion to cancer, which grants an excellent window for clinical prevention, diagnosis, and treatment. Nonetheless, despite several preventions and treatments like HPV screening, prophylactic HPV vaccines, surgery, radiotherapy, and chemotherapy, the burden of disease remains heavy globally. Presently, three classes of prophylactic vaccines, quadrivalent HPV vaccine, bivalent HPV vaccine, and a novel nonavalent HPV vaccine, are commercially available. Even though these vaccines are efficacious in protecting against 90% of HPV infection, they grant limited advantages to remove pre-existing infections. Hence, new advances have been made in the establishment of therapeutic vaccines. Therapeutic vaccines vary from prophylactic vaccines in that they aim to induce cell-mediated immunity and destroy the infected cells rather than neutralizing antibodies.[146]

HPV is known to be required for causing several gynecologic malignancies and is also linked to a subgroup of head and neck malignancies. This understanding has created the chance to regulate these HPV-linked carcinomas via vaccination. Nevertheless, despite the availability of prophylactic HPV vaccines, HPV infections stays extremely prevalent globally. Additionally, while prophylactic HPV vaccines have been efficient in preventing infection, they are not effective at eliminating pre-existing HPV infections. Therefore, there is an urgent requirement for therapeutic and

T cell-based vaccines to resolve existing HPV infections and HPV-linked lesions and cancers. On the contrary prophylactic vaccines, which produce neutralizing antibodies, therapeutic, and T cell-based vaccines amplify cell-mediated immunity against HPV antigens. Due to the increased availability of HPV infections globally, there is an urgent requirement to establish effective treatments for previously developed HPV infections and HPV related diseases. One potential treatment strategy involves the use of therapeutic vaccines. Contrary to preventative vaccines, which are intended to synthesis neutralizing antibodies against viral particles, therapeutic vaccines are meant to induce cell-mediated immune responses to particularly target and kill infected cells. In many instances, as HPV linked lesions progress into cancer, the HPV viral DNA will be merged with the host's genome. [147,148]

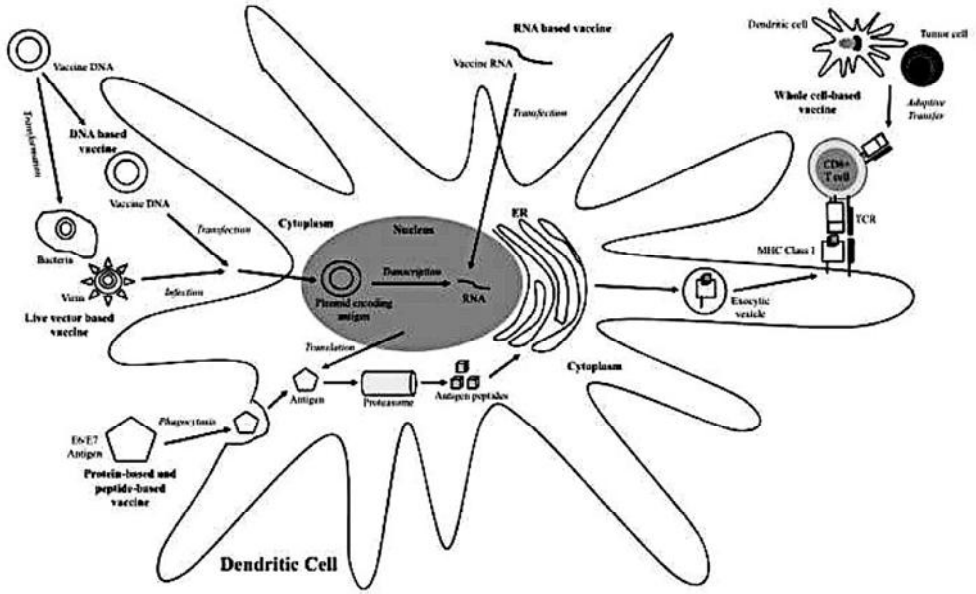


Figure 14. Immune activation by therapeutic HPV vaccination.

(ER – Endoplasmic Reticulum; MHC – Major Histocompatibility Complex; TCR – T Cell Receptor; E6/E7 – Human Papillomavirus E6 and E7 Protein.).

Administering of several strains of therapeutic HPV vaccines results in the introduction of antigen into the body in different configurations. DNA plasmids coding for antigens (HPV oncoprotein E6 and E7) can be transferred into dendritic cells via direct DNA vaccination or via infection of altered live vector vaccine. The DNA coding for antigens will be transcribed into RNA, which can also be introduced into the cell via RNA vaccination. The transcribed RNA will be subsequently translated into antigen proteins, or long peptides, which can be taken up by the dendritic cells via phagocytosis following protein or peptide-based vaccination. The antigen proteins or long peptides are later processed by the proteasomes into short peptides, loaded onto class one major histocompatibility complex (MHC I) inside the endoplasmic reticulum (ER) to be presented to T cell receptors (TCR) of CD8+ T cells. Alternately, autologous dendritic or tumor cells can be retrieved and prepared ex vivo to express target antigen on MHC I molecules as well as the required co-stimulatory molecules and be introduced back to the body (a process called adoptive transfer) as whole cell-based vaccines for the priming of T cells.

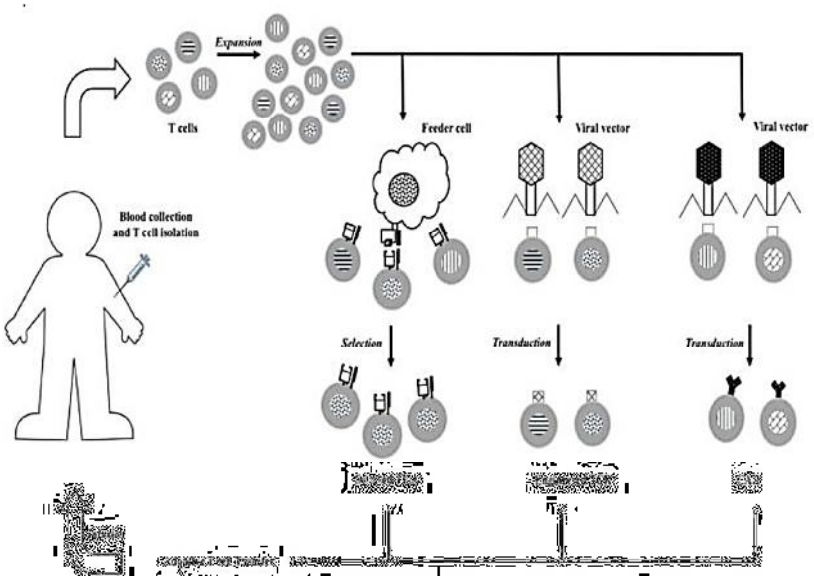


Figure 15. Generation of Passive Immunity via adoptive T cell transfer

A schematic representation of the process of T cell-based vaccination is given in figure 15. In short, autologous T cells are derived from patients via PBMC collection. The T cells are non-specifically proliferated (typically with CD3 and CD28 agonistic antibodies), followed by several processes to induce antigen-specific T cells: 1) proliferated, heterogeneous T cell population can be co-cultured with antigen pulsed feeder cells (generally dendritic cells or irradiated tumor cells) to select for antigen-specific T cells, 2) expanded T cells can be transduced with DNA coding for engineered T cell receptor that is specific to the antigen using viral vectors, and 3) proliferated T cells can be transduced with DNA coding for chimeric antigen receptor that is specific to the antigen. The resultant, selected or modified T cells can then be introduced back into the patient for the generation of immune responses against antigen-expressing cells. [148]

MATERIALS AND METHODS

Clinical Materials

Blood (Whole blood, Serum), vaginal Swabs.

Laboratory Materials

HIV detection kits (ELISA/ Spot), Viral Transport medium, PCR, Reagents.

Study design

The human research ethics committee at KLE University in Belagavi, India, gave its approval for this cross-sectional study with the Institutional Ethical Committee Ref no KLEU/Ethic/2012-13/D-4573 dated 18.03.2013. The HIV Positive and HIV Negative women aged 18–45 years (reproductive age) who attended the department of Obstetrics and Gynecology were included in the study group and control group respectively. Women were excluded from study group if they did not have HIV, were under the age of 18 or were beyond the age of 45, were unable to give informed consent, or were unwilling to provide samples if they were terminally sick and had low CD4 counts. All study participants gave their informed consent after receiving the study fact sheet and agreeing to provide samples.

Data and Sample Collection

Demographic data of all the participants were collected and recorded in a structured questionnaire which was approved by the ethics committee through an interview by a gynecologist. After collecting data and informed consent, participants were subjected to a routine gynecological examination which included general, pelvic,

and speculum examination and sample collection. Based on the data provided by the participants, they were counseled on the risk of sexual behavior and provided with contraceptives if needed. Return appointments and referrals were made as required by the gynecologists.

Sample collection and methods

HIV Testing

HIV Tridot is an immunoassay that uses a blood sample from an individual to detect HIV infection. This is a sensitive and rapid diagnostic test used to detect HIV1 and HIV2 antibodies in serum and blood using specific HIV-1 and HIV-2 antigens.

CD4 cell count

A CD4 cell count is a laboratory test that measures the number of CD4 T-cells. The normal count is between 500-1500 cells/mm^3. This test is used by clinicians to track the demolition of CD4 cells as well as the efficacy of antiretroviral therapy (ART). The CD4 cell count has evolved as the best indication of disease progression for physicians, and it is used to stage disease and guide medical therapy. As per the Center for Disease Control and Prevention (CDC), it is one of the indications for the diagnosis of AIDS when the CD4 cell count falls below 200 cells/mm^3. The reduction in CD4 T cells can lead to opportunistic infections, and it increases mortality.

Procedure

A CD4 count requires a blood sample, which is obtained by a routine blood draw. The blood specimens must be processed within 18 hours of the sample collection. The absolute number of CD4 T cells can be calculated using a variety of

methods, but the immunofluorescence-based flow cytometry analysis is the gold standard. Flow cytometry uses the binding of fluorochrome-labelled probes to specific receptors present on the cell surface. CD4 T cells are stained with fluorescent-labeled monoclonal antibodies that especially bind to the CD4 receptor present on the cell surface and can be detected by a flow cytometer as a relative percentage of cells expressing the receptor on their surface. The results are reported as CD4 percentages, and the absolute number is calculated by multiplying the percentage by the total white cell count. The CD4 count is used to determine how far HIV has progressed. CD4 counts should be performed on all patients who have been diagnosed with HIV for the first time. [(149)]

Pap test

This screening is used to identify the pre-cancerous and cancerous processes in the endocervical canal (transformation zone) of the female reproductive system. In this test, the cells are scraped from the cervix for examination under a microscope. As per American Society of Cytopathology guidelines, the smears are collected with the help of Ayer's spatula and cytobrush from the squamo-columnar junction. The cellular material obtained is quickly smeared on a clean, grease free glass slide. Two smears are prepared for each case. The glass slides are then fixed by using 95% ethyl alcohol. The smears are stained with Papanicolaou stain and visualised under a light microscope and are reported by a pathologist according to the 2001 Bethesda system.

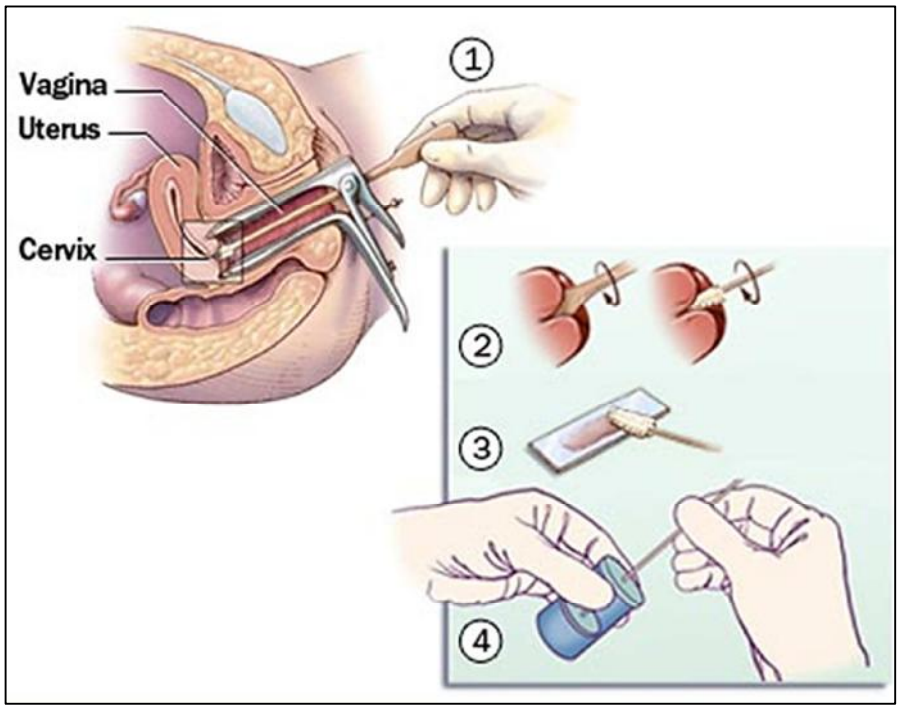


Figure 16. Procedure of PAP Smear test

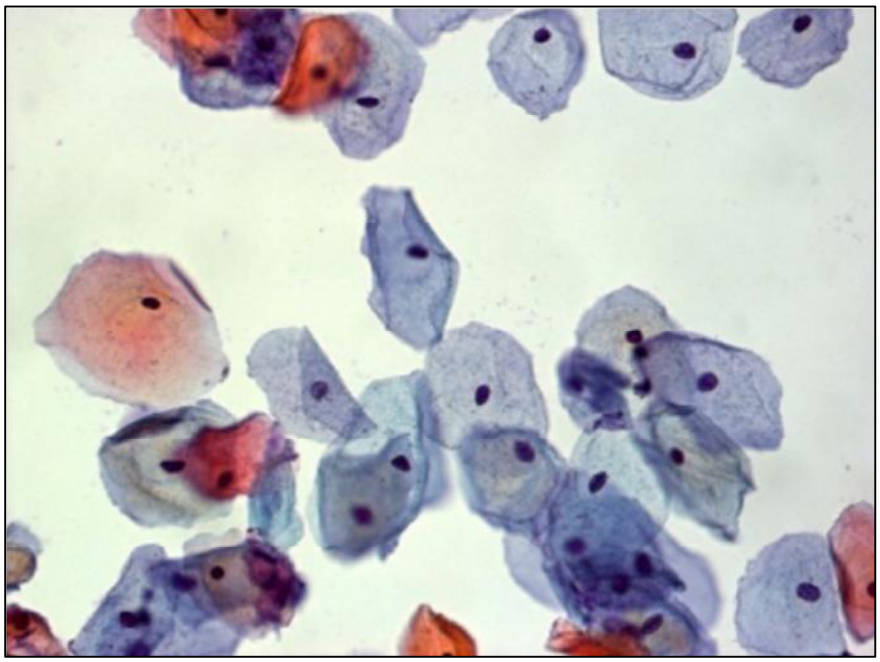


Figure 17. Normal Sqaumous Epithelial cells Stained with Papanicolaou stain.

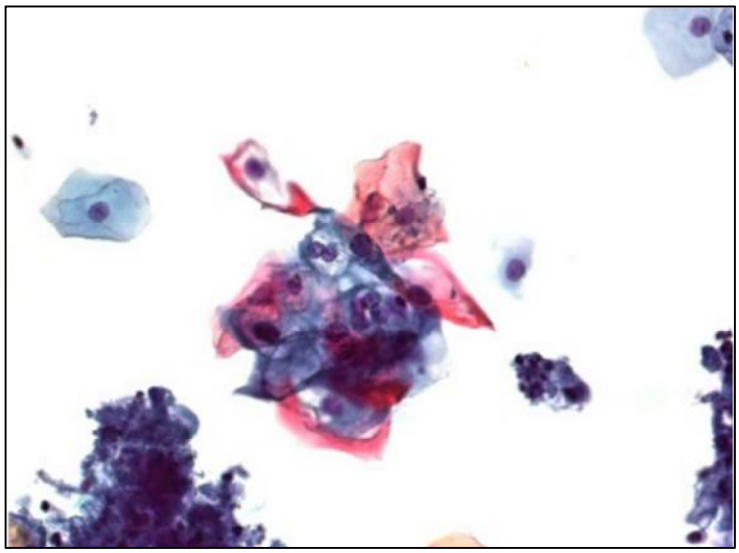


Figure 18. LSIL-Abnormal squamous epithelial cells stained with Papanicolaou stain.

Figure 19. Collection and storage of samples.

Cervical samples were collected from the transformation zone of the cervix using the DNA collection device (cytobrush), which was rinsed in 20ml of PreservCyt® vial (Hologic, Inc.). Samples were immediately stored at 4°C by transporting them to the laboratory at the department of Microbiology, where they were further stored at -20°C until DNA extraction.

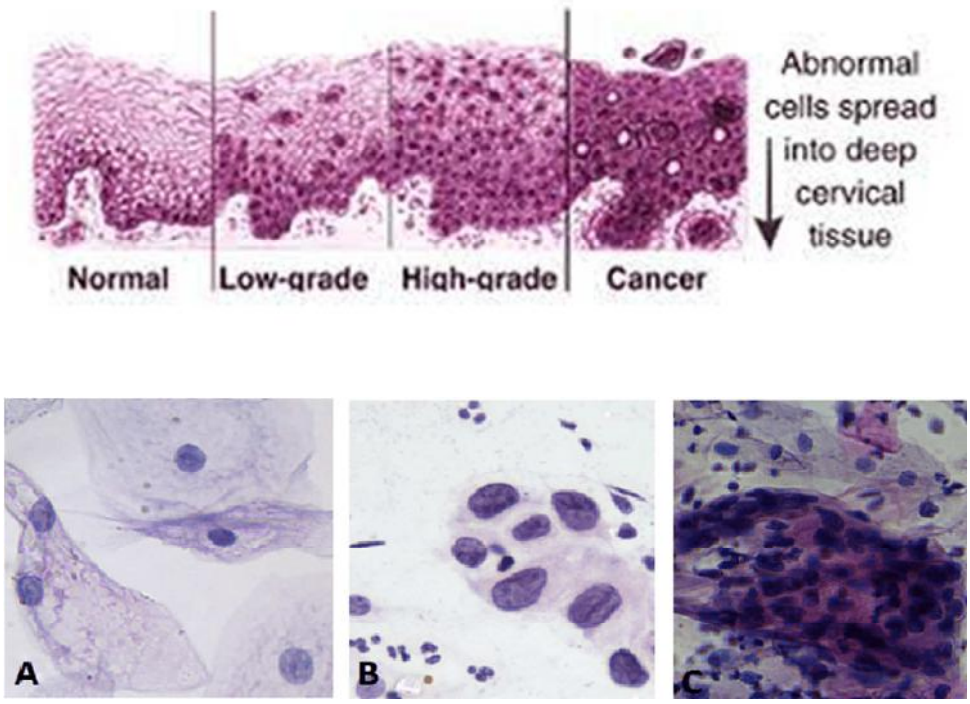


Figure 20. (a) Normal Pap smear image (b) Image of Pap smear with malignant cells (c) overlapped cell clusters and artifacts

Colposcopy

Colposcopy and colposcopy-directed biopsies are usually used to evaluate patients with abnormal Pap smear findings who do not have a gross cervical lesion. Following the treatment of 3% acetic acid solution, the cervix is examined under a bright filtered light at 10- to 15-fold magnification. Acetowhitening as well as the vascular patterns characteristic associated with dysplasia or carcinoma can be detected. Colposcopy can detect both low-grade and high-grade dysplasia.

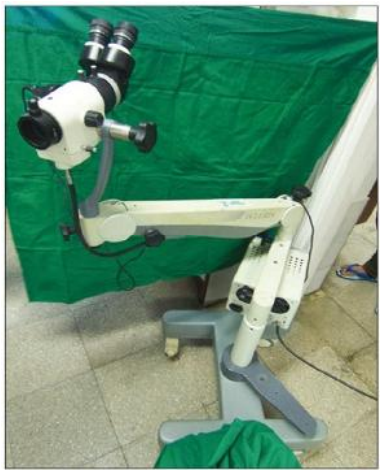


Figure 21. Colposcopy: A colposcope is a low-power, binocular field, stereoscopic microscope having a powerful light source that is used for magnified visual examination of the uterine cervix to diagnose cervical neoplasia. [(133)]

DNA extraction

DNA extraction from cervical samples was performed using the QIAamp DNA Mini Kit (Qiagen Ltd, Crawley, UK). At 56ºC, aliquots of 200 µl of samples were treated for 10 minutes with 20 µl of proteinase K and 200 µl of AL buffer. To execute DNA precipitation, 200 µl of ethanol (96%) was added to the digested sample. The DNA was eluted in 200 µl of AE buffer and stored at -20ºC until further use. Out of 214 HIV positive samples, 197 samples were considered for further analysis, as the remaining 17 samples were contaminated. Out of 100 HIV negative samples, 96 samples were considered for further analysis, as the remaining 04 samples were contaminated.

The QIAamp DNA Mini Kit was used to extract DNA from cervical tissues (Qiagen Ltd, Crawley, UK). At 56oC, aliquots of 200 l of samples were treated for 10 minutes with 20 l of K proteinase and 200 l of AL buffer. To execute DNA precipitation, 200 l of ethanol (96%) was added to the digested sample. The DNA was

eluted in 200 l of AE buffer and kept at -20oC until needed. Because the remaining 17 HIV positive samples were tainted, only 197 were chosen for further study. Because the remaining four samples were compromised, only 96 HIV-negative samples were chosen for further investigation.

DNA Amplification

All the pre and post DNA extraction processes were carried out in separate rooms and cabinets at the National Aids Research Institute (NARI), Pune, which were located in different spaces so as to avoid further errors due to contamination. Contamination and errors were also monitored during the extraction process using blank controls.

Sample processing for DNA extraction

DNA extraction of the cervical specimens was carried out at the National Aids Research Institute (NARI), Pune. Cervical lavage samples that were collected in PreservCyt® and stored at -20°C were processed in a biosafety cabinet in a laboratory physically separated from where the PCR amplification was performed. DNA of both HPV infected and non-infected cells was released by lysing cervical cell specimens using lysis buffer supplied with the Roche Amplicor HPV kit under denaturing conditions at elevated temperatures in the presence of proteinase K, followed by DNA purification in columns with a silica-based membrane using vacuum processing.

Roche Linear array for HPV genotyping

The procedures were followed according to manufacturer's instruction by following manual provided with the kit.

The PCR-based amplification of target DNA using the Linear Array® HPV genotyping test (LA-HPV) (Roche Molecular Systems, Pleasanton, CA, USA) is an improved and commercialised version of the PGMY line blot assay (PGMY-LB). This technique utilizes a pool of consensus L1 PGMY09/11 primers which design to amplify HPV-DNA from 37 genotypes. Following PCR amplification, biotin-labelled amplicons were transferred to a well of the typing tray containing a genotype strip. The strips were washed and hybridised using the Profiblot T48 automatic system. The target DNA was detected by enzymatic and colorimetric development, and the Linear Array HPV Genotyping Strip was visibly read by comparing the pattern of blue lines to the Linear Array HPV genotyping test reference guide.

All the extracted DNA samples were subjected to linear array genotyping assay (Roche Diagnostics, Indianapolis, IN, USA) for further amplification and identification of the HPV genotype. This assay depends on PCR amplification of target DNA using HPV primers followed by hybridization of the amplified product to oligonucleotide probes and their colorimetric detection. Particularly, the master mix contains the primers for the amplification of a 450-bp fragment of the L1 region of more than 37 HPV genotypes and a 268-bp fragment of the human β globin gene. The detection and genotype determination were performed using the denaturated amplified DNA and an array of oligonucleotide probes, located in the polymorphic region of L1 region that permitted independent identification of individual HPV genotypes. The specificity of the test was established to include negative samples in each assay.

Table 4. Thermocycler program "RLA Assay"

Step	Temp., Time	Number of Cycles
Hold	50 ℃, 2 min	1
Hold	95 ℃. 9 min	1
Cycle (set ramp rate at 50%)	95 ℃, 30 Sec 55 ℃, 1 min 72 ℃, 1 min	40
Hold	72 ℃, 5 min	1
Hold	72 ℃, ∞	1

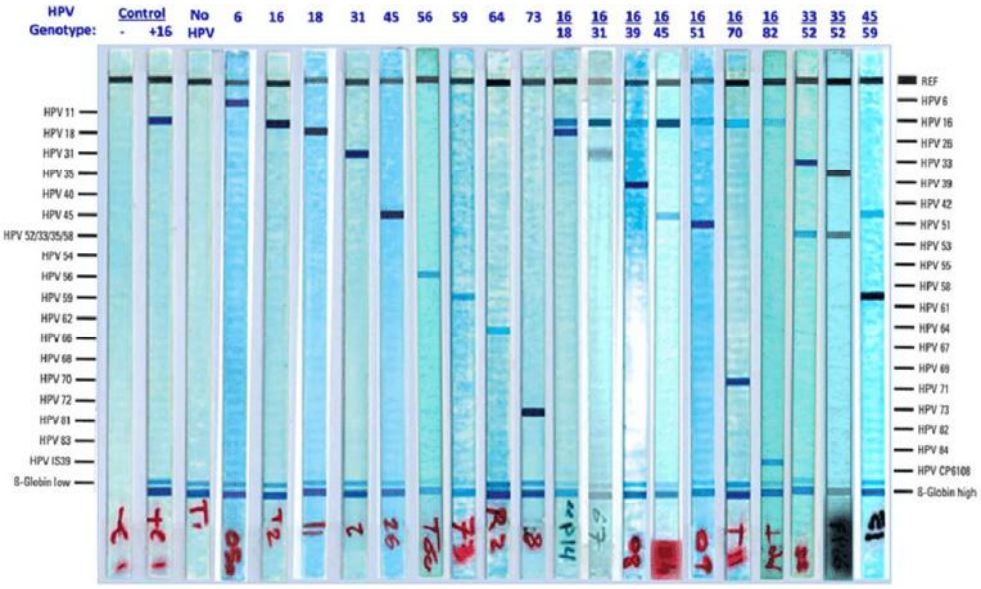
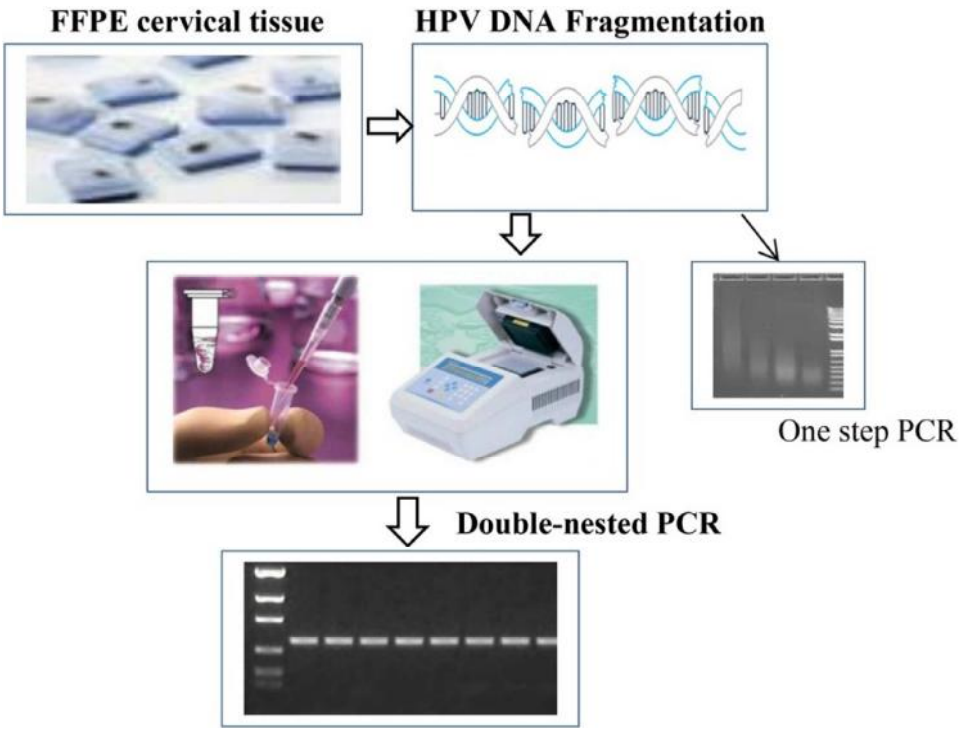


Figure 22. Roche Linear Array HPV genotyping test (LA-HPV) for HPV

Polymerase chain reaction (PCR)



FFPE : Formalin-Fixed Paraffin-Embedded (FFPE Sample)

(Source :https://www.sciencedirect.com/science/article/pii/S2215016118300852)

Figure 23. Polymerase chain reaction (PCR)

At present, the vast majority of PCR investigations have employed consensus primers to amplify a wide range of HPV types in a single PCR amplification. The L1 capsid gene and other conserved sections of the HPV genome are targeted by these primers. The MY09 and MY11 primers are designed to target a 450-bp segment of the HPV L1 ORF. [15,150] With an analytical sensitivity of 0.5 to 10 fg (10–200 copies), the GP5+ plus GP6+ primers target a fragment inside the area targeted by MY09 and MY11.[15,151]

- **Sample selection for testing with two different primers**: Among the 214 women who attended the Obstetrics & Gynecology clinic, 33 samples yielded positive results in the linear array genotype assay, and these samples were selected to test for HPV-DNA by PCR using the different primers MY09/11 and G5/G6.

- **Amplification of DNA with MY09/11 and G5/G6 primers:** Two different pairs of oligonucleotide primers were used in this study to detect HPV in cervical samples that were MY09/MY11 and G5/G6. In order to standardize the amount of material used, equal volumes of DNA extract were used for PCR with two different primers. PCR reactions for both the primers were carried out separately by following the standardized protocol according to the manufacturer's instructions.

An independent PCR reaction was carried out to amplify the L1-region of the HPV genome, using MY09/11 and G5/G6 primers. In the first reaction, MY09/11 primers were used in a mix containing Taq DNA Polymerase Master Mix (QIAGEN®) and 10 pmol of PC04/GH20 primers. The PC04/GH20 primers amplify a 248bp product of the human-globin housekeeping gene. Amplification was performed with an initial denaturation step of 15 min at 95°C, followed by 40 cycles (1 min of denaturation at 94°C, annealing at 55°C/1 min, and an extension step at 72°C/5 min) and a final extension of 72°C/10 min. In another set of PCR, G5/G6 primers were used in a mix containing Taq DNA Polymerase Master Mix (QIAGEN®) and 10 pmol of PC04/GH20 primers. Amplification was performed with an initial denaturation step of 15 min at 95 °C, followed by 40 cycles (4 °C/1 min, 55 °C/1 min, and 72 °C/1 min) with a final extension of 72 °C/5 min. Commercially

available positive controls and water as a negative control were used for both the PCR reactions. After amplification, the products were then visualized on 2% agarose gels containing 10 µL of ethidium bromide/100 mL agarose, under UV light. [152]

Table 5: General primers sequences for HPV DNA detection.

Primer	Sequences* (5'-3')	Size (bp)
MY11	GCMCAGGGWCATAAYAATGG	~ 450
MY 09	CGTCCMARRGGAWACTGATC	
G5	TTTGTTACTGTGGTAGATAC	~ 150
G6	GAAAAATAAACTGTAAATCA	

MY09/MY11 and G5/G6 primers are commonly used in PCR assays to amplify the L1 region of the HPV genome. These primers effectively amplify a broad spectrum of HPV genotypes from cells obtained in cervical smears. The MY09-MY11 primer set is a combination of 25 primers, each of which is produced with multiple defective nucleotides and amplified into a 450bp product.[121,152,153] The G5/G6 primer set consists of a fixed nucleotide sequence for each primer and produces an approximately 150bp product and detects a broad range of HPV types by using a lowered annealing temperature during PCR. [152,153]

The degenerated MY09/MY11 oligonucleotide primer uses a high annealing temperature (55ºC) and can amplify multiple types of HPV infections. However, the G5/G6 oligonucleotide primer has a lower annealing temperature (42ºC) as compared to the MY09/11 primer, allowing the best amplification by single genotype HPV infections.[121,152]

Table 6. Detection of HPV using MY09/MY11 and G5/G6 primer sets.

Process	Temperature (°C)	Duration	Number of cycles
MY09/11 primers			
Pre-denaturation	95	5min	1
Denaturation	95	15s	40
Annealing	55	1min	
Extension	72	1min	
Final extension	72	10min	1
G5/6 primers			
Pre-denaturation	95	10min	1
Denaturation	95	30s	40
Annealing	42	40s	
Extension	72	30s	
Final extension	72	10min	1

As an internal control, DNA samples were subjected to PCR for human-globin gene amplification, which uses PCO3 (5'CTTCTGACAACAACTGTGTTCACTAGC3') and PCO4 (5'TCACCACAACTTCATCCACGTTCACC3') oligonucleotides.

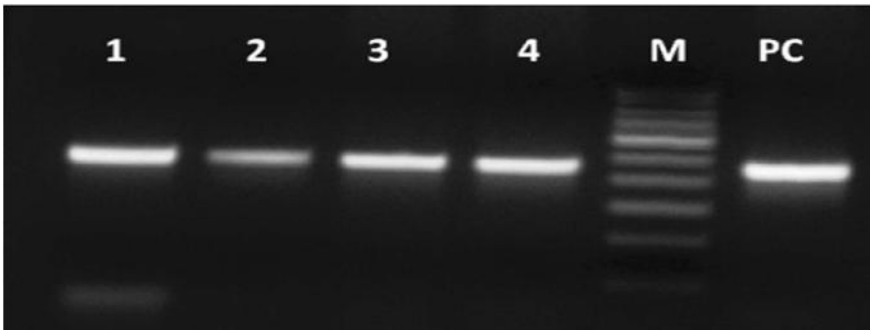


Figure 24. Detection of HPV using consensus MY09/MY11 primer targeting 450bp L1 region. (Lane 1-4= Positive samples, M= 100bp marker, PC= Positive control).

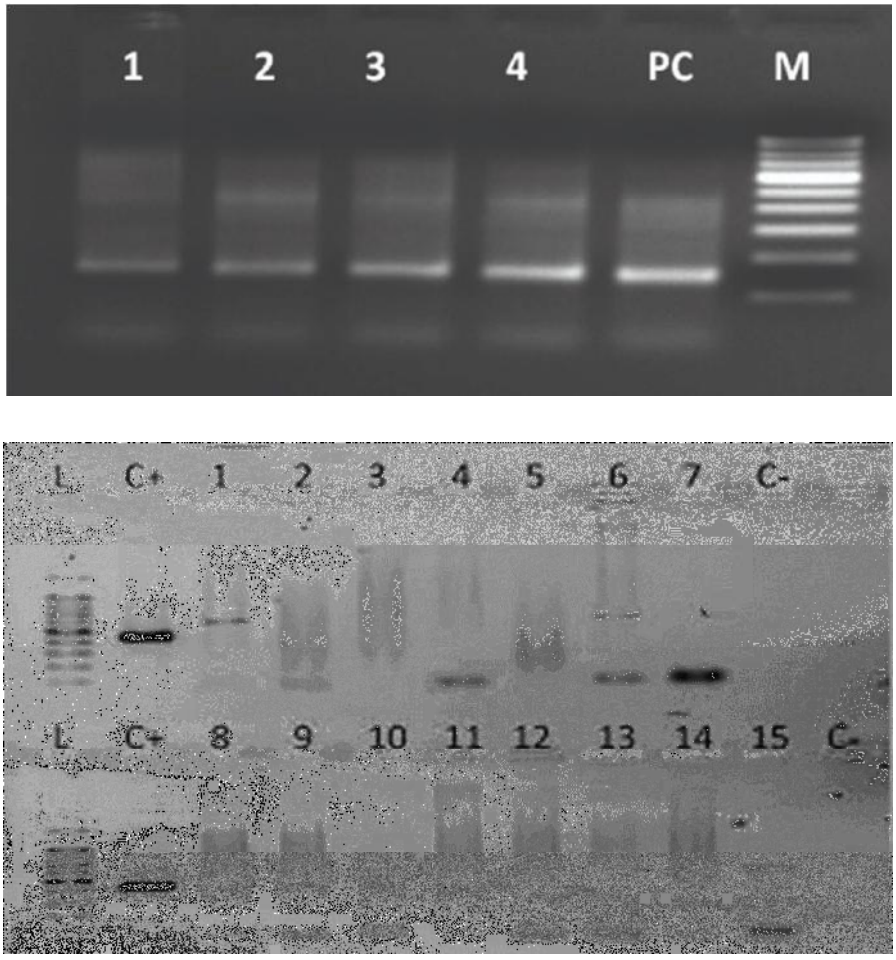


Figure 25. PCR using G5/G6 primers amplifies a 150bp region. (Lane 1-4 = Positive samples, PC = Positive control, M = 100bp marker).

Statistical Analysis

HIV positive

The statistical programme R i386.3.5.1 was used to analyse the data. Continuous data was represented in the form of mean ± SD and the categorical variables were represented by the frequency table. The association between categorical variables is studied using the chi-square test. The variables that influence HPV infection are studied using logistic regression. The level of significance was considered, $p \leq 0.05$.

HIV Negative

The statistical programme R i386.3.5.1 was used to analyse the data. Continuous data was represented in the form of mean ± SD and the categorical variables were represented by the frequency table. The Cochran Armitage trend test is used to analyse trends. The crude odds ratio is used to investigate the variables that influence HPV. The level of significance was considered, $p \leq 0.05$.

RESULTS

Age and CD4 counts

- The mean age
    - 33.93 ± 5.7 years for HIV positive women
    - 32.07 ± 6.79 years for HIV negative women
- The median CD4 count in HIV positive women was 468 cells/mm^3 with interquartile range of 344-629 cells/mm^3.
- ART (antiretroviral therapy) was used by 26% of HIV-positive women.

HIV Positive patients

Table 7. Socio-demographic details of patients tested for HIV

Variables		Group		Total	p-value
		HIV Positive (n=197)	HIV negative (n=96)		
Age (in years)	≤ 20	0(0)	8(8.33%)	8	0.002[C]
	21-30	52(26.39%)	31(32.29%)	83	
	31-40	120(60.91%)	45(46.87%)	165	
	≥ 41	25(12.69%)	12(12.5%)	37	
Age (in years)		33.93±5.6 (21-49)	32.07±6.78 (19-44)		0.01[t]
Literacy	Illiterate	52(26.4%)	8(8.33%)	60	0.001[C]
	1-4 years	139(70.55%)	12(12.5%)	151	
	5-10 years	0	47(48.95%)	47	
	College	1(0.5%)	24(25%)	25	
	Graduate & above	5(2.53%)	5(5.2%)	10	
Occupation	Housewife	58(29.4%)	52(54.16%)	110	<0.0001[C]
	Salaried	139(70.5%)	21(21.8%)	160	
	Labour	0	23(23.95%)	23	
Marital status	Married	197(100%)	80(83.33%)	277	
	Divorced	0	7(7.29%)	7	
	Widow	0	3(3.12%)	3	
	Non disclosed	0	5(5.2%)	5	

A total of 293 patients were screened in the study, out of which 197 were HIV-positive and 96 were HIV-negative (Table 7).

Out of 197 HIV-positive groups, 120 (60.91%) were aged between 31-40 years. 52 (26.39%) were between the ages of 21-30 and 25 (12.69%) were ≥41 years old. The mean value was 33.93±5.6 (21-49 years of age). Out of 96 HIV-negative groups, 45 (46.87%) were aged between 31–40 years. 31 (32.29%) were between the ages of 21 and 30 years, 12 (12.5%) were ≥41 years of age and 8 (8.33%) were ≤20 years of age. The mean value was found to be 32.07±6.78 (19-44 years of age). The p-value was found to be 0.01t. The highest number, i.e., 120 (60.91%) were found to be HIV-positive in the age group 31–40 years and none were found in the age group ≤20. The highest number, i.e., 45 (46.87%) were in the HIV-negative group falls in the age group of 31–40 and the lowest, i.e., 8 (8.33%) falls in the age group ≤20.

Out of 197 HIV-positive groups, 139 (70.55%) had literacy levels of 1-4 years. 52 (26.4%) were illiterate, and 5 (2.53%) were graduates or above. Out of 96 HIV-negative women, 47 (48.95%) had a literacy rate of between 5-10 years, and 12 (12.5%) were 1-4 years. 24 (25%) studied in college, 5 (5.2%) were graduates or above, and 8 (8.33%) were illiterate, and the p-value was found to be 0.001C.

Out of 197 HIV-positive women, 139 (70.5%) were salaried and 58 (29.4%) were housewives. Out of 96 HIV-negative women, 52 (54.16%) were housewives, 23 (23.95%) were laborers, and 21 (21.8%) were salaried. The p-value was > 0.0001c.

None were found to be divorced, widowed, or non-discourse in an HIV-positive group. In the HIV-negative group, 7 (7.29%) were divorced, 3 (3.12%) were widowed and 5 (5.2%) were non-disclosed (Table 7).

Table 8. Demographic details of HIV-positive patients

Variable	Mean	Median	Range
Number of family members	4.6 ±2.7	4	1-28
Number of sexual partners	1.09 ±0.3	1	0-3
Number of pregnancies	2.3 ±1.3	2	0-9

The demographic details of HIV-positive patients, such as the number of family members (Mean value-4.6±2.7), the number of sexual partners (Mean value-1.09±0.3), and the number of pregnancies (Mean value-2.3±1.3) are tabulated in Table 8.

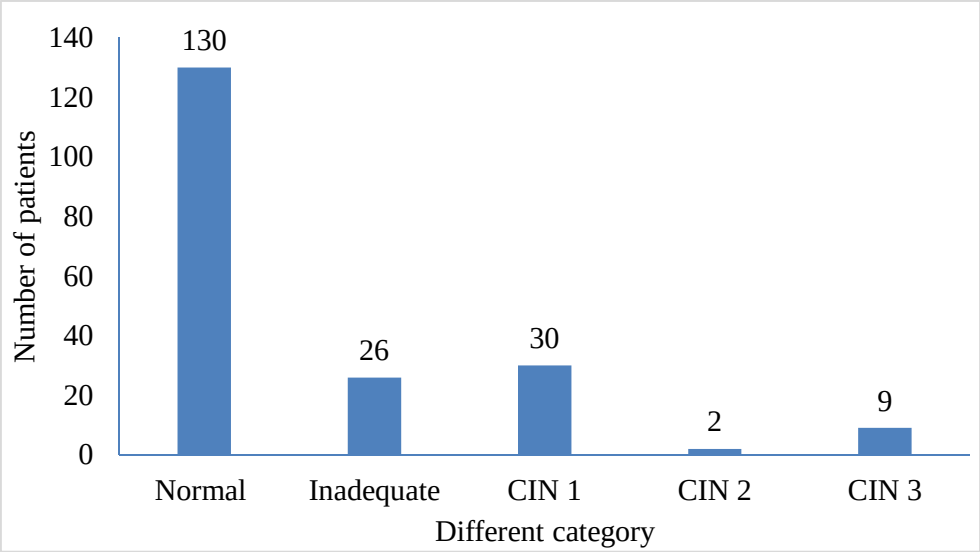


Figure 26. Graphical representation of Colpo findings among HIV-positive patients

Out of 197 HIV-positive patients, the colpo findings were as follows: 130 were normal, 26 were inadequate. The Cervical Intraepithelial Neoplasia 1 (CIN1) was found to be 30, CIN2 was found to be 2 and CIN3 was found to be 9 (Figure 26).

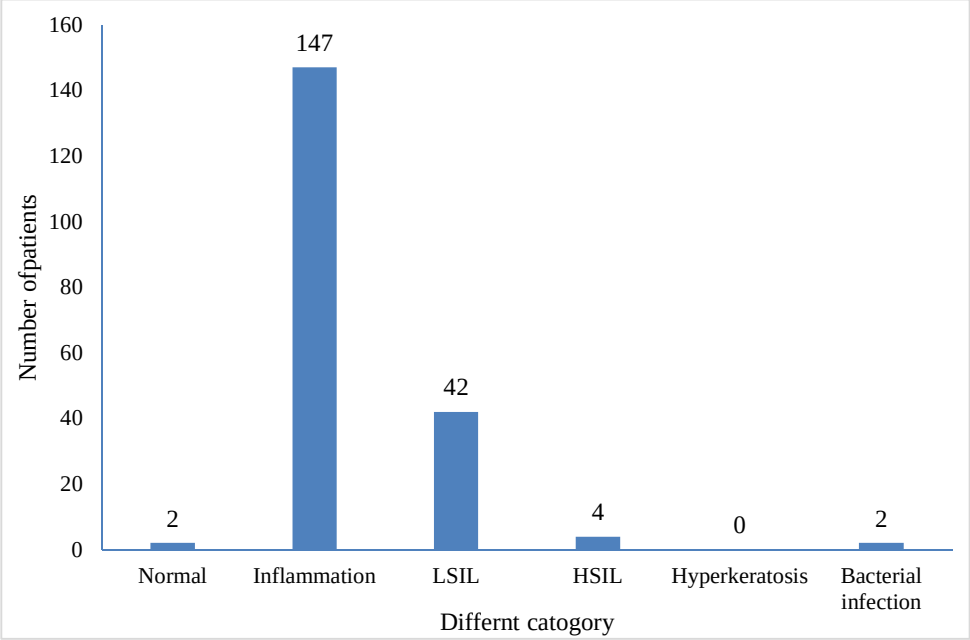


Figure 27. Graphical representation of PAP report among HIV-positive patients

A Papanicolaou (PAP Test) was conducted among 197 HIV-positive patients. The results were as follows: 147 showed inflammatory lesions. 42 cases were of the low-grade squamous intraepithelial lesion (LSIL), and 4 cases were of the high-grade intraepithelial lesion (HSIL). 2 cases were normal, 2 cases showed bacterial infection, and 0 cases showed hyperkeratosis (Figure 27).

Table 9. Prevalence of HPV genotypes in HIV-positive patients, overall and stratified by CIN status

	Overall (n=86) N% (95% CI)	Normal (n=53) % (95% CI)	Inadequate (n=13) % (95% CI)	CIN 1 (n=13) % (95% CI)	CIN 2 (n=2) % (95% CI)	CIN 3 (n=5) % (95% CI)	P-value
Carcinogenic	51 59.3(48.92,69.69)	60.38(47.21,73.55)	46.15(19.05 ,73.25)	53.85(26.75,80. 95)	100(100,100)	80(44.94,10 0)	0.5757
Possibly carcinogenic	14 16.28(8.48,24.08)	15.09(5.46,24.73)	0	30.77(5.68,55.8 6)	0	40(0,82.94)	0.1194
Non/Un carcinogenic	47 54.65(44.13,65.17)	52.83(39.39,66.27)	69.23(44.14 ,94.32)	53.85(26.75,80. 95)	50(0,100)	40(0,82.94)	0.8511
Single	47 54.65(44.13,65.17)	58.49(45.22,71.76)	61.54(35.09 ,87.98)	38.46(12.02,64. 91)		60(17.06,10 0)	0.2693
Multiple	39 45.35(34.83,55.87)	41.51(28.24,54.77)	38.46(12.02 ,64.91)	61.54(35.09,87. 98)	100(100,100)	40(0,82.94)	0.2683
Carcinogenic HPV genotypes							
HPV 16	17 19.77(11.35,28.18)	26.42(14.54,38.28)	0	0	50(0,100)	40(0,82.94)	0.04
HPV 18	5 5.81(0.87,10.76)	3.77(0,8.90)	0	15.38(0,35)	0	20(0,55.06)	0.2294
HPV 31	3 3.49(0,7.36)	5.66(0,11.88)	0	0	0	0	0.6517
HPV 33	17 19.77(11.35,28.18)	13.21(4.09,22.32)	23.08(0.02, 45.98)	38.46(12.02,64. 91)	50(0,100)	20(0,55.06)	0.2159
HPV 35	15 17.44(9.42,25.46)	11.32(2.79,19.85)	23.08(0.02, 45.98)	30.77(5.68,55.8 6)	50(0,100)	20(0,55.06)	0.2764
HPV 39	8 9.3(3.16,15.44)	11.32(2.79,19.85)	7.69(0,22.1 8)	7.69(0,22.18)	0	0	0.959
HPV 51	6 6.98(1.59,12.36)	7.55(0.04,14.66)	15.38(0,35)	0	0	0	0.5287
HPV 52	15 17.44(9.42,25.46)	11.32(2.79,19.85)	23.08(0.02, 45.98)	30.77	50(0,100)	20(0,55.06)	0.2924
HPV 56	4 4.65(0.02,9.10)	7.55(0.04,14.66)	0	0	0	0	0.6107
HPV 58	17 19.77(11.35,28.18)	13.21(4.09,22.32)	30.77(5.68, 55.86)	30.77(5.68,55.8 6)	50(0,100)	20(0,55.06)	0.3173
HPV 59	6 6.98(1.59,12.36)	5.66(0,11.88)	0	15.38(0,35)	50(0,100)	0	0.093
HPV 68	2 2.33(0,5.51)	3.77(0,8.90)	0	0	0	0	>0.99
Possibly' carcinogenic HPV genotypes							
HPV 53	6 6.98(1.59,12.36)	7.55(0.04,14.66)	0	7.69(0,22.18)	0	20(0,55.06)	0.5982
HPV 66	2 2.33(0,5.51)	3.77(0,8.90)	0	0	0	0	>0.99
HPV 67	2 2.33(0,5.51)	0	0	7.69(0,22.18)	0	20(0,55.06)	0.1209
HPV 70	2 2.33(0,5.51)	1.89 (0,5.54)	0	7.69(0,22.18)	0	0	0.6282
HPV 73	1 1.16(0,3.43)	1.89 (0,5.54)	0	0	0	0	>0.99
HPV 82	4 4.65(0.02,9.10)	3.77(0,8.90)	0	15.38(0,35)	0	0	0.2899
Individual 'Non-carcinogenic' or 'Unknown carcinogenic' HPV genotype							
HPV 6	1 1.16(0,3.43)	1.89 (0,5.54)	0	0	0	0	>0.99
HPV 11	1 1.16(0,3.43)	0	7.69(0,22.1 8)	0	0	0	0.3918
HPV 40	1 1.16(0,3.43)	0	7.69(0,22.1 8)	0	0	0	0.3983
HPV 42	10 11.63(4.85,18.40)	9.43(1.56,17.30)	23.08(0.02, 45.98)	15.38(0,35)	0	0	0.5917

HPV 54	2 2.33(0,5.51)	3.77(0,8.90)	0	0	0	0	>0.99
HPV 55	5 5.81(0.87,10.76)	7.55(0.04,14.66)	7.69(0,22.18)	0	0	0	0.8721
HPV 61	8 9.3(3.16,15.44)	13.21(4.09,22.32)	0	7.69(0,22.18)	0	0	0.4993
HPV 62	6 6.98(1.59,12.36)	5.66(0,11.88)	7.69(0,22.18)	7.69(0,22.18)	0	20(0,55.06)	0.8901
HPV 71	3 3.49(0,7.36)	3.77(0,8.90)	7.69(0,22.18)	0	0	0	>0.99
HPV 72	6 6.98(1.59,12.36)	3.77(0,8.90)	0	15.38(0,35)	50(0,100)	20(0,55.06)	0.0495
HPV 81	1 1.16(0,3.43)	1.89 (0,5.54)	0	0	0	0	>0.99
HPV 83	2 2.33(0,5.51)	1.89 (0,5.54)	0	7.69(0,22.18)	0	0	0.6277
HPV 84	8 9.3(3.16,15.44)	9.43(1.56,17.30)	7.69(0,22.18)	15.38(0,35)	0	0	0.8921
HPV CP6108	2 2.33(0,5.51)	1.89 (0,5.54)	0	7.69(0,22.18)	0	0	0.6172
HPV IS39	2 2.33(0,5.51)	1.89 (0,5.54)	7.69(0,22.18)	0	0	0	0.5952

A total of 197 cases with a mean age of 33.93±5.69 years were considered for the study, with 86 HPV-positive and 111 HPV-negative cases. Out of 86 HPV-positive cases, 53 had normal CIN and 13 were inadequate. 13 showed CIN 1, 2 had CIN 2 and 5 had CIN 3.

It has been observed that, out of 53 normal CIN, 60.38% showed carcinogenic HPV whereas 53.85% and 10% of the 13 CIN-1 and 2 cases had carcinogenicity, respectively. 47 (54.65%) of 86 HPV-positive cases had single HPV, whereas 39 (45.35%) had multiple HPV. The HPV genotypes identified in descending order of persistency were HPV 16, HPV 33, HPV 35, HPV 52, and HPV 58. Using the chi-square test with stimulation, it has been observed that HPV 16 and 72 are significant genotypes detected in women with CIN lesions as compared to those with normal cervical status. HPV 53, 66, 67, 70, 73, and 82 are the HPV genotypes that are known to be possibly carcinogenic (Table 9).

Table 10. Association between participants' characteristics and prevalence of HPV among HIV positive patients.

Factor		Any HPV	Single HPV	Multiple HPV	HPV 16	Non-HPV 16
Age (in years)		0.99(0.94,1.05)	0.97(0.91,1.03)	1.02(0.96,1.09)	0.99(0.90,1.09)	1.01(0.92,1.11)
Education	Literate	0.50(0.02,5.66)	1.57(0.07,18.25)	-	-	-
	Illiterate	Reference				
Occupation	Salaried	0.90(0.48,1.72)	0.63(0.31,1.30)	1.59(0.71,3.86)	3.30(0.87,21.69)	0.30(0.05,1.15)
	House wife	Reference				
Ever pregnancy	Yes	**4.47(1.01,31.58)**	-	1.01(0.21,7.37)	-	-
	No	Reference				
Number of pregnancies	>3	0.86(0.39,1.87)	1.14(0.45,2.73)	0.73(0.25,1.91)	0.88(0.18,3.19)	1.13(0.31,5.43)
	3 or less	Reference				
Number of sex partners	2 or more	1.80(0.73,4.59)	1.34(0.47,3.51)	1.41(0.47,3.83)	2.12(0.53,7.11)	0.47(0.14,1.90)
	1	Reference				
Use of contraception	Yes	0.73(0.37,1.42)	0.89(0.43,1.90)	0.90(0.40,2.10)	1.42(0.45,5.41)	0.71(0.18,2.22)
	No	Reference				
Screened for CC	Yes	0.96(0.16,5.53)	1.06(0.85,1.28)	0.95(0.68,1.18)	1.11(0.78,1.35)	0.93(0.74,1.28)
	No	Reference				
STI symptom	Yes	1.10(0.52,2.34)	1.30(0.5,2.93)	0.95(0.35,2.37)	0.92(0.20,3.20)	1.08(0.31,5.11)
	No	Reference				
History of opportunistic infection	Yes	1.64(0.55,5.57)	1.45(0.42,6.71)	1.83(0.45,12.39)	-	-
	No	Reference				
CD4 (in %)		1(0.97,1.02)	0.99(0.96,1.01)	1.01(0.99,1.04)	1.01(.97,1.05)	0.98(0.95,1.03)

**Absence of outcome like any HPV, single HPV is a taken as a reference; 197 subjects were considered; "-" indicates that variables are excluded as there are zero counts.*

Note: 197 subjects were considered for the above table. For single HPV, multiple and HPV-negative cases are considered non-single HPV.

Using logistic regression, it has been concluded that every pregnancy significantly affects any HPV. Using the adjusted odds ratio, it has been observed that the odds of any HPV are 4.47 times higher for subjects with a history of pregnancy than for other patients (Table 10).

Table 11. The association of prevalent carcinogenic HPV genotypes (present alone or in combination with carcinogenic types) with the risk of CIN2 and CIN3 in HIV-positive patients.

	CIN 2 (vs ≤CIN1)	**CIN 3 (vs ≤CIN2)**
	OR [95% CI]	**OR [95% CI]**
Carcinogenic	4.31[0.67,84.07]	2.37(0.29,49.12)
Possibly carcinogenic	2.22[0.26,14.56]	2.60(0.27,20.92)
Non/Un carcinogenicity	0.63(0.11,3.33)	0.76(009,5.80)
Single	0.54(0.09,2.71)	1.22(0.18,10.49)
Multiple	1.86(0.37,10.62)	0.82(0.10,5.69)

**OR is adjusted for age, Number of pregnancies, use of contraception and number of CD4 cells*

From multivariable logistic regression, it has been concluded that the appearance of carcinogenic, possibly carcinogenic, non/un carcinogenic, and single HPV and multiple HPV is not significantly associated with CIN 2 as well as CIN 3, respectively, keeping other terms constant (Table 11).

Note: Normal and inadequate CIN is considered as "≤CIN1"

Table 12. Comparison of the PAP test with MY9/11 and G5/G6 primer sets among HIV-positive patients.

	Positive	**Negative**	**P-Value**
MY9/11	64	132	0.62^{McNem}
G5/G6	66	130	

The MY9/11 and G5/G6 oligonucleotide primers were used for HPV-DNA PCR analysis. Out of 196 HIV-positive cases, 64 were detected as positive by MY9/11 and 132 were detected as negative. 66 were detected as positive for the G5/G6 primer, and 130 were detected as negative. As shown in Table 12, no significant difference was observed when the p-value was compared between the 2 groups (the p-value was 0.62McNem). 86 cases were found to be positive for HPV and 110 cases were found negative in the Pap test when compared with MY9/11 (64 cases positive and 132 negative) and G5/G6 tests (66 cases positive and 130 negative).

Table 13. Diagnostic efficacy of MY9/11 and G5/G6 primers

HIV positive cases	Sensitivity	Specificity	PPV	NPV
MY9/11	72.42 % (63.87% to 83.22%**)**	100.00% (96.70% to 100.00%)	100.00%	17.06% (12.55% to 22.78%)
G5/G6	76.74% (66.39% to 85.18%)	100.00% (96.70% to 100.00%)	100.00%	18.45% (13.36% to 24.94%)

The sensitivity in detecting HPV DNA between two primer sets, MY9/11 and G5/G6, was found to be 72.42% and 76.74%, respectively. The specificity for both primer sets was 100.00% among HIV-positive cases. As tabulated in Table 13, the PPV (Positive predictive value) and NPV (Negative predictive value) were found to be 100%, 17.06%, and 18.45%, respectively.

HIV Negative patients

Table 14. Demographic details of HIV-negative patients

Variable	Mean	Median	Range
Number of family members	4.8 ±1.9	4	1-13
Number of sexual partners	1.03 ±0.26	1	0-2
Number of pregnancies	1.84 ±1.02	2	0-4

The demographic details of HIV-negative patients, such as the number of family members (Mean value: 4.8±1.9), number of sexual partners (Mean value: 1.03±0.26), and number of pregnancies (Mean value: 1.84±1.02) are tabulated in Table 14.

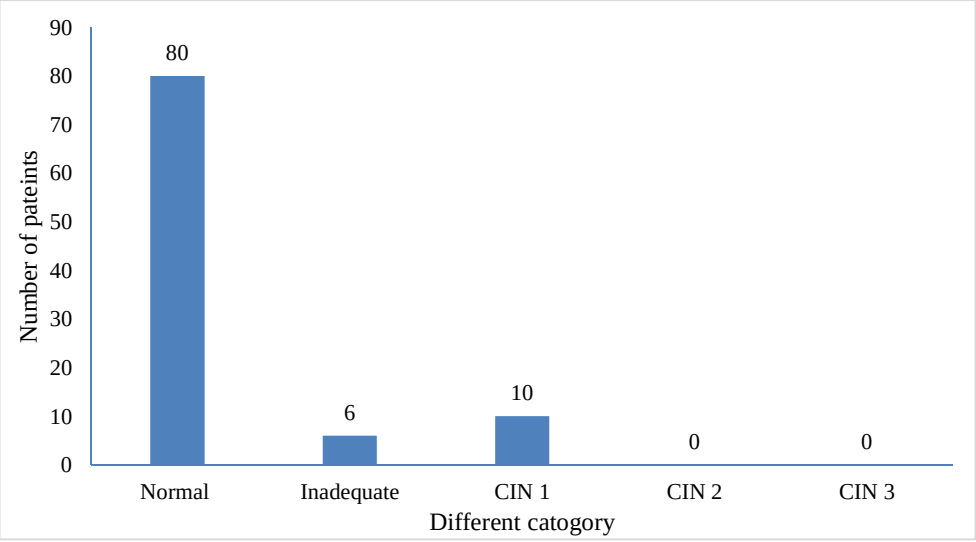


Figure 28. Graphical representation of colposcopy finding among HIV-negative patients

Out of 96 HIV-negative patients, the colposcopy findings were as follows: 80 were normal, 6 were inadequate. Cervical Intraepithelial Neoplasia 1 (CIN1) was found to be 10. None was found for CIN2 and CIN 3 (Figure 28).

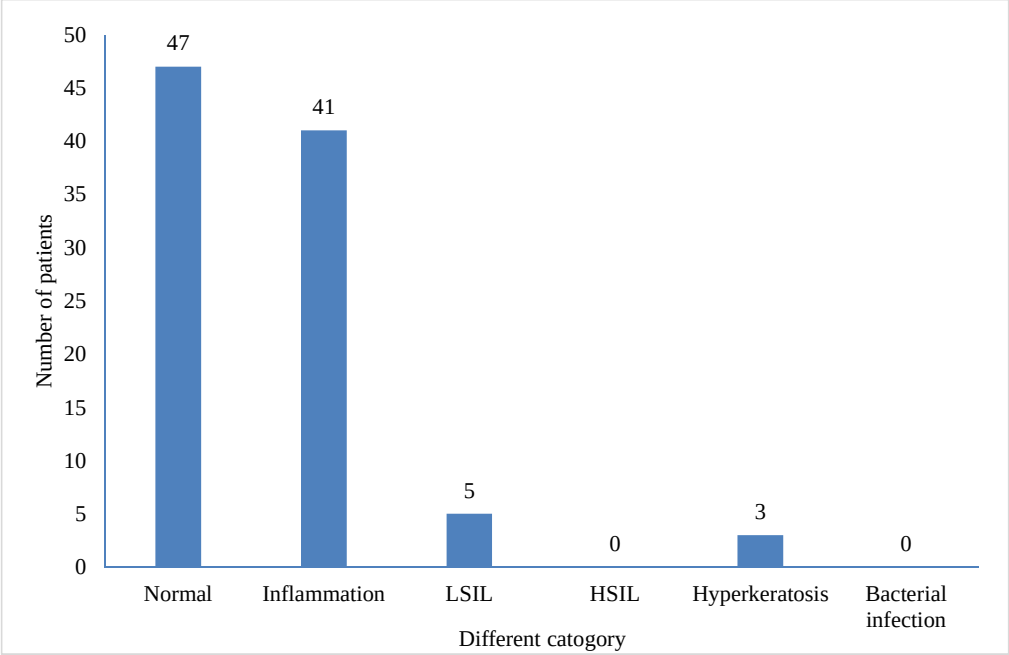


Figure 29. Graphical representation of PAP test among HIV-negative.

A Papanicolaou (PAP Test) was conducted among 96 HIV-negative patients. The results were as follows: 47 showed normal, 41 inflammatory lesions, 5 cases were of the low-grade squamous intraepithelial lesion (LSIL), and none of the high-grade intraepithelial lesion (HSIL), bacterial infection, and 3 cases showed hyperkeratosis (Figure 29).

Table 15. Summary of the Socio-demographic data

Factor	Sub-category	Overall	HPV (based on genotype result)	
			Yes(n=13)	No
Age group	19-24	14 (14.74%)	3 (21.43%)	11 (78.57%)
	25-29	14 (14.74%)	2 (14.29%)	12 (85.71%)
	30-34	29 (30.53%)	4 (13.79%)	25 (86.21%)
	35-39	20 (21.05%)	1 (5%)	19 (95%)
	≥40	18 (18.95%)	2 (11.11%)	16 (88.89%)
Marital status	Married	81 (84.38%)	12 (14.81%)	69 (85.19%)
	Divorced	7 (7.29%)	0 (0%)	7 (100%)
	Widow	3 (3.13%)	0 (0%)	3 (100%)
	Not disclosed	5 (5.21%)	1 (20%)	4 (80%)
No. of family members	01-Feb	5 (5.21%)	1 (20%)	4 (80%)
	03-Apr	45 (46.88%)	7 (15.56%)	38 (84.44%)
	05-Jun	32 (33.33%)	3 (9.38%)	29 (90.63%)
	≥7	14 (14.58%)	2 (14.29%)	12 (85.71%)
Education	Literate	88 (91.67%)	11 (12.5%)	77 (87.5%)
	Illiterate	8 (8.33%)	2 (25%)	6 (75%)
Addiction	Yes	12 (12.5%)	2 (16.67%)	10 (83.33%)
	No	84 (87.5%)	11 (13.1%)	73 (86.9%)
Ever pregnant	Yes	87 (90.63%)	12 (13.79%)	75 (86.21%)
	No	9 (9.38%)	1 (11.11%)	8 (88.89%)
No. of pregnancies (Among ever pregnant women)	1	26 (29.89%)	6 (23.08%)	20 (76.92%)
	2	38 (43.68%)	4 (10.53%)	34 (89.47%)
	3	17 (19.54%)	2 (11.76%)	15 (88.24%)
	4	6 (6.9%)	0 (0%)	6 (100%)
Number of live births (Among ever pregnant women)	0	2 (2.3%)	0 (0%)	2 (100%)
	1	40 (45.98%)	8 (20%)	32 (80%)
	2	30 (34.48%)	2 (6.67%)	28 (93.33%)
	3	14 (16.09%)	2 (14.29%)	12 (85.71%)
	4	1 (1.15%)	0 (0%)	1 (100%)
Age of 1st vaginal intercourse	Minor	7 (7.29%)	3 (42.86%)	4 (57.14%)
	18-22	65 (67.71%)	10 (15.38%)	55 (84.62%)
	23-27	18 (18.75%)	0 (0%)	18 (100%)

	28 and above	4 (4.17%)	0 (0%)	4 (100%)
	Never	2 (2.08%)	0 (0%)	2 (100%)
No. of sexual partners (Excluded 2 subjects)	1	89 (92.71%)	12 (13.48%)	77 (86.52%)
	2	5 (5.21%)	1 (20%)	4 (80%)
Use of Contraception	Yes	70 (74.47%)	10 (14.29%)	60 (85.71%)
	No	24 (25.53%)	3 (12.5%)	21 (87.5%)
Type of contraceptive (Among subjects who used contraception)	Birth control pill	14 (20%)	0 (0%)	14 (100%)
	Injectable contraceptive	6 (8.57%)	2 (33.33%)	4 (66.67%)
	Condom	51 (72.86%)	9 (17.65%)	42 (82.35%)
	Diaphragm	0 (0%)	-	-
	Copper-t	39 (55.71%)	6 (15.38%)	33 (84.62%)
	Others	1 (1.43%)	0 (0%)	1 (100%)
Period of using contraception	Month	14 (20%)	3 (21.43%)	11 (78.57%)
	Year	56 (80%)	7 (12.5%)	49 (87.5%)
Use of contra. In last month	Yes	31 (44.29%)	2 (6.45%)	29 (93.55%)
	No	39 (55.71%)	8 (20.51%)	31 (79.49%)
Screened for cervical cancer			-	-
How many times screened			-	-

Genotype Result: "No" is considered as HPV absent.

A total of 96 cases, with a mean age of 32.07±6.79 years, consisting of 13 HPV-positive cases and 83 HPV-negative cases, were considered for the study.

It has been observed that most of the cases are in the "30–34" age group, followed by "35–39" years. About 81 (84.38%) women were married, of which 12 (14.81%) had HPV. 88 (91.67%) been literate, of which 11 (12.5%) had HPV. 8 (8.33%) been illiterate, of which 2 (22.5%) had HPV. 12 (12.5%) women had some addiction, of which 2 (16.67%) had HPV. Out of 96 cases, 87 (90.63%) had pregnancy at least once, of which 12 (13.79%) had HPV and 75 (86.21%) had no HPV. Among ever-pregnant women, 26 (29, 89%) had one pregnancy, of which 6

(23.08%) had HPV, and 38 (43.68%) had two pregnancies, with four (10.53%) having HPV. Only six (6.97%) had four pregnancies. 89 (92.71%) had only 1 sexual partner, of which 12 (13.48%) had HPV (2 cases excluded). 70 of 94 cases who had sex at least once used contraception. 14 (20%) used birth control pills, 6 (8.57%) used injectable contraceptives, of which 2 (33.33%) had HPV. Different types of contraceptives such as condoms, 51 (72.86%) of which 9 (17.65%) had HPV, followed by Copper-t 39 (55.71%) of which 6 (15.38%) had HPV. Condoms and Copper-t are the common types of contraceptives used (Table 15).

Table 16. Prevalence of HPV genotypes in HIV-negative patients, overall and stratified by CIN status

Genotype	Overall N % [95% CI]	Grading				P-value
		1+(n=7)	2+(n=5)	3+(n=9)	4+(n=2)	
Carcinogenic	12 52.17(32.52,71.31)	28.57(6.47,64.77)	20(2.25,62.86)	88.89(58.55,98.77)	50(6.08,93.92)	0.0396
Possibly' carcinogenic	3 13.04(3.81,30.87)	0	20(2.25,62.86)	11.11(1.23,41.45)	50(6.08,93.92)	0.1485
Non-carcinogenic	8 34.78(18.02,55.11)	71.43 (35.23,93.52)	60(20.94,90.56)	0	0	0.0024
Carcinogenic HPV genotypes						
HPV 16	2 8.7(1.85,25.09)	0	0	22.22(4.93,54.38)	0	0.268
HPV 59	10 43.48(24.99,63.50)	28.57(6.47,64.77)	20(2.25,62.86)	66.67(34.78,89.58)	50(6.08,93.92)	0.1487
Possibly' carcinogenic HPV genotypes						
HPV 53	2 8.7(1.85,25.09)	0	20(2.25,62.86)	0	50(6.08,93.92)	0.268
HPV 70	1 4.35(0.47,18.5)	0	0	11.11(1.23,41.45)	0	0.4442
Individual 'Non-carcinogenic' or 'Unknown carcinogenic' HPV genotype						
HPV 42	1 4.35(0.47,18.5)	14.29(1.59,50.08)	0	0	0	0.1918
HPV 54	1 4.35(0.47,18.5)	14.29(1.59,50.08)	0	0	0	0.1918
HPV 61	1 4.35(0.47,18.5)	14.29(1.59,50.08)	0	0	0	0.1918
HPV 62	2 8.7(1.85,25.09)	14.29(1.59,50.08)	20(2.25,62.86)	0	0	0.2542
HPV 72	2 8.7(1.85,25.09)	14.29(1.59,50.08)	20(2.25,62.86)	0	0	0.2542
CP6108	1 4.35(0.47,18.5)	0	20(2.25,62.86)	0	0	0.7871

*Cases with multiple genotypes are considered individually as a grade is provided for all genotypes in a case with multiple genotypes.

It has been observed that out of 23 HPV-positive cases, 7 cases had a grade of 1+, 5 cases had a grade of 2+, 9 cases had a grade of 3+, and 2 cases had a grade of 4+. Out of 7 grade 1+ cases, 28.57% had carcinogenic HPV, whereas 20% and 89% of grade 2+ and grade 3+ cases had carcinogenic HPV, respectively. It has been observed that HPV 59 is the most common genotype, followed by 16, 53, 62, and 72. Using the Cochran Armitage trend test, it has been concluded that there is a significant linear increasing trend in the proportion of carcinogenic and non-carcinogenic genotypes over grade. p-value of the carcinogenic HPV genotype, i.e., 0.0396, is considered significant over the non-carcinogenic HPV genotype (p-value-0.0024) (Table 16).

Table 17. Association between participants, characteristics, and presence of HPV among HIV-negative patients.

Factor		Any HPV	Single HPV	Multiple HPV	HPV 16	Non-HPV 16
Age (in years)		0.92[0.83,1.01]	0.93[084,1.03]	0.85[0.64,1.05]	1.16[0.95,1.55]	**0.88[077,0.98]**
Education	literate	0.43[0.08,3.17]	0.34[0.06,2.63]	0.49[0.02,11.09]	0.49[0.02,11.09]	0.34[0.06,2.63]
	Illiterate	Reference				
Addiction	Yes	1.33[0.26,6.88]	1.67[0.23,7.84]	1.32[0.06,29.12]	7.55[0.27,209]	0.67[0.03,4.13]
	No					
Ever pregnancy	Yes	1.28[0.15,11.17]	2.86[0.16,52.46]	0.09[0.01,1.63]	0.56[0.02,12.45]	1.04[0.16,21.21]
	No	Reference				
Number of sex partners	2	1.65[0.08,12.61]	2.03[0.21,19.95]		3.25[0.14,76.41]	2.03[0.09,15.81]
	1 or less	Reference				
Use of contraception	Yes	1.28[0.35,6.19]	1.77[0.35,8.80]	0.36[0.01,9.81]	1.93[0.09,41.64]	0.99[0.24,4.05]
	No	Reference				

Using univariate logistic regression, age has been found to be significantly related to non-HPV 16 genotypes. The odds of the absence of non-HPV 16 genotypes increase by a factor of 1.14 for every one-year increase in age, according to the odds ratio (Table 17).

Note: The analysis of the above table includes all 96 cases. The analysis of the above table includes all 96 cases. In the single HPV column, only HPV cases with a single genotype are considered, and the remaining (including multiple HPV) are considered absent.

Some factors have been left out because further information is needed. All analysis is done with the assumption that no objectives or synopsis are present.

Table 18. Comparison of the PAP test with MY9/11 and G5/G6 primer sets among HIV-negative patients

	Positive	**Negative**	**p-value**
MY9/11	11	85	1.0
G5/G6	11	85	

The two oligonucleotide primers used in HPV-DNA PCR (polymerase chain reaction) analysis are MY09/11 and G5/G6. Out of 96 HIV-negative cases, 11 were detected as positive and 85 were detected as negative by both the primer sets. When the p-value was compared between the two groups, there was no significant difference (p-value was 1.0), as shown in Table 18. There were 13 cases reported to be positive for HPV and 83 cases were found negative in Pap test when compared with MY9/11 (11 cases positive and 85 negative) and G5/G6 tests (11 cases positive and 85 negative).

Table 19. Diagnostic efficacy of MY9/11 and G5/G6 among HIV negative patients

HIV Negative cases	Sensitivity	Specificity	PPV	NPV
MY9/11	84.62% (54.55% to 98.08%)	100.00% (95.65% to 100.00%)	100.00%	25.49% (8.73% to 55.04%)
G5/G6	84.62% (54.55% to 98.08%)	100.00% (95.65% to 100.00%)	100.00%	25.49% (8.73% to 55.04%)

In HIV-negative patients, the sensitivity and specificity of two primer sets, MY9/11 and G5/G6, in detecting HPV DNA were reported to be 84.62 % and 100.00 %, respectively. As shown in Table 19, the PPV (Positive predictive value) and NPV (Negative predictive value) were determined to be 100 % and 25.49 %, respectively.

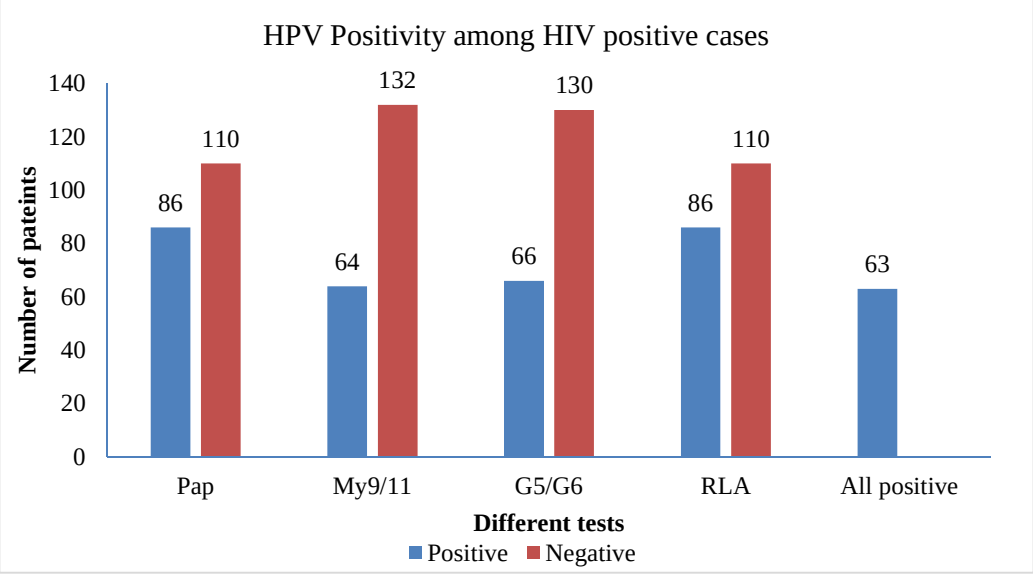


Figure 30. Determination of HPV positivity among HIV-positive cases

Different tests were conducted for the detection of HPV positivity among HIV-positive cases, such as the Pap test, PCR by My9/11, G5/G6, and RLA (Roche linear array). The results shown in figure 30 are as follows; a total of 197 cases were considered, of which the Pap test showed 86 positive and 110 negative, the My9/11 test showed 64 positive and 132 negative, the G5/G6 test showed 66 positive and 130 negative, and the RLA test showed 86 positive and 110 negative.

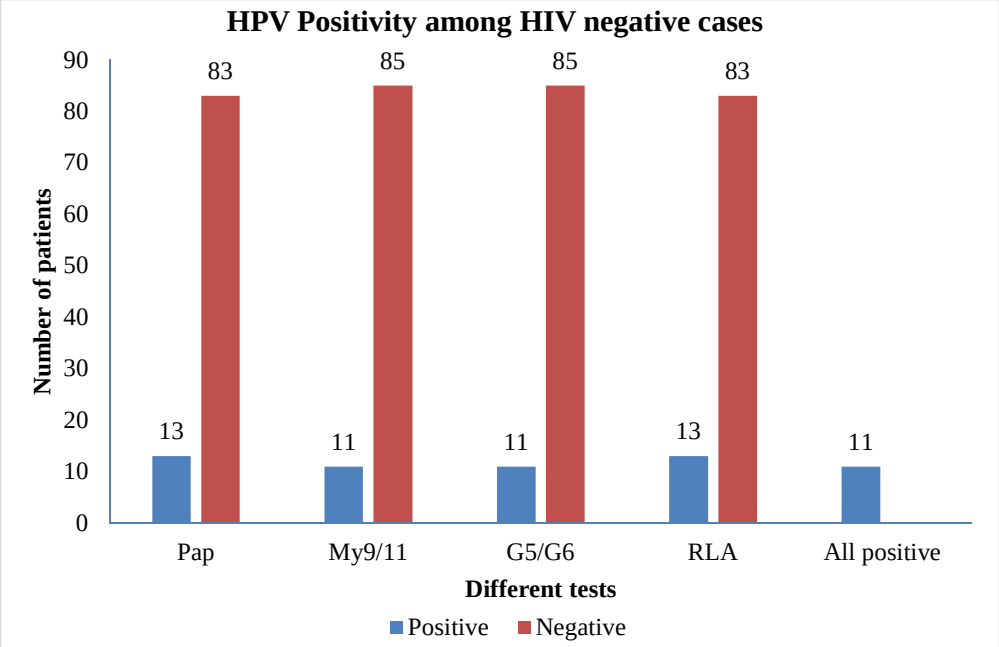


Figure 31. Determination of HPV Positivity among HIV-negative cases.

Different tests were conducted for the detection of HPV positivity among HIV-negative cases, such as the Pap test, My9/11, G5/G6, and RLA (Roche linear array). The results shown in figure 31 are as follows: A total of 96 cases were considered, of which the Pap test showed 13 positive and 83 negative, the My9/11 and G5/G6 tests showed 11 positive and 85 negative, and the RLA test showed 13 positive and 83 negative.

Table 20. Overall results for HPV positivity among HIV-positive and negative cases

Variable	PAP		MY9/11		G5/G6		RLA		P-value
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	
HIV Positive	86	110	64	132	66	130	86	110	0.022^{C}
HIV Negative	13	83	11	85	11	85	13	83	0.94^{C}

Different tests for HPV positivity among HIV-positive and negative cases are tabulated in table 20. The p-value for HIV-positive 4 was found to be 0.022^{C}, which is significant, and for the HIV-negative as found to be 0.94^{C}, which is non-significant.

DISCUSSION

During the last few years, Papanicolaou smear is being replaced with HPV genotyping due to its high sensitivity and specificity. Linear Array genotyping and PCR testing with several primers were most commonly used assays for HPV detection.[154,155]

Agnes *et al.,* 2020,[156] conducted a study in which 217 women with an average age of 35.73 years were considered. Out of 217 women, 68 (31.3%) tested positive for HPV DNA, 29 (13.36%) tested positive for HIV, and 14 (6.5%) tested positive for both HIV and HPV infection. In HIV-positive women, HPV infection was significantly more prevalent ($p = 0.031$). Also, HPV 16 was found to be the most prevalent among other genotypes. In the present study, a total of 197 HIV-positive patients were evaluated, with an average age of 33.93±5.6. Out of 197 HIV-positive patients, 64 (32.48%) and 66 (33.5%) were HPV DNA positive, respectively, by MY9/11 and G5/G6 primers. The most common genotypes were HPV 16 and HPV 72.

In another report, the prevalence of HPV infection in Central and Eastern Italy was determined by Edoardo *et al.,* 2017.[157] A total of 200 swabs were analyzed for cytological screening and for HPV-DNA-genotyping testing. In total, 66/200 swabs resulted in HPV-positive, whereas in our study, 86/197 cases were detected as HPV-positive. The overall HPV prevalence was 33%, with a higher prevalence in the HIV-positive group (48%). In our study, overall HPV prevalence was found to be 45.35% (multiple HPV), 54.65% (single HPV), and 59.3% (carcinogenic) out of 86 HPV-positive cases. The most frequent genotypes were HPV 16, 31, 52, 58, 66, 73, and 89

when compared with our studies. The HPV genotypes detected in descending order of frequency were HPV 16, 33, 35, 52, and 58.

In a study conducted by Hong-Yun *et al.* 2012,[158] the colposcopic-histopathological diagnosis was performed where 67 out of 83 women (80.7%) revealed no evidence of CIN. CIN1 was reported in 9 women (9.5%), CIN2 in 5 women (5.3%), and CIN3 in 2 women (2.1%). 16/83 (19.3%) had evidence of CIN1+ lesions and 7/83 (8.4%) had evidence of CIN2+ lesions, whereas in our study, out of 86, 53 (61.62%) had normal CIN, 13 were inadequate. CIN1 was reported in 13 women, CIN2 in 2 women, and CIN3 in 5 women.

In another study, Rodolfo *et al*. 2018 [50] detected 59 (73.75%) HPV DNA out of 80 cervical samples. Patients who were HIV-infected presented a high prevalence of HPV co-infection. High-risk HPVs were predominant (59.3%). Also, HPV 56 (17%) was the most prevalent genotype, followed by HPV 16 (15.3%). In our study, out of 197 HIV-positive cases, HPV DNA was detected in 64 cases by the MY9/11 primer and in 66 by the G5/G6 primer. HPV 16 and HPV 72 were the most prevalent genotypes.

One of the studies conducted by Maria *et al.,* 2011,[159] determined the rate of high-risk-HPV genotypes in low and high grade squamous intraepithelial lesions (LSIL and HSIL) as well as in cervical carcinoma among Venezuelan women. HPV DNA was detected in 68%, 95%, and 98.7% of LSIL, HSIL, and cervical carcinoma cases, respectively. HPV types 16 and 18 were the most common high-risk HPV types observed, followed by HPV types 52, 33, 45, and 31. In our study, 42 cases out of 197 were found to be LSIL, 4 cases were found to be HSIL, and 51 out of 86 HPV-positive cases showed carcinogenicity. The most prevalent genotypes were HPV16

and 72, followed by HPV33, 35, 52, and 58. Women with CIN lesions were found to have HPV 16 and 72 genotypes compared to those with normal cervical status.

In our study, out of 86 HPV-positive cases, 51 (59.3%) showed carcinogenic HPV compared to another study conducted by Arati *et al.,* 2012.[155] Here, carcinogenic HPV genotypes were present in 35.3% (98/278) of HIV-infected women. 'Possibly carcinogenic' and 'unknown carcinogenic' genotypes were present in 14.7% and 29.5% of patients, respectively. Multiple HPV genotypes were found to be 50.7%, and a single HPV genotype was found to be 52.5%. HPV16 was the most common HPV genotype. This is in contrast with our study, i.e., 14/86 (16.28%) were found to be "Possibly carcinogenic" and 47/86 (54.65%) were found to be "Unknown carcinogenic." Single HPV was found to be 47/86 (54.65%) and multiple HPV was found to be 39 out of 86 (45.35%). Here, HPV 16, 33, 35, 52, and 58 were the common HPV genotypes.

Jaya *et al.*, 2016, [160] screened a total of 216 HIV women, of whom 58 were HPV-positive and 56 were high-risk HPV types. HPV 16 was the most prevalent type. They concluded that HIV-positive women over 35 years of age had a higher risk of HPV infection. These findings were very much similar to our study, where out of 293 patients, 197 were screened as HIV-positive and 86/197 cases were HPV-positive. HPV genotypes 16, 33, 35, 52, and 58 were the most common. A high-risk of HPV infection has been seen in the age group of 21-40 years old.

Nowadays, PCR is the most commonly used technique in clinical research as well as in diagnostic laboratories to detect HPV infections. For the detection of the L1 region, two oligonucleotide primer systems, MY09/MY11 and G5/G6, are commonly used. Both of these primers detect a wide spectrum of HPV DNA.

The degenerated MY09/MY11 oligonucleotide primers use a high annealing temperature (55ºC) and can amplify multiple HPV infections, whereas G5/G6 primers anneal at a lower temperature (42ºC) and only amplify a single HPV genotype. As previously demonstrated with the MY09/11 and G5/G6 assays in different laboratories, each method showed a high level of reproducibility when compared to itself.[161]

In one of the studies conducted by Milena *et al*., 2014 [192], a total of 1375 women aged 14–76 were involved, out of which 216 were HIV-positive, whose ages ranged from 20–73 years old. 69.0% of HIV-positive women (n = 149: 62.3–75.0, 95% CI) were HPV positive, and multiple HPV infections were found in 78.5% of the samples (n = 117: 71.0–84.8, 95% CI). When compared with our study, 86 cases were found to be positive for HPV. Carcinogenic and multiple HPVs were found to be 51 (59.3%) and 39 (45.35%), respectively. These results indicate that HIV-positive women are more prone to HPV infection and that multiple HPV infections occur more frequently in HIV-positive women because of a deficiency in the immune system. In that study, HPV 16 was the most prevalent genotype, which is in contrast to our study where HPV 16, 33, 35, 52, and 58 genotypes were detected.

In a similar study, the prevalence of HPV and cervical cytologic abnormalities were reported in HIV-infected women in Uganda. The HPV prevalence was found to be 46.2% (49/106) and HPV 52, 18, and 16 were the most commonly detected high-risk genotypes. The majority of HPV positive women were infected with high-risk HPV genotypes 16 and 18.[163] In our study, 51/86 (59.3%) were found to be carcinogenic. The HPV genotypes detected were 16, 33, 35, 52, and 58.

In our study, the HPV genotypes detected were HPV 16, 33, 35, 52, and 58, which is similar to the results conducted by Lynette *et al*., 2014,[164] where the most commonly detected HPV types were HPV 16, 18, 35, 45, 33, and 52, respectively. In conclusion, HPV infections were more common in HIV-positive women compared to HIV-negative women, and the prevalence of single and multiple HPV infections seemed higher among HIV-positive women than among HIV-negative women.

On univariate analysis, the significant predictors of HPV infection were HIV, age, and duration of sexual life. Bivariable and multivariable logistic regression analyses were conducted to analyze data. In bivariable models, HPV was significantly predicted by HIV infection. In the study conducted by Kamlesh *et al*., 2011 [165] the prevalence of HPV 16, 18 among HIV-positive females was higher than that of HIV-negative females. Similarly, in our study, HPV 16, 33, 35, 52, and 58 were prevalently detected HPV genotypes. This data concludes that HIV positive women have a greater risk of cervical cancer and HPV infection.

Ibrahima *et al.*, 2017 [166] identified HPV 16 and 18 in cervical cancer biopsy samples by PCR. Both HPV 16 and 18 were significantly more frequent in women over 50 years of age than in younger women. When compared to our study, HPV 16 and 72 were significant genotypes detected in women aged 21–49 years with CIN lesions. These findings imply that greater co-infection with high-risk HPV genotypes might be a risk factor for sustained exposure over time as women grow older.

In our study, HPV16 and 72 were the significant genotypes detected in women with CIN lesions (p-value found to be 0.04 and 0.0495, respectively), which were statistically significant. Out of 197/293 HIV-positive cases, 53 had normal CIN, 13 showed CIN 1, 2 had CIN 2, and 5 had CIN 3, while out of 96/293 HIV-negative

cases, 23 were found to be HPV-positive cases. The p-value of carcinogenic HPV was found to be 0.0396, which was statistically significant. These results were in contrast with the study conducted by Ongeziwe *et al.*, 2021 [(167)] where out of 193 women, 93.5% screened with CIN 2 and 96.6% screened with CIN 3 had HPV infection (p-value 0.012). These findings show that HIV-positive women had a much greater incidence of any HPV infection as compared to HIV-negative women.

In one more study reported by Bariki *et al.*, 2021[(168)] suggested that HPV 52 was the most commonly acquired HPV genotype in HIV positive women, followed by HPV 16, HPV 56, HPV 58, and HPV 35. The same acquisition patterns were found in HIV-negative women, whilst HPV16 was as commonly acquired as HPV52, and the relative importance of HPV 45 and 58 increased among HIV-positive women. Similar patterns have also been described in our study where HPV16, followed by HPV33, 35, 52, and 58, were the HPV genotypes detected among 197 HIV-positive cases and HPV 59, followed by 16, 53, 62, and 72, were the HPV genotypes found among 96 HIV-negative cases. These findings conclude that HPV 16 is more common in both HIV-positive and HIV-negative women. HPV acquisition has also been linked to young age, the number of lifetime partners, and HIV positivity.

In one of the studies done by Kirubel *et al*., 2019,[(169)] the overall HR HPV burden was 13.7%. "Other HR HPV" genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68 types) were the most frequent (76%) genotypes identified, followed by HR HPV 16 (16%). The overall frequency of abnormal cytology was 13.1%. Around three-fourths (72%) of the HR HPV-infected women were in the age range of 31 to 60 years, and this was remarkably related with abnormal cytology. HPV 16 and HPV 72 were significant genotypes detected in our study, followed by HPV 33, 35, 52, and 58.

Of 197 HIV-positive cases, 86 were HPV-positive, with a mean age of 33.93±5.6 (21–40 years of age). These results conclude that age-specific HPV infection was found in women in the age group of 21–40 years. A high risk of HPV infection is found in HIV-positive women in this age group.

In one of the studies conducted by Oppah *et al*., 2021 [(170)] of the 258 cervical cancer patients enrolled in this study had 45% (n = 116) confirmed HIV positive. The median age of the participants was 51 years old. The overall median age between HIV-positive and HIV-negative cervical cancer groups was significantly different (p = 0.001). In univariate regression analysis, the HR-HPV genotypes or multiple HR-HPV genotypes were not found to be significantly associated with any of the HPV-related risk factors (i.e., age, sexual debut, parity, and STI history). Out of 258 cervical cancer patients, 96% (n = 248) were screened positive for HR-HPV DNA, constituting 14 HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). HPV16 was the frequently identified genotype detected in 48% (n = 123) of the participants, with high frequencies for the following genotypes: HPV 35 (26.4%), HPV 18 (25.2%), HPV 58 (10.5%), HPV 33 (9.7%), and HPV 31 (6.6%). In comparison with our study, 197 HIV-positive cases were considered, of which 86 cases were HPV-positive. HPV 16 and HPV 72 were significant genotypes detected in women with CIN lesions. The HPV genotypes detected in descending order of frequency were HPV 16, 33, 35, 52, and 58. Out of 96 HIV-negative cases, 13 were found to be HPV-positive. HPV 59 is a common genotype, followed by 16, 53, 62, and 72. This suggests that HPV 16 and HPV 72 were the two most prevalent genotypes found irrespective of HIV status.

According to Monique et al., 2018 [171] the most prevalent HR-HPV types were 56/59/66 (32.2%), 35/39/68 (28.0%), 52 (21.5%), 16 (19.4%), and 45 (12.9%). 43.0% of the women with HR-HPV infection (n = 93) had multiple infections. Cervical abnormalities were more common in HPV-positive women than in HPV-negative women. The multivariable analysis revealed that increasing age was associated with a lower risk of HR-HPV infection. In our study, out of 197 HIV-positive cases, 86 were found to be HPV-positive. In descending order of frequency, HPV 16, 33, 35, 52, and 58 genotypes were discovered. Of these genotypes, HPV 16 and 72 were found to be significant genotypes. Out of 96 HIV-negative cases, 13 were found to be HPV-positive. Here, HPV 59 was found to be the most common genotype, followed by HPV 16, 53, 62, and 72. This study suggests that women leaving with HIV have a higher prevalence of HPV infection compared to HIV-negative patients.

In one of the studies conducted by Eun *et al*., 2013,[172] HPV 16 was the most prevalent HR-HPV type detected in both HIV-positive as well as HIV-negative women. HPV 18, 52, 31, 39, 45, and 56 were also common among HIV-infected women, with prevalence ranging from 3.3% to 6.7%. Other commonly detected HR-HPV types in HIV-negative women were 51, 58, 18, 35, 45, and 52, with prevalence ranging from 0.4% to 0.7%. There were some differences in the distribution of HPV genotypes between HIV-positive and HIV-negative women, except for HPV 16, which was similar to our study, where HPV 16 was the predominant genotype found in both HIV-positive and negative women. Using logistic regression, it has been observed that every pregnancy significantly affects any HPV in HIV-positive women.

Cervicovaginal HPV DNA was detected in 141 out of 145 (97.2%) participants, while four (2.8%) women had no evidence of cervicovaginal HPV DNA. High-risk HPV DNA was detected in 131 (90.3%) women. We found a single HPV genotype in 14 (9.7%) women and multiple (⩾2) HPV genotypes in 127 (87.5%) women. Among the HR types, HPV 52 was found to be the most prevalent (37.2%). 145 women had cytologic results within normal limits; 34 reported LSIL and 49 HSIL.[173] In our study, cytological results were found to be 42/197 LSIL, 4/147 HSIL, and 2 normal. Out of 86 HPV-positive cases 47 (54.65%) had a single HPV, whereas 39 (45.35%) had multiple HPV. In HIV-negative patients, 47 were normal, 5 were of LSIL, and none were of HSIL. These results conclude that HIV-positive women are prone to HPV infection compared to HIV-negative women.

HPV 16 was the most prominent HR-HPV genotype in both HIV positive and negative groups. This finding is in agreement with the study conducted by Leabaneng *et al.*, 2020.[174] This result suggests a direct relationship between co-infection of HIV and HPV 16.

In one of the studies conducted by H De Vuyst *et al.*, 2012,[175] cervical intraepithelial neoplasia (CIN) 1 was detected in 186/498 women (39.5%); CIN2 in 66 (14.0%); and CIN3 in 47 (10.0%). Women with higher education had a significantly lower HPV prevalence, compared with women with primary education only or no education (PR=0.74; 95% CI: 0.58–0.95). Compared to our study, out of 197 HIV-positive cases, 86 were found to be HPV positive. 53/86 showed CIN 1, 2/86 showed CIN2, and 5/86 showed CIN 3. Positivity for HR-HPV was not associated with marital status, a number of lifetime or recent partners, the use of hormonal contraceptives, or a number of pregnancies.

A study conducted by Philip *et al*., 2011[176] where among the 608 women diagnosed with CIN3, 601 (98.8%) cases were positive for any HPV genotype and 95.4% for any carcinogenic HPV. HPV16 (59.9%), HPV31 (18.1%), HPV52 (14.8%), HPV51 (14.0%), and HPV18 (13.2%) were the five most common HPV genotypes detected. In our study out of 197 HIV positive cases 86 found to be HPV-positive were 53 out of 86 had normal CIN, 5 had CIN 3. Out of 53 normal CIN 60.38% showed carcinogenic HPV. Carcinogenic and uncarcinogenic HPV is not

Of 126 specimens, 88 (69.8%) were from HIV-infected women, and 38 (30.2%) were from HIV-uninfected women. The HIV-infected patients were younger than their HIV-uninfected counterparts.[174] When compared to our study, 293 patients were considered where 197 were HIV-positive and 96 were HIV-negative. 120 out of 197 women were in the middle age group (31–40). The number of HIV-positive women was higher than that of HIV-negative women.

In one of the studies conducted by Gary et al., 2006,[177] the six most common high-risk HPV types were 16 (4.5%), 58 (3.6%), 18 (3.1%), 52 (2.8%), 31 (2.0%), and 33 (2.0%). In addition, HPV 16 was also the most common type, which was similar to our study, where HPV 16, 33, 35, 52, and 58 were detected. Women having CIN lesions were significantly detected for HPV16 and 72 as compared to normal cervical status.

The HPV prevalence was higher among HIV-positive women (52.4%) than among HIV-negative women (20.8%) overall and in all age groups. HPV prevalence in younger women aged 17–19 years, regardless of HIV status. HIV-positive women were more likely than HIV-negative women to have CIN 2 or 3. The most prevalent high-risk HPV types were HPV 16, 35, and 58, with no significant changes in type

distribution by HIV status.[178] This is in contrast with our study, where HPV prevalence was higher among HIV-positive women who fall into the age group of 21–49 years regardless of HIV status. HPV 16 and 72 were the prevalent genotypes found in HIV-positive patients, whereas HPV 59 was the prevalent genotype in HIV-negative women.

Andrew *et al.*, 2009 [179] found that HPV 45 and 58 were the most common high-risk variants. Thin prep abnormalities are more common in HIV-positive women than in HIV-negative women. These results are contradictory to our study, where HPV 16 was the most common high-risk HPV genotype found in both HIV-positive and HIV-negative women. The variation in this result may be due to the prevalence of varying genotypes of HPV from country to country as well as to HIV status.

In our study, HPV16 and 72 were the most prevalent genotypes found in HIV-positive women, while HPV59 was the most common in HIV-negative women. This is in agreement with the previous, study conducted by Ngugi *et al.*, 2011[180] where HPV 16 was the prevalent.

The majority of infections regress spontaneously by natural immune responses that appear to develop during the course of infection. As a result, the rate of prevalent infections in women over 30 years of age drops significantly, ranging between 5 and 10%.[13] The current study showed that age was significantly associated with non-HPV 16 genotypes, which contradicted the findings of Teixeira *et al.*, [171] who stated that multiple infections had a higher HR-HPV prevalence in older age groups than in younger age groups.

In our study Pap smear test results were as follows; out of 197 HIV-positive patients, 42 cases were LSIL and 4 cases were HSIL and out of 96 HIV-negative patients 5 cases were of LSIL and none were of HSIL which was similar with a study conducted by Milena *et al*., 2014.[162] where more lesions were detected in the HIV-positive group (mainly LSIL), this concludes that women having this type of immunosuppression have a higher incidence and prevalence of premalignant lesions caused by the immune system becoming unable to efficiently eliminate HPV infection. Type-specific distribution revealed that HPV-16 was the most prevalent type in the population being studied which was consistent with worldwide results to date. [162,181–183]

In a study by Petros *et al.,* 2013, 95 women with a median age of 38 years (IQR: 33–41) were screened for HPV DNA on Pap smear. 30/94 women (32%) and 18/94 women (19%) had low-grade and high-grade squamous intraepithelial lesions (LSIL/HSIL). In all, more than half of the participants experienced cervical inflammatory responses, including STIs. In 43 women who received cervical biopsies, eight (8.4%) had CIN-1, five (5.3%) had CIN-2, and two (2.1%) had carcinomas in situ. In our study, a total of 293 patients were considered, where 197 cases were HIV-positive and 96 were HIV-negative. The mean age was 33.93±5.69 and 32.07± 6.78 for HIV positive and negative women. LSIL was found in both HIV-positive and negative cases, whereas HSIL was observed only in HIV-positive cases. This concludes that HIV-positive women are more prone to HPV infection than HIV-negative women.

According to Myassa *et al*., 2013 [184] HIV status was found to be the strongest risk factor for HR HPV infection. HIV-positive women have a significantly higher risk of HR HPV infection as compared with HIV-negative women. The odds of being HR HPV positive among HIV-positive women decreased with age. Among the 3699 women, 751 (20.3%) were HR HPV positive, which included 427 women with a single-type HPV infection and 240 women with multiple HPV types. A total of 349 women (9.4%) were HIV positive. Among 349 HIV-positive women, 45.9% were HR HPV positive, whereas among HIV-negative women, the HR HPV prevalence was only 17.3%. HIV-positive women aged 50 or older have a significantly higher risk of HPV infection. In our study, 197 HIV-positive women detected with single and multiple HPV were not significantly associated with CIN 2 as well as CIN 3. Also women over 45 years of age have a greater risk of HPV infection.

In one of the studies conducted by Smita *et al*.,2005,[185] the mean age of the participants was 29.1 years (SD 6.14, range 18–60). 269 (93.7%) of the 287 Pap smears were found to be negative for intra-epithelial lesion or malignancy, while 18 (6.3%) had squamous cell abnormalities. There were 10 low-grade squamous intraepithelial lesions (LSIL) among the 18 smears with squamous cell abnormality. None of the participants had high-grade squamous intraepithelial lesions (HSIL) or squamous cell carcinoma in cytology. Infection with the HPV 16 genotype was the most prevalent infection in the present study population. In our study, the PAP test was conducted among 197 HIV-positive patients and 96 HIV-negative patients. LSIL was found in both HIV-positive and negative women, whereas HSIL was found only in HIV-positive patients. This leads to the conclusion that women with HIV are at a higher risk of HPV infection than women who are not.

In a study reported by Vikrant *et al.*,2010 [186] a total of 303 women were enrolled, with a median age of 30 years. Most of the participants were widowed or separated (187/303, 61.7%). More than one-third (114/302, 37.7%) were not educated beyond primary school. The final composite colposcopic-histopathologic diagnoses revealed no abnormality (no evidence of CIN) in 220 out of 303 (72.6%) women. CIN 1 was reported in 33 (10.9%) women, CIN 2 in 31 (10.2%) women, and CIN 3 in 18 (5.9%) women, while 1 (0.3%) woman was diagnosed with ICC. In our study, the mean age of the enrolled HIV-infected women was 33.93±5.69 (n = 293). In HIV-positive patients, none were found to be widowed or separated, whereas only 3 out of 7 cases were found to be widowed or separated in HIV-negative cases. The majority of the HIV-positive women were less literate (139/197). In total, 30 were found to be CIN 1, and 9 were found to be CIN 2 and CIN 3, respectively, whereas in HIV negative cases, only 10 were found to be CIN 1, and none were found to be CIN 2 or CIN 3. This indicates that HIV-positive patients are prone to CIN lesions compared to HIV-negative ones.

A total of 445 women (255 HIV-positive and 191 HIV-negative) were included in the study conducted by Jaquet *et al.,* 2012.[187] A total of 18 CIN were identified, including 16 in grade 1, one in grade 2 and one ICC. In HIV-positive women, HPV 35 (15.7%), 16 (14.2%), 18 (11.4%), and 58 (11.4%) were the four most common HPV types identified. In HIV-negative women, HPV 35 (10.5%), 16 (10.0%) and 59 (10.0%) were the three most frequent types identified, followed by HPV 18 (5.8%). In our study, a total of 293 (197 HIV-positive and 96 HIV-negative) were included in the study. HPV 16, and 72 were found to be significant genotypes in HIV-positive women, whereas HPV 59 was found to be a common genotype found in HIV-negative women.

In one of the studies conducted by Fatma *et al.*, 2018, [188] out of 201 women, HPV DNA was detected only in 91 (45.2%). The median ages of HPV positive and negative women were 40 and 44 years, respectively. According to cytological screening, 72 patients had normal cytologic findings, 94 patients had ASCUS, 29 had LSIL, and 6 had HSIL. The most commonly identified HPV types were HPV 58, HPV 16, HPV 31, HPV 33, HPV 11, and HPV 35. In our study, HPV DNA was detected by PCR, where 64 were detected as positive (MY9/11) and 66 (G5/G6). The age of the participants was between 18-45 years. According to cytological screening, LSIL was found in both HIV-positive and negative individuals, whereas HSIL was found only in HIV-positive individuals. The most common HPV genotypes found were HPV 16, 72 (HIV-positive) and 59 (HIV-negative).

In a study conducted by Dawit *et al.*,2018, [189] 233 HIV-negative women were enrolled, who were evaluated for cytology examination. The results revealed that 141 (60.5%) samples were normal, while 92 (39.5%) were abnormal. The prevalence of HPV16 was significantly higher among women with abnormal cytology. HPV16 was the most prevalent of all the HR-HPV genotypes. Other common HR-HPV types were HPV 35, HPV 45, HPV 18, and HPV 31, which are similar to our study. In particular HPV 16 and 72 were significant genotypes detected in women with CIN lesions as compared to those with normal cervical status in HIV-positive women, while HPV 59 was common among HIV-negative women.

A study was conducted by Suchitra *et al.*, 2016 [190] where HPV 16 was the commonest genotype found (38.5%); HPV 16 and 18 put together contributed to 73.3% of HPV infection; 27.5% of HIV-infected women had squamous cell abnormalities. The prevalence of high-risk HPV infection was higher in women with

high-grade squamous intraepithelial lesions or greater lesions (85.7%) as compared to women with normal cytology (52.1%), which is relatable to our study where HPV 16 and 72 were significant genotypes detected in women with CIN lesions compared to normal cervical status. The high-grade squamous intraepithelial lesions were found in HIV-positive women. The high prevalence rate of HPV infection and CIN in HIV-positive women requires frequent Pap smear screening.

In a study of Rashmirani *et al.*, 2017,[(191)] the most prevalent genotype was HPV16 (87.28%), followed by HPV18 (24.56%) and HPV 51 (3.46%). Compared to our study, HPV 16 and HPV 71 were significant genotypes detected, followed by 33, 35, 52, and 58 in HIV-positive women.

In our study, HPV DNA was detected by PCR where two primers were used, MY9/11 and G5/G6 in both HIV-positive and HIV-negative cases to detect HPV. All samples were amplified for HPV DNA and genotypes were identified by a linear genotype assay. Out of 197 HIV-positive cases, 64 were detected as HPV-positive by MY9/11 and 132 as negative. 66 were In our study, HPV DNA was detected by PCR using two primer sets, MY9/11 and G5/G6, which were used to confirm HPV in both HIV-positive and HIV-negative cases. All samples were also identified as having the genotype of HPV by a linear genotype assay. Out of 197 HIV-positive cases, 64 were detected as HPV-positive by MY9/11 and 132 as negative. 66 were detected as positive for G5/G6 and 130 as negative. Of the HIV-negative out of 96 cases, 11 were detected as HPV-positive and 85 were negative by both the primer sets. This concludes that both the primer sets MY09/MY11 and G5/G6 amplified a wide range of HPV genotypes in cervicovaginal samples and detected similar results for HPV positivity. Since G5/G6 primers detected an additional 2 positive samples ,our study

suggests that more than one type of oligonucleotide primer should be used in clinical samples to increase the sensitivity of HPV detection, which is similar to a study conducted by Vinay *et al.*, 2016,[152] wherein of the 33 samples that were positive in RLA, HPV DNA was detected in 63.7% (21 of 33) and 72.73% (24 of 33) of the samples by using the MY09/11 and the G5/G6 primer sets, respectively. Among the 24 HPV-positive samples, 21 (63.7%) were detected by one or both methods, and 3 (9.09%) were detected only by the G5/G6 primer set.

SUMMARY

The main goal of this study was to identify the HPV high-risk genotypes in HIV-infected women of childbearing age by molecular methods. This study was a hospital-based case report at Belagavi in Karnataka. Women infected with Human Immunodeficiency Virus (HIV) show a higher risk of Human Papilloma Virus (HPV) infection and cervical cancer. In this study, a total of 293 women were screened, out of which 197 were HIV-positive and 96 were HIV-negative. The highest number of HIV-positive women fell into the age group of 31–40 years, and 70.55% were less literate.

Colposcopy was conducted among 197 HIV-positive women to detect the CIN lesions, where 30 were found to be CIN 1, 2 were found to be CIN 2, and 9 were found to be CIN 3. The remaining 130 were normal. A Papanicolaou (PAP test) was also conducted where 147 showed inflammatory lesions. 42 cases were of LSIL and 4 cases were of HSIL. The HPV positivity among 197 HIV-positive cases resulted in 86 HPV-positive and 111 HPV-negative cases. Out of 86 HPV-positive cases, 53 had normal CIN, while 60.38% showed carcinogenic HPV. 47 had a single HPV, whereas 39 had multiple HPV.

A higher prevalence of multiple opportunistic infections and a broad spectrum of HPV genotypes were reported in HIV-infected women. According to the findings, HIV positive women with various genotypes had a greater overall prevalence of HPV, suggesting that they are more likely to develop cervical cancer.

The HPV genotypes detected were HPV 16, 33, 35, 52, and 58. HPV 16 and HPV 72 were significant genotypes. The p-value was found to be 0.04 and 0.0495,

respectively. Using logistic regression analysis, it has been concluded that every pregnancy significantly affects any HPV. Using the adjusted odds ratio, the odds of any HPV are 4.47 times higher for subjects with a history of pregnancy than for other patients. Using multivariable logistic regression, it has been concluded that the presence of carcinogenic, possibly carcinogenic, noncarcinogenic, single HPV and multiple HPV is not significantly associated with CIN results. The HPV DNA was detected by PCR using two primers (MY9/11 and G5/G6). 64 were detected as positive by MY9/11 and 132 as negative. 66 were detected as positive for the G5/G6 primer and 130 as negative. No significant difference was observed (p-value = 0.2McNem).

The colposcopy was conducted among 96 HIV-negative patients to detect CIN lesions, where 10 were found to be CIN 1, and none were found for CIN 2, or CIN 3. 80 were found to be normal. A PAP test was also conducted where 47 showed normal, 5 cases were of LSIL, and none were found for HSIL. The majority of the cases were in the age group of 30-34 years of age. Of 96 HIV-negative cases, 13 were found to be HPV-positive and 83 were HPV-negative. HPV 59 was the most common genotype, followed by 16, 53, 62, and 72. Out of 13 HPV positive cases, 7 had a grade of 1+, 5 had a grade of 2+, 9 had a grade of 3+ and 2 had a grade of 4+. Out of 7 grade 1+ cases, 28.57% had carcinogenic HPV, whereas 20% and 89% of grade 2+ and grade 3+ cases had carcinogenic HPV, respectively. Using the Cochran Armitage trend test, it has been observed that there is a significant linear increasing trend in the proportion of carcinogenic and non-carcinogenic genotypes over grade. Using univariate logistic regression, it has been concluded that age is significantly associated with non-HPV 16 genotypes. Out of 96 HIV-negative cases, 11 were detected as positive by MY9/11 and 85 were detected as negative. 11 were detected as positive

for the G5/G6 primer and 85 were detected as negative. When the p-value was compared between the two groups, no significant difference was observed (p-value was 1.0). Different tests for HPV positivity among HIV-positive and negative cases were conducted, which resulted in the p-value for HIV-positive being 0.022C, which is significant, and HIV-negative being found to be 0.94, which is non-significant.

HPV infections were more common in HIV-positive women when compared to HIV-negative women. Women with HIV infection present a higher risk of infection by HPV and cervical cancer. HPV 16 and 72 were found to be common genotypes in both HIV-positive and HIV-negative women. Young age, an increasing number of lifetime partners, and HIV positivity were all linked to an increased risk of HPV infection.

CONCLUSION

The main aim of the study was to detect the HPV high-risk genotypes in HIV-infected women of childbearing age by molecular methods. This study was a hospital-based case study at Belagavi in Karnataka. Women infected with the Human Immunodeficiency Virus (HIV) are more likely to have Human Papilloma Virus (HPV) infection and develop cervical cancer. Low-grade squamous intraepithelial lesions were found in both HIV-positive and HIV-negative women. However, high-grade intraepithelial lesions were found only in HIV-positive women, suggesting that HIV-positive women are more susceptible to HPV infection than HIV-negative women.

Human immunodeficiency virus (HIV)-infected women represent the highest risk population subgroup for the higher frequency and rapid development of HPV-induced cervical intraepithelial neoplasia (CIN). HPV 16 and 72 were the common genotypes found in HIV-positive women, whereas HPV 59 was the common genotype in HIV-negative women. HPV-DNA was analyzed by PCR where two primers were used; MY09/11 and G5/G6. When the p-values of the two groups were examined, no significant difference was found between HIV-positive and HIV-negative patients.

Different tests, such as Pap test, PCR, and RLA, were conducted for HPV detection among HIV-positive and HIV-negative cases. The Pap test is being replaced by HPV genotyping due to its high sensitivity and specificity. HIV-positive women present a higher risk of infection by HPV and cervical cancer. Cervical cancer should be avoided in HIV-negative women using HPV genotyping and vaccination techniques.

These findings suggest that HIV-positive women are at a greater risk of cervical cytological abnormalities as compared to HIV-negative women. Cervical cancer can be prevented by vaccination in both HIV-positive and HIV-negative women.Newer vaccinations, such as the nonavalent HPV vaccine, may provide improved coverage for women, but they must be examined. Increased accessibility to screening programmes, adherence to follow-up among those with lesions, and intensified health education for HIV-positive women are all critical steps in improving early diagnosis.

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ANNEXURES

Annexure I: Ethical Clearance Letter

KLE UNIVERSITY
(Formerly known as KLE Academy of Higher Education & Research, Belgaum)
[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956 vide Government of India Notification No.F.9-19/2000-U.3(A)]
'Accredited 'A' Grade by NAAC
Office of the Registrar, KLE University,
JNMC Campus, Nehru Nagar, Belgaum-590 010, Karnataka State, India
☎: 0831-2444444/2493779 **FAX:** 0831-2493777 **Web:** http://www.kleuniversity.edu.in **E-mail:** info@kleuniversity.edu.in

Ref.No.KLEU/Ethic/2012-13/D-4573. Date:18-3- 2013

To,
Mr. Vinay P.S.
Ph.D.Scholar 2012-13 Batch,
KLE University,
Belgaum.

Dear Research Scholar,

The KLE University Ethics Committee on Human Subjects for Ph. D Research Project met on 8th March 2013 to consider your application for approval of the research project **"DETECTION OF HUMAN PAPILLOMAVIRUS SEROTYPES IN HIV INFECTED WOMEN OF CHILD BEARING AGE".**

As there are no ethical issues involved in your proposed research project., the committee has provided approval for this research project.

You are requested to report to Ethical Committee of the following:

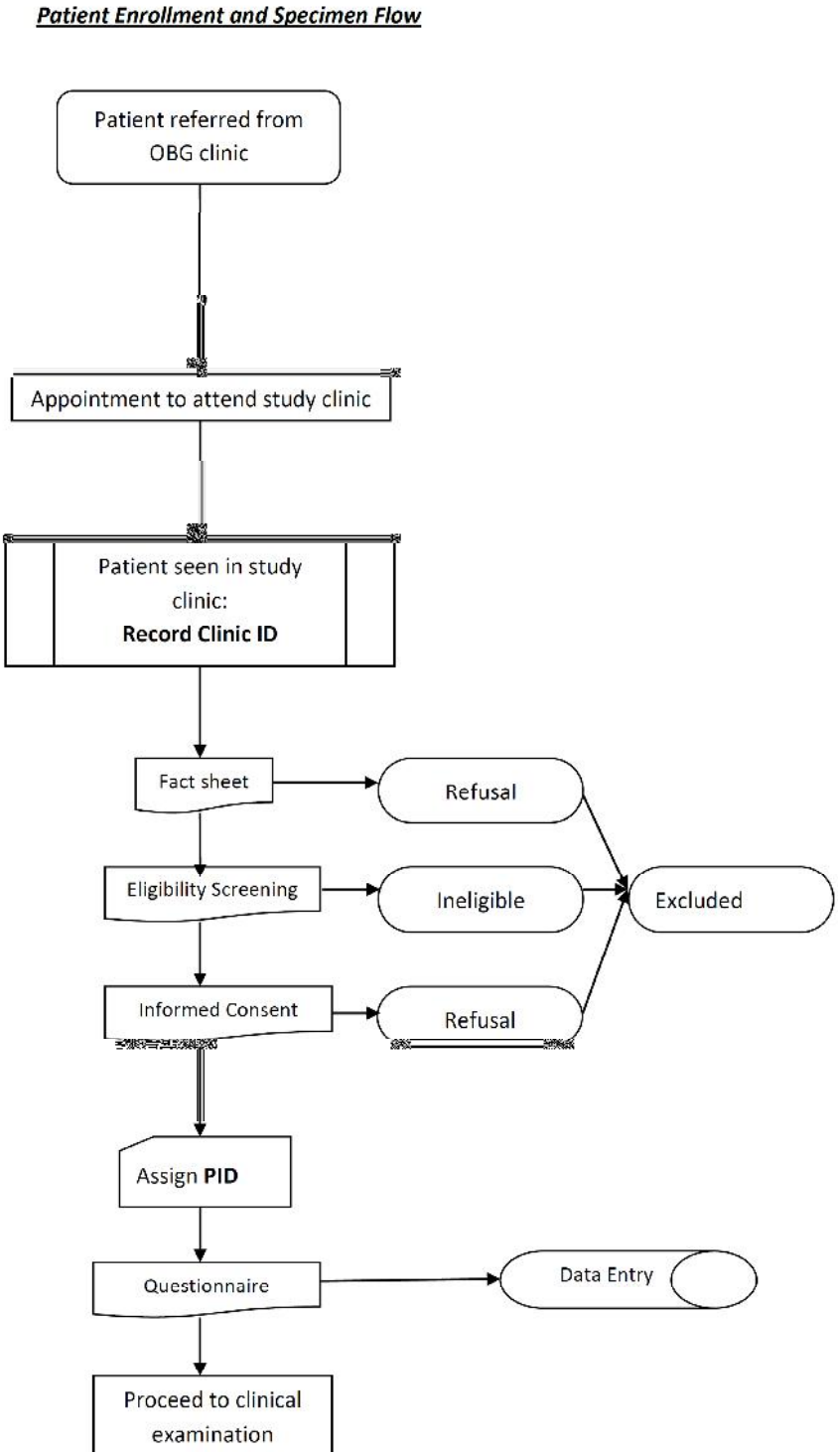
1. Any deviation from or change of the protocol.
2. All serious adverse events.
3. Any changes in study documents.

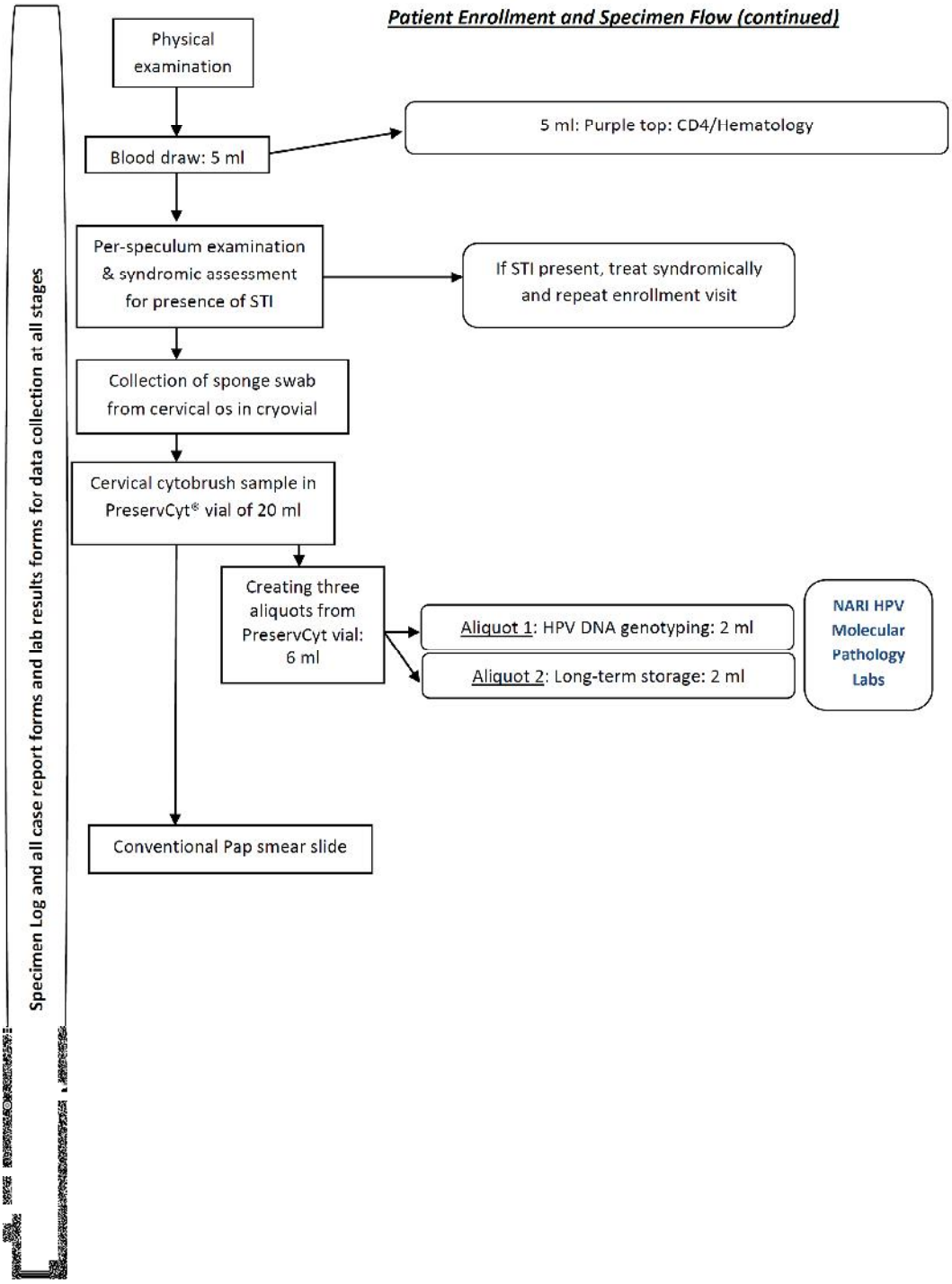
(Dr. Hema Dhumale)
Member Secretary,
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belgaum.

(Prof. Sudha A.Raddi)
Chairman
Ph.D. Ethical Committee(Human),
K.L.E. University,
Belgaum.

CC to: - The Director Academic Affairs, KLE University
- The Director Research Foundation, KLE University
- The Registrar, KLE University
- Special Officer to Hon. Vice Chancellor, KLE University, Belgaum

Annexure II: Protocol flow diagram




Patient Enrollment and Specimen Flow (continued)
Specimen Log and all case report forms and lab results forms for data collection at all stages
Physical examination
Blood draw: 5 ml
5 ml: Purple top: CD4/Hematology
Per-speculum examination & syndromic assessment for presence of STI
If STI present, treat syndromically and repeat enrollment visit
Collection of sponge swab from cervical os in cryovial
Cervical cytobrush sample in PreservCyt® vial of 20 ml
Creating three aliquots from PreservCyt vial: 6 ml
Aliquot 1: HPV DNA genotyping: 2 ml
Aliquot 2: Long-term storage: 2 ml
NARI HPV Molecular Pathology Labs
Conventional Pap smear slide

Annexure III: Information Sheet and Patient consent

INFORMED CONSENT

DETECTION OF HUMAN PAPILLOMAVIRUS HIGH RISK GENOTYPES IN HIV INFECTED WOMEN OF CHILD BEARING AGE

RESEARCHERS– Vinay. P. S, Dr. C. S. Patil, Dr. Anita Dalal

Introduction:

This study is done to identify Human Papillomavirus (HPV) that causes different conditions like Genital warts to Cancer. These are caused by certain types of Human papillomaviruses. In this study we are concentrating on the various Human papillomavirus types that may cause cervical cancer if not diagnosed early. It may sometimes present with vaginal bleeding, but symptoms may be absent when the cancer is in early stages therefore, if these are detected in early stage they can be treated and hence prevent them from progressing to cancer. You have been invited to participate in this study to find out the types of Human papillomavirus (HPV).

Explanation of procedures:

In this study you will have to answer, few prepared questions about family, marriage, history of cancer, HIV and other sexually transmitted. We will do examination of your cervix with simple instruments like vaginal speculum which will not harm you in any way. Also we will be taking 5 ml of blood and vaginal swabs for further investigation and identification.

Vaginal swab:

"Vaginal swab" is usually used to collect sample from woman's genitals. To collect this, a trained medical professional uses a cotton swab to collect a small amount of fluid/ scrapings from the inside of the vagina. Using this type of test, we can determine whether a woman has certain types of infections or check for the presence of foreign organisms like HPV. Once the vaginal swab is collected, the swab is usually sealed inside a collection container and sent for a lab's analysis.

A vaginal swab is usually used in gynecological diagnostic procedures. A doctor or other health care professional may use a vaginal swab to investigate a possible sexually transmitted disease (STD), for example HPV. This type of test is also used to analyze the organisms that are present inside the vagina. For example, a woman may have

harmless or harmful bacteria in her vagina, and this test makes it possible to identify them.

The doctor/ nurse will collect vaginal samples with an elongated q-tip looking tool, as well as a small bristled brush-looking tool. Also doctor can examine around the inside of the vagina to ensure that there are no lumps or abnormalities with the ovaries or uterus.

Possible Benefits:

You will come to know whether you have HPV infection which may lead to several associated conditions. If detected positive the lesions and conditions can be treated immediately. Hence, they can be prevented from transforming into cancerous lesions.

Possible harms:

The test applied on you will not have any serious effects and harms. Only you may feel slightly uncomfortable while taking swab.

Confidentiality:

Your identity and the results of tests will not be revealed. All information collected will be coded, so that no one will know your identity. During publication also the identity is not disclosed.

Withdrawal:

Participation in this study is voluntary. You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected

Costs of participation:

The cost of the study will be borne by the researcher. There will be no additional cost to you for participating in this study.

Payment for participation:

There will be no incentives to you for participating in this study.

Questions:

If you have any questions about this study or a research-related injury, you can contact:

- **Mr. Vinay. P. S**, Research scholar, Lecturer, Dept. of Microbiology, USM-KLE International Medical Programme, Belgaum. Ph: 8970313466
- **Dr. C. S. Patil**, Research guide, HOD, Dept. of microbiology, USM KLE International Medical programme, Belgaum. Ph: 9448072678
- **Dr. Anita Dalal,** Research Co-guide, Professor of Obstetrics and Gynecology, JN Medical College, Belgaum. Ph: 9448140343
- **Dr. Sudha A. Raddi,** Chairman, Ethical Committee for Ph.D. Research, KLE University, Belgaum. Ph: 9448354712

Signatures:

You are not waiving any of your legal rights by signing this consent form. You are free to refuse all or any part of these activities, without any problem. When you sign or make your thumb print on the form below, that means that your questions and concerns have been answered and you willingly agree to undergo the study procedures.

Date:

Participant Full Name with signature/thumb impression

Witness Name with signature

Name of the person obtaining informed consent with signature

Name of the investigator or designee with signature

KLE's PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM

STATEMENT CONCERNING PARTICIPATION IN RESEARCH WORK

CONSENT FORM

DETECTION OF HUMAN PAPILLOMAVIRUS HIGH RISK GENOTYPES IN HIV INFECTED WOMEN OF CHILD BEARING AGE

I have read the information on the aims and objectives of the proposed research work and was provided the opportunity to ask questions and given adequate time to think over the issue. The aim and objectives of the study are clear to me. I have not been pressurized to participate in any way.

I understand that participation in this research work is completely voluntary and that I may withdraw from it at any time and without providing reasons. This will have no influence on the regular treatment of my condition neither will it influence the care that I receive from my doctor.

I know that this research work has been approved by Research, Ethics and Publications committee of the KLE University.

I am fully aware that the results of this research will be used for scientific purposes and may be published.

I agree to this. I am assured that my personal details will be kept confidential and privacy guaranteed.

Therefore I hereby give consent to participate in this research work.

Date: **Name and Signature / left hand Thumb mpression of Patient**

Place:

Name and signature of witness

STATEMENT BY THE RESEARCHER

I have provided verbal and written information regarding this research work to the study participant. I agree to answer any further questions concerning the research work as best as I am able. I will adhere to the protocol which is approved by the KLE University.

Date: **Signature of the researcher**

(VINAY. P. S)

ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿ ಪತ್ರ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ: ಹೆಚ್.ಐ.ವಿ ಸೋಂಕಿತ ಗರ್ಭಧಾರಣೆಯ ವಯಸ್ಸಿನ ಮಹಿಳೆಯರಲ್ಲಿ ಮಾನವ ಪಪಿಲ್ಲೋಮ ವೈರಸ್ ಸೆರೋಟೈಪ್ಗಳನ್ನು **ಪತ್ತೆ** ಹಚ್ಚಲು

ಸಂಶೋಧಕ-ವಿನಯ್. ಪಿ ಎಸ್, ಕೆ.ಎಲ್.ಇ University, ಬೆಳಗಾವಿ

ಪರಿಚಯ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನರೂಲಿಗಳು, ಜನನಾಂಗದ ಕ್ಯಾನ್ಸರ್ ಹಾಗೂ ಮುಂತಾದ ವಿವಿಧ ಸ್ಥಿತಿಗಳನ್ನು ಉಂಟುಮಾಡುವ ಹ್ಯೂಮನ್ ಪ್ಯಾಪಿಲೋಮವೈರಸ್ (HPV) ಗುರುತಿಸಲು ಮಾಡಲಾಗುತ್ತದೆ. ಈ ಸ್ಥಿತಿಗಳನ್ನು ಕೆಲವು ರೀತಿಯ ಹ್ಯೂಮನ್ ಪ್ಯಾಪಿಲೋಮವೈರಸ್ (HPV) ಉಂಟಾಗುತ್ತವೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಾವು ಆರಂಭಿಕ ಹಂತದ (ರೋಗನಿರ್ಣಯ ಆಗುವ ಮೊದಲೇ) ಗರ್ಭಕಂಠದ ಕ್ಯಾನ್ಸರ್ (Cervical cancer) ಉಂಟುಮಾಡುವ ವಿವಿಧ ಬಗೆಯ ಹ್ಯೂಮನ್ ಪ್ಯಾಪಿಲೋಮವೈರಸ್ ಗಮನಿಸಲಾಗುತ್ತದೆ. ಕೆಲವೊಮ್ಮೆ ಯೋನಿ ಸ್ರಾವ ಅಸ್ತಿತ್ವವನ್ನು ಕಾಣಬಹುದು, ಆದರೆ ಈ ಲಕ್ಷಣಗಳು ಕ್ಯಾನ್ಸರ್ ನ ಆರಂಭಿಕ ಹಂತದಲ್ಲಿ ಇಲ್ಲದಿರಬಹುದು, ಆದ್ದರಿಂದ ಇವು ಆರಂಭಿಕ ಹಂತದಲ್ಲಿ ಪತ್ತೆಯಾದಲ್ಲಿ ಅವುಗಳಿಗೆ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಬಹುದು ಮತ್ತು ಅವುಗಳನ್ನು ಕ್ಯಾನ್ಸರ್ ಗೆ ಮುಂದುವರೆಯದಂತೆ ತಡೆಯಬಹುದು. ನೀವು ಹ್ಯೂಮನ್ ಪ್ಯಾಪಿಲೋಮವೈರಸ್ (HPV) ವಿಧಗಳನ್ನು ಕಂಡುಹಿಡಿಯಲು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಮಂತ್ರಿಸಲಾಗಿದೆ.

ವಿಧಾನಗಳ ವಿವರಣೆ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಕುಟುಂಬ, ಮದುವೆ, ಕ್ಯಾನ್ಸರ್, ಎಚ್ಐವಿ ಮತ್ತು ಇತರೆ ಲೈಂಗಿಕವಾಗಿ ಹರಡುವ ರೋಗಗಳ ಇತಿಹಾಸ, ಬಗ್ಗೆ ಸಿದ್ಧಪಡಿಸಿದ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸಬೇಕಾಗುತ್ತದೆ. ನಾವು ನಿಮ್ಮ ಗರ್ಭಕಂಠದ ಪರೀಕ್ಷೆಯನ್ನು ಸರಳ ಉಪಕರಣ ಯೋನಿ ಸ್ಪೆಕ್ಯುಲಮ್ ಉಪಯೋಗಿಸಿ ಮಾಡುತ್ತೇವೆ ಇದು ನಿಮಗೆ ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ನೀವು ಹಾನಿ ಮಾಡುವುದಿಲ್ಲ. ಹಾಗೂ ನಾವು ಮತ್ತಷ್ಟು ತನಿಖೆಗಾಗಿ 15 ಮಿಲಿ ರಕ್ತ ಮತ್ತು ಯೋನಿ ಸ್ವೇದರೋಧಕಗಳನ್ನು ತೆಗೆದುಕೊಳ್ಳುತ್ತೇವೆ.

ಯೋನಿ ಹೀರೊತ್ತಿಗೆ: "ಯೋನಿ (ಸ್ತ್ರೀ ಜನನಾಂಗದ) ಹೀರೊತ್ತಿಗೆ" ಸಾಮಾನ್ಯವಾಗಿ ಮಹಿಳೆಯ ಜನನಾಂಗಗಳ ಮಾದರಿ ಸಂಗ್ರಹಿಸಲು ಬಳಸಲಾಗುತ್ತದೆ. ಇದನ್ನು ಸಂಗ್ರಹಿಸಲು ಒಬ್ಬ ತರಬೇತಿ ಪಡೆದ ವೈದ್ಯಕೀಯ ಸಿಬ್ಬಂದಿ ಹತ್ತಿ ಹೀರೊತ್ತಿಗೆ ಬಳಸಿಕೊಂಡು ಒಂದು ಸಣ್ಣ ಪ್ರಮಾಣದ ದ್ರವ / ಯೋನಿಯ ಒಳಗಿನ ಪದರವನ್ನು ಕೆರೆದು ಸಂಗ್ರಹಿಸಲಾತ್ತದೆ. ಈ ರೀತಿಯ ಪರೀಕ್ಷೆಯಿಂದ, ನಾವು ಒಂದು ಮಹಿಳೆಯ ಕೆಲವು ಬಗೆಯ ಸೋಂಕುಗಳ ನಿರ್ಧಾರ ಅಥವಾ HPV ನಂತಹ ವಿದೇಶಿ ಜೀವಿಗಳ ಇರುವಿಕೆಯನ್ನು ಪರಿಶೀಲಿಸಬಹುದು, ಒಮ್ಮೆ ಸಂಗ್ರಹಿಸಿದ ಯೋನಿ ಹೀರೊತ್ತಿಗೆ , ಸಾಮಾನ್ಯವಾಗಿ ಹೀರೊತ್ತಿಗೆ ಸಂಗ್ರಹಣೆಯ ಕಂಟೇನರ್ ಒಳಗೆ ಮೊಹರು ಮಾಡಿ ಲ್ಯಾಬ್ ನ ವಿಶ್ಲೇಷಣೆಗೆ ಕಳುಹಿಸಲಾಗುತ್ತದೆ.

ಒಂದು ಯೋನಿ ಹೀರೊತ್ತಿಗೆ ಸಾಮಾನ್ಯವಾಗಿ ಸ್ತ್ರೀ ಸಂಬಂಧಿ ರೋಗಶಾಸ್ತ್ರೀಯ ರೋಗನಿರ್ಣಯದ ವಿಧಾನಗಳಲ್ಲಿ ಬಳಸಲಾಗುತ್ತದೆ. ವೈದ್ಯರು ಅಥವಾ ಇತರ ಆರೋಗ್ಯ ವೃತ್ತಿಪರರು ಲೈಂಗಿಕವಾಗಿ ಹರಡುವ ರೋಗವನ್ನು (STD), ಉದಾಹರಣೆಗೆ HPV ತನಿಖೆಗೆ ಯೋನಿ ಹೀರೊತ್ತಿಗೆ ಬಳಸಬಹುದು. ಈ ರೀತಿಯ ಪರೀಕ್ಷೆಯನ್ನು ಸ್ತ್ರೀ ಜನನಾಂಗದ ಒಳಗೆ ಇರುವ ಜೀವಿಗಳ ವಿಶ್ಲೇಷಣೆಗೆ ಬಳಸಬಹುದು. ಉದಾಹರಣೆಗೆ, ಮಹಿಳೆಯೊಬ್ಬಳು ತನ್ನ ಜನನಾಂಗದಲ್ಲಿ ತೊಂದರೆಯಿಲ್ಲದ ಅಥವಾ ಅಪಾಯಕಾರಿ ಬ್ಯಾಕ್ಟೀರಿಯಾವನ್ನು ಹೊಂದಿರಬಹುದು, ಮತ್ತು ಈ ಪರೀಕ್ಷೆ ಅದನ್ನು ಗುರುತಿಸಲು ಸಾಧ್ಯಮಾಡುತ್ತದೆ.

ವೈದ್ಯರು ಅಥವಾ ದಾದಿಯರು ಉದ್ದನೆಯ Q-ತುದಿಯಂತೆ ಕಾಣುವ ಸಲಕರಣೆ ಮತ್ತು ಸಣ್ಣ ಬಿರುಸು ಕೂದಲುಳ್ಳ ಸಲಕರಣೆಯಿಂದ ಸ್ತ್ರೀ ಜನನಂಗದ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸುತ್ತಾರೆ. ಅಲ್ಲದೆ ವೈದ್ಯರು ಜನನಾಂಗದ ಒಳಗೆ ಅಂಡಾಶಯದಲ್ಲಿ ಅಥವಾ ಗರ್ಭಾಶಯದಲ್ಲಿ ಯಾವುದೇ ಉಂಡೆಗಳು ಅಥವಾ ವೈಪರೀತ್ಯಗಳು ಇವೆಯೇ ಎಂಬುದನ್ನು ಖಚಿತಪಡಿಸಿಕೊಳ್ಳಲು ಜನನಾಂಗದ ಒಳಗೆ ಪರೀಕ್ಷಿಸ ಮಾಡಬಹುದು.

<u>ಸಂಭಾವ್ಯ ಲಾಭಗಳು:</u> ನಿಮಗೆ ವಿವಿಧ ಸ್ಥಿತಿಗಳನ್ನು ಉಂಟು ಮಾಡುವ ಹೆಚ್.ಪೀ.ವೀ ಸೋಂಕು ಇದೆಯೇ ಎಂದು ತಿಳಿಯುತ್ತದೆ. ಒಂದು ವೇಳೆ ಪತ್ತೆಮಾಡಲಾದಲ್ಲಿ ಗಾಯಗಳು ಮತ್ತು ಪರಿಸ್ಥಿತಿಗಳಿಗೆ ತಕ್ಷಣವೇ ಚಿಕಿತ್ಸೆ ನೀಡಬಹುದು. ಆದ್ದರಿಂದ, ಅವುಗಳನ್ನು ಕ್ಯಾನ್ಸರ್ ಗಡ್ಡೆಗಳಾಗಿ ಪರಿವರ್ತಿತವಾಗುವುದರಿಂದ ತಡೆಗಟ್ಟಬಹುದು .

<u>ಸಂಭಾವ್ಯ ತೊಂದರೆಗಳು</u>: ಈ ಪರೀಕ್ಷೆಯಿಂದ ನಿಮಗೆ ಯಾವುದೇ ಗಂಭೀರ ಪರಿಣಾಮಗಳು ಮತ್ತು ತೊಂದರೆಗಳು ಆಗುವುದಿಲ್ಲ. ಕೇವಲ ಹೀರೊತ್ತಿಗೆ ಪಡೆಯುವಾಗ ನೀವು ಕೊಂಚ ಅಹಿತಕರ ಅಭಿಪ್ರಾಯ ಹೊಂದಬಹುದು.

<u>ಗೋಪ್ಯತೆ:</u> ನಿಮ್ಮ ಗುರುತನ್ನು ಮತ್ತು ಪರೀಕ್ಷೆಗಳ ಫಲಿತಾಂಶಗಳನ್ನು ಬಹಿರಂಗವಾಗಿ ತೋರಿಸಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ಯಾರೂ ತಿಳಿಯುವುದಿಲ್ಲ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಕೋಡ್ ಮಾಡಲಾಗುತ್ತದೆ. ಪ್ರಕಟಣೆ ಸಂದರ್ಭದಲ್ಲಿ ಗುರುತನ್ನು ಬಹಿರಂಗ ಮಾಡುವುದಿಲ್ಲ.

<u>ವಾಪಸಾತಿ:</u> ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವಿಕೆ ಐಚ್ಛಿಕವಾಗಿದೆ. ನೀವು ಹಾಗೆ ಇಚ್ಛಿಸದಿದ್ದರೆ ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ನೀವು ಭಾಗವಹಿಸದಿರಬಹುದು. ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಸಂಶೋಧನೆಯಲ್ಲಿನ ಭಾಗವಹಿಸುವಿಕೆ ನಿಲ್ಲಿಸಬಹುದು. ನಿಮ್ಮ ಆಯ್ಕೆಯನ್ನು ಮತ್ತು ನಿಮ್ಮ ಎಲ್ಲಾ ಹಕ್ಕುಗಳನ್ನು ಗೌರವಿಸಲಾಗುವುದು.

<u>ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ವೆಚ್ಚ:</u> ಅಧ್ಯಯನದ ವೆಚ್ಚ ಸಂಶೋಧಕರು ಭರಿಸುತ್ತಾರೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಭಾಗವಹಿಸಲು ಯಾವುದೇ ಹೆಚ್ಚುವರಿ ವೆಚ್ಚಗಳನ್ನು ಇರುವುದಿಲ್ಲ.

<u>ಭಾಗವಹಿಸುವಿಕೆ ಪಾವತಿ:</u> ಈ ಅಧ್ಯಯನದಲ್ಲಿನ ಭಾಗವಹಿಕೆಯಿಂದ ನಿಮಗೆ ಯಾವುದೇ ಪ್ರೋತ್ಸಾಹಧನ ಇರುವುದಿಲ್ಲ.

<u>ಪ್ರಶ್ನೆಗಳು:</u> ಈ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ, ನೀವು ಸಂಪರ್ಕಿಸಬಹುದು

•ಶ್ರೀ ವಿನಯ್. ಪಿ ಎಸ್, ಸಂಶೋಧನೆ ವಿದ್ಯಾರ್ಥಿ, ದೂರವಾಣಿ: 8970313466

•ಡಾI ಸಿಎಸ್ ಪಾಟೀಲ್, ಸಂಶೋಧನಾ ಮಾರ್ಗದರ್ಶಿ, ಸೂಕ್ಷ್ಮ ಜೀವವಿಜ್ಞಾನದ ಇಲಾಖೆ, ಯು.ಎಸ್.ಎಮ್- ಕೆ.ಎಲ್.ಇ ಐ.ಎಂ.ಪಿ, ಬೆಳಗಾವಿ. ದೂರವಾಣಿ: 9448072678

•ಡಾI ಅನಿತಾ ದಲಾಲ್, ಸಂಶೋಧನಾ ಸಹ ಮಾರ್ಗದರ್ಶಿ, ಪ್ರಾಧ್ಯಾಪಕ, ಪ್ರಸೂತಿ ಮತ್ತು ಗರ್ಭಶಾಸ್ತ್ರ, ಜೆಎನ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು, ಬೆಳಗಾವಿ. ದೂರವಾಣಿ: 9448140343

•ಡಾI ಸುಧಾ ಎ ರಡ್ಡಿ, ಅಧ್ಯಕ್ಷರು, ಪಿಎಚ್ ಸಂಶೋಧನೆ ಸಂಬಂಧಿಸಿದ ಎಥಿಕಲ್ ಸಮಿತಿ, ಕೆ.ಎಲ್.ಇ ವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ. ದೂರವಾಣಿ: 9448354712

ಸಹಿಗಳು:

ಈ ಸಮ್ಮತಿಯ ನಮೂನೆಯನ್ನು ಸಹಿ ಮಾಡುವುದರಿಂದ, ನಿಮ್ಮ ಯಾವುದೇ ಕಾನೂನು ಹಕ್ಕುಗಳನ್ನು ಬಿಟ್ಟುಕೊಡುವುದಿಲ್ಲ. ನೀವು ಯಾವುದೇ ಸಮಸ್ಯೆ ಇಲ್ಲದೆ, ಈ ಎಲ್ಲಾ ಚಟುವಟಿಕೆಗಳನ್ನು ಅಥವಾ ಯಾವುದೇ ಭಾಗವನ್ನು ನಿರಾಕರಿಸುವಂತೆ ಮುಕ್ತವಾಗಿರುತ್ತದೆ. ನೀವು ಕೆಳಗಿನ ಫಾರ್ಮ್ಲ್ಯಾನಲ್ಲಿ ನಿಮ್ಮ ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆರಳು ಮುದ್ರಣ ಮಾಡಿದಲ್ಲಿ, ನಿಮ್ಮ ಕಾಳಜಿಗಳು ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಉತ್ತರಿಸಿದೆ ಎಂದರ್ಥ ಮತ್ತು ನೀವು ಸ್ವಇಚ್ಛೆಯಿಂದ ಅಧ್ಯಯನದ ಕಾರ್ಯವಿಧಾನಗಳಲ್ಲಿ ಒಳಗಾಗಲು ಒಪ್ಪುತ್ತೀರಿ.

ದಿನಾಂಕ:

ಭಾಗವಹಿಸುವವರ ಹೆಸರು ಮತ್ತು ಸಹಿ/ ಎಡಗೈ ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು

ಸಾಕ್ಷಿಯ ಹೆಸರು ಮತ್ತು ಸಹಿ

ಅನುಮೋದನೆಯನ್ನು ಪಡೆಯುವ ವ್ಯಕ್ತಿ ಹೆಸರು ಮತ್ತು ಸಹಿ

ಸಂಶೋಧಕ ಅಥವಾ ನಿಯುಕ್ತಗಾರನ ಹೆಸರು ಮತ್ತು ಸಹಿ

ಕೆ.ಎಲ್.ಇ ನ ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆ ಮತ್ತು ವೈದ್ಯಕೀಯ ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಬೆಳಗಾವಿ
ಸಂಶೋಧನಾ ಕಾರ್ಯದಲ್ಲಿ ಹೇಳಿಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಸಮ್ಮತಿಯ ನಮೂನೆ
ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ: ಹೆಚ್.ಐ.ವಿ ಸೋಂಕಿತ ಗರ್ಭಧಾರಣೆಯ ವಯಸ್ಸಿನ ಮಹಿಳೆಯರಲ್ಲಿ ಮಾನವ ಪಪಿಲ್ಲೋಮ ವೈರಸ್ ಸೆರೋಟೈಪ್ಗಳನ್ನು **ಪತ್ತೆ** ಹಚ್ಚಲು

ನನಗೆ ಉದ್ದೇಶಿತ ಸಂಶೋಧನಾ ಕೆಲಸದ ಗುರಿಗಳನ್ನು ಮತ್ತು ಉದ್ದೇಶಗಳ ಮಾಹಿತಿಯನ್ನು ಓದಲು, ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಹಾಗೂ ಕುರಿತು ಯೋಚಿಸಲು ಸಾಕಷ್ಟು ಸಮಯದ ಅವಕಾಶವನ್ನು ಒದಗಿಸಲಾಗಿತ್ತು. ಅಧ್ಯಯನದ ಗುರಿಗಳು ಮತ್ತು ಉದ್ದೇಶಗಳು ನನಗೆ ಸ್ಪಷ್ಟವಾಗಿದೆ. ನನಗೆ ಈ **ಕಾರ್ಯದಲ್ಲಿ** ಭಾಗವಹಿಸಲು ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ಒತ್ತಡಕ್ಕೇರಲಾಗಿಲ್ಲ.

ಈ ಸಂಶೋಧನೆಯಲ್ಲಿನ್ನು ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ವೈಯಕ್ತಿಕವಾಗಿದ್ದು ಹಾಗೂ ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಕಾರಣಗಳನ್ನು ನೀಡದೆಯೇ ಹಿಂದೆಗೆದುಕೊಳ್ಳಬಹುದು . ಇದು ನನ್ನ ವೈದ್ಯರು ನೀಡುವ ನಿಯಮಿತ ಚಿಕಿತ್ಸೆ ಮತ್ತು ಕಾಳಜಿಯ ಮೇಲೆ ಯಾವುದೇ ಪ್ರಭಾವವನ್ನು ಹೊಂದಿರುವುದಿಲ್ಲ.

ಈ ಸಂಶೋಧನೆಯ ಕೆ.ಎಲ್.ಇ ವಿಶ್ವವಿದ್ಯಾಲಯ ಸಂಶೋಧನೆ, ನೀತಿಶಾಸ್ತ್ರ, ಮತ್ತು ಪ್ರಕಟಣೆಗಳ ಸಮಿತಿಯ ಅನುಮೋದನೆ ಪಡೆದಿದೆ ಎಂದು ತಿಳಿದಿದೆ.

ಈ ಸಂಶೋಧನೆಯ ಫಲಿತಾಂಶಗಳು ವೈಜ್ಞಾನಿಕ ಉದ್ದೇಶಗಳಿಗಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ ಮತ್ತು ಪ್ರಕಟಿಸ ಬಹುದು ಎಂದು ಸಂಪೂರ್ಣ ಅರಿವಿದೆ.

ನಾನು ಇದಕ್ಕೆ ಒಪ್ಪುತ್ತೇನೆ. ನನ್ನ ವೈಯಕ್ತಿಕ ವಿವರಗಳನ್ನು ಗೌಪ್ಯವಾಗಿ ಇಡಲಾಗುತ್ತದೆ ಮತ್ತು ಗೌಪ್ಯತೆ ಖಾತರಿ ಮಾಡಲಾಗಿದೆ ಎಂದು ನನಗೆ ಭರವಸೆ ಇದೆ.

ಆದ್ದರಿಂದ ನಾನು ಇದರ ಪ್ರಕಾರವಾಗಿ ಈ ಸಂಶೋಧನೆಯ**ಲ್ಲಿ** ಭಾಗವಹಿಸಲು ಅನುಮತಿ ನೀಡಿರುತ್ತೇನೆ.

ದಿನಾಂಕ:
ಭಾಗವಹಿಸುವವರ ಹೆಸರು ಮತ್ತು ಸಹಿ
ಸ್ಥಳ: **ಎಡ ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು**

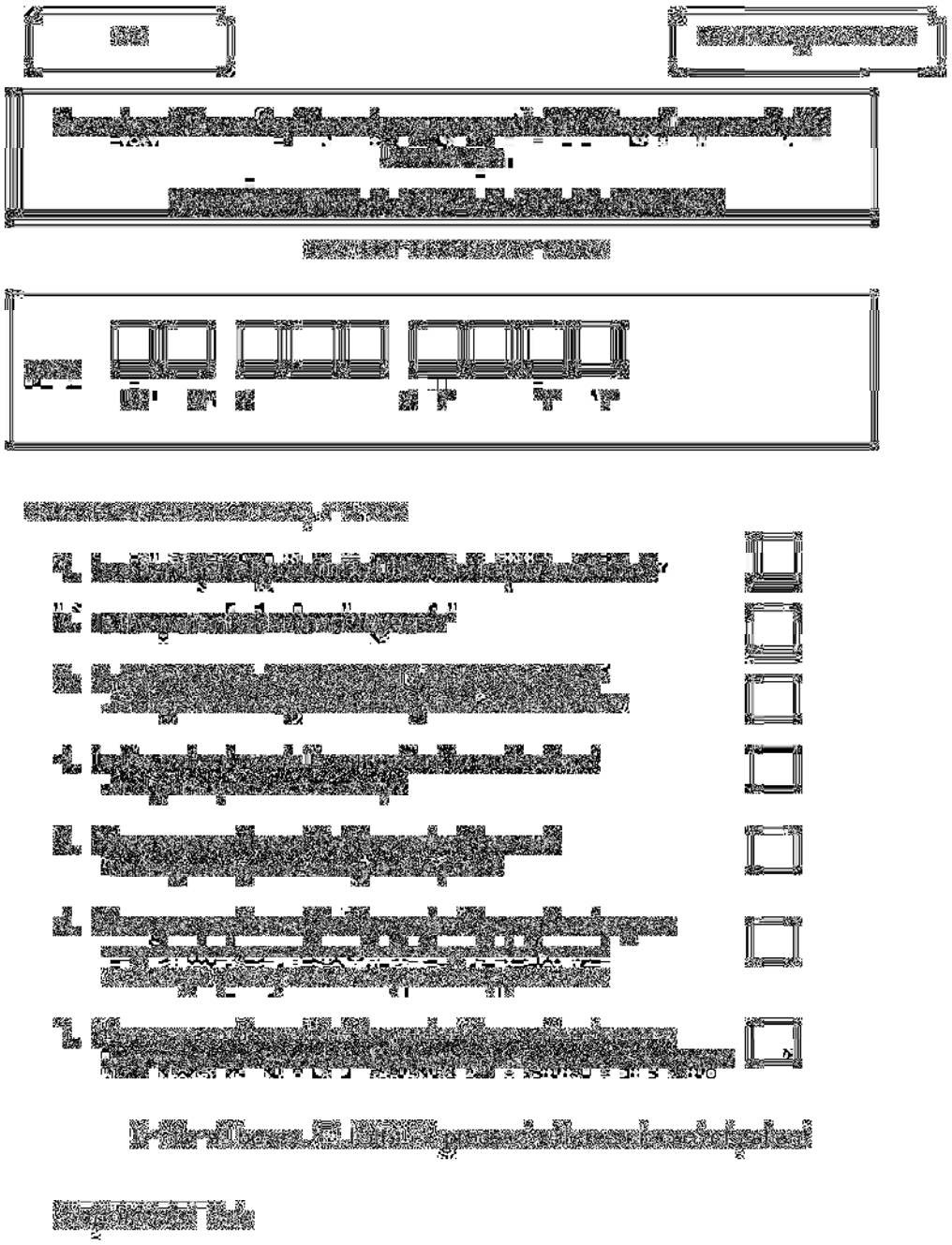
ಸಾಕ್ಷಿಯ **ಹೆಸರು ಮತ್ತು ಸಹಿ**

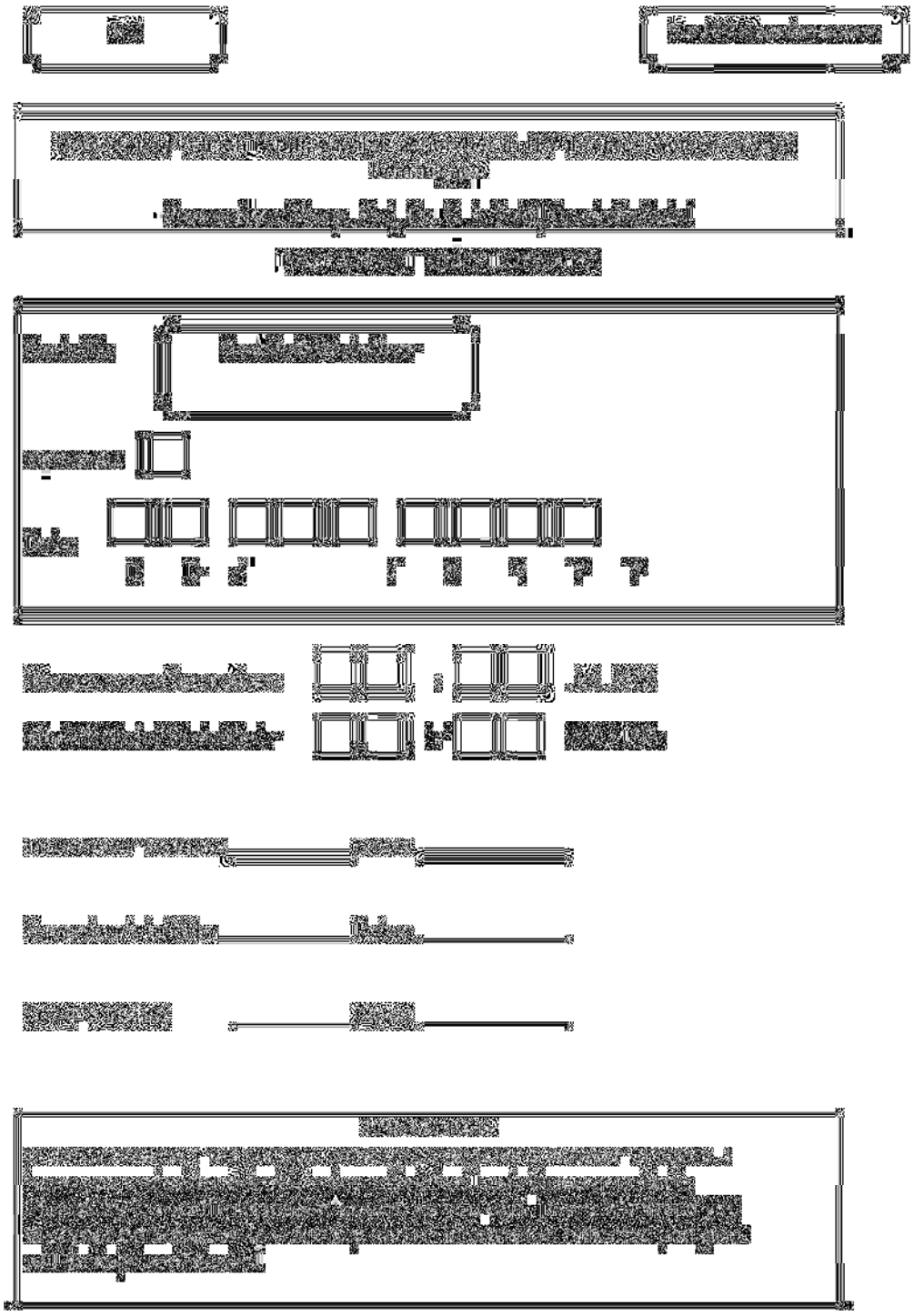
ಸಂಶೋಧಕರ ಹೇಳಿಕೆ

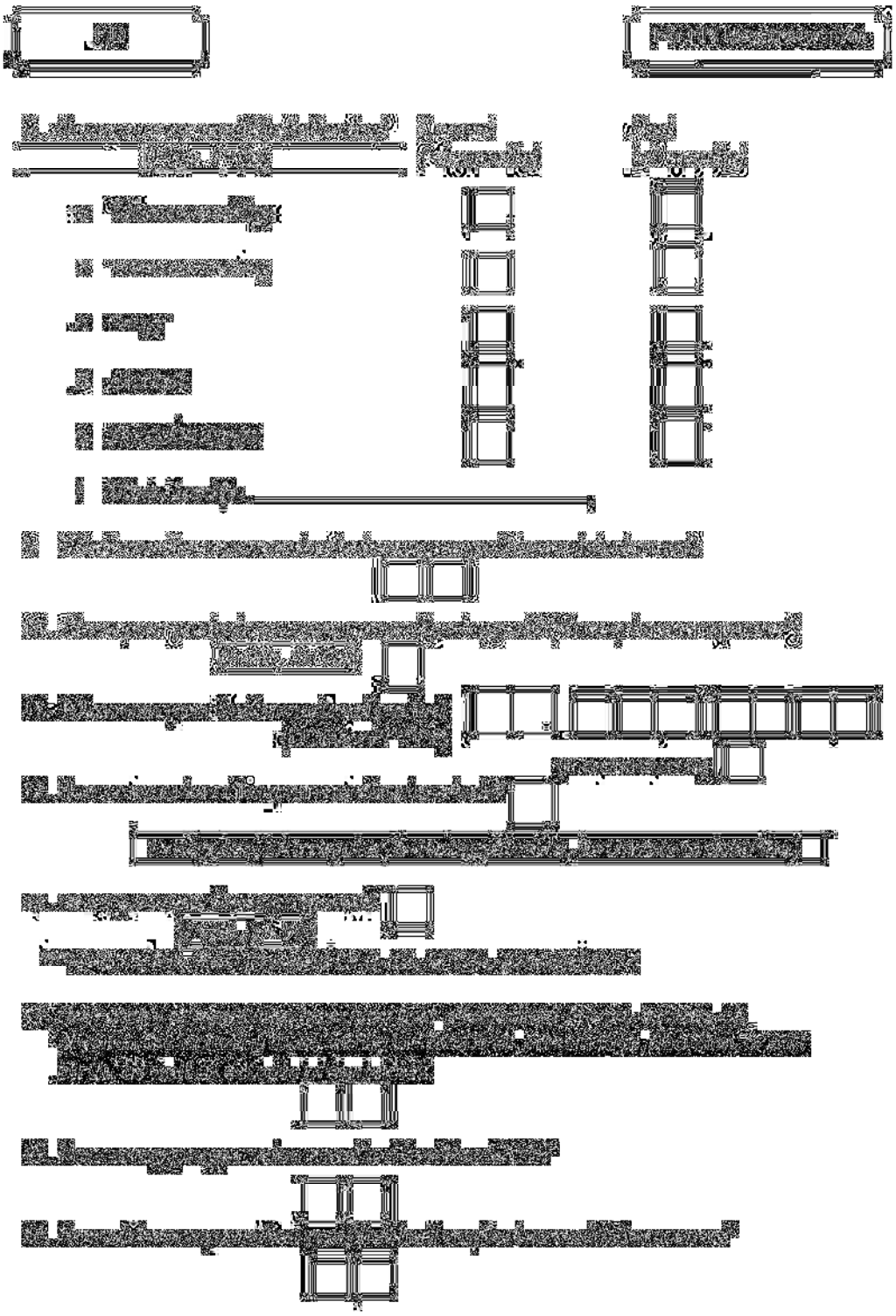
ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಿದ ಸ್ವಯಂಸೇವಕರಿಗೆ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ಮೌಖಿಕ ಮತ್ತು ಲಿಖಿತ ಮಾಹಿತಿಯನ್ನು ಒದಗಿಸಿದ್ದೇನೆ. ನಾನು ಸಂಶೋಧನೆಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸಲು ಒಪ್ಪುತ್ತೇನೆ. ನಾನು ಕೆ.ಎಲ್.ಇ ವಿಶ್ವವಿದ್ಯಾಲಯ ಅನುಮೋದನೆ ಪಡೆದ ಪ್ರೋಟೋಕಾಲ್‌ಗೆ ಅಂಟಿಕೊಳ್ಳುತ್ತೇನೆ.

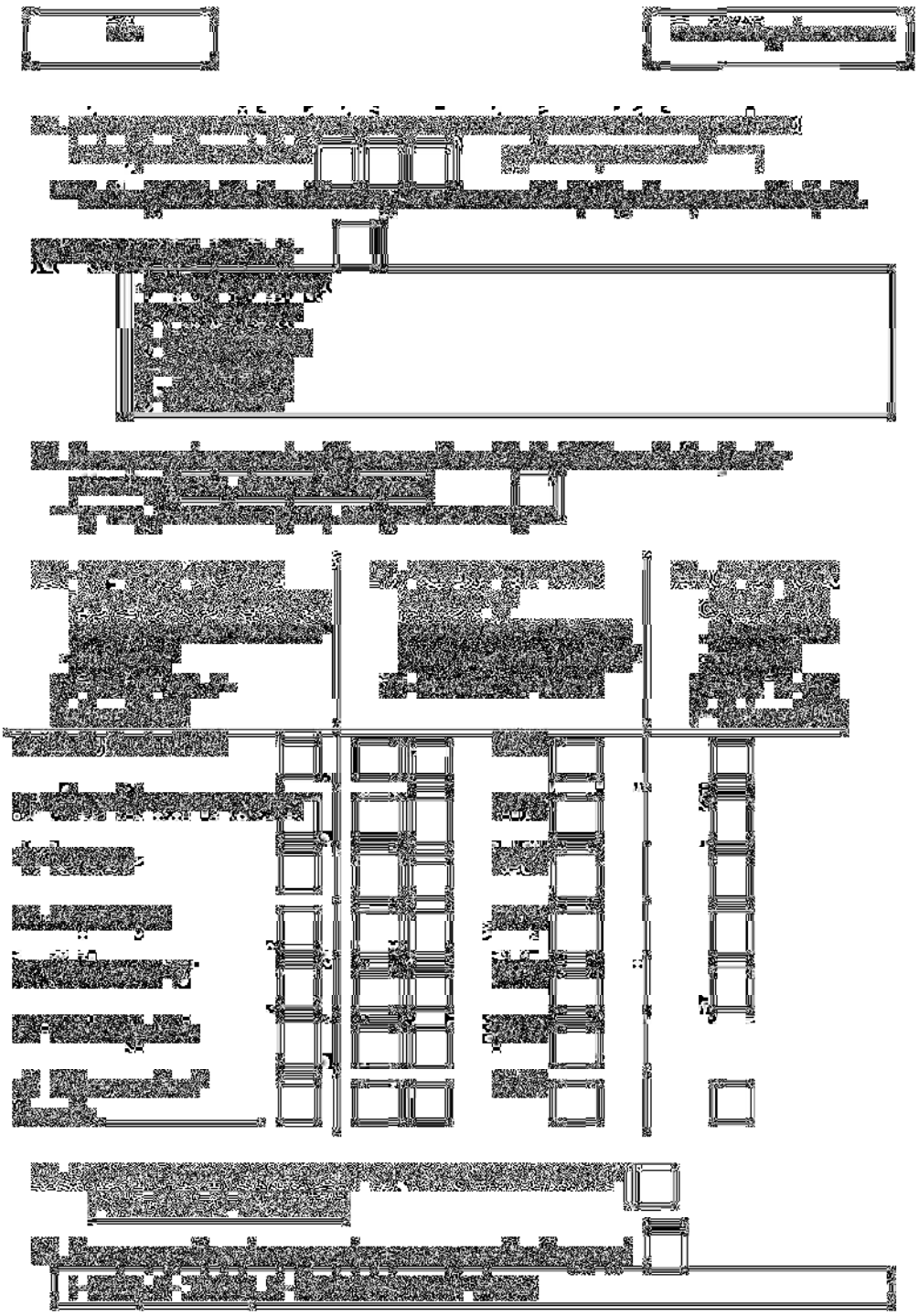
ದಿನಾಂಕ: **ಸಂಶೋಧಕರ ಸಹಿ**
(ವಿನಯ್. ಪಿ. ಎಸ್)

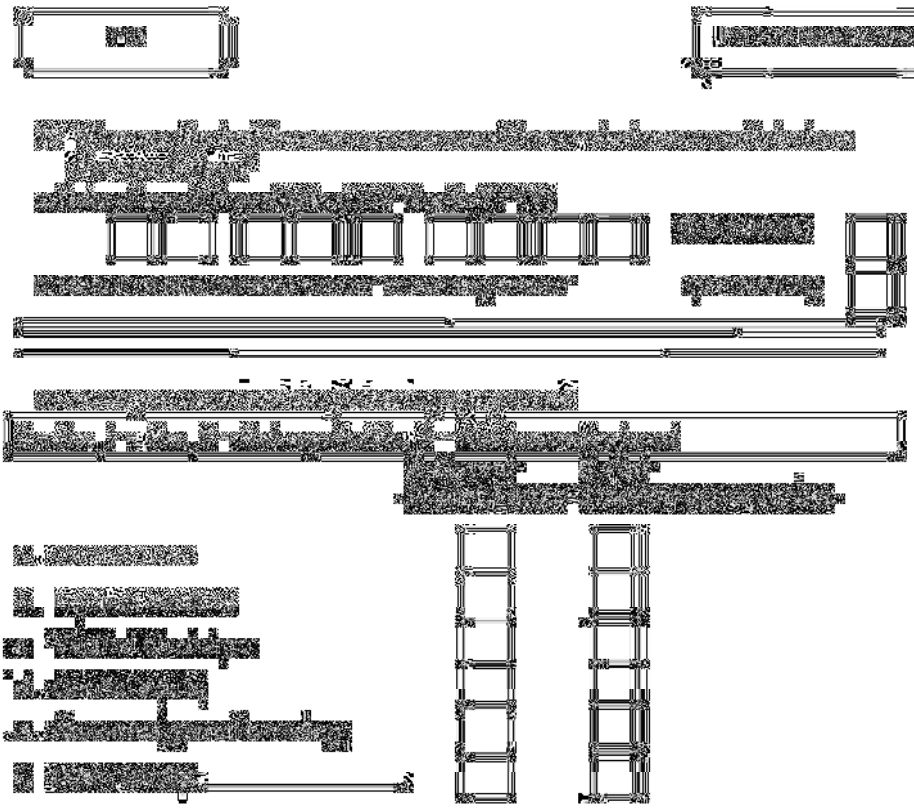
Annexure IV: Demographic data collection form

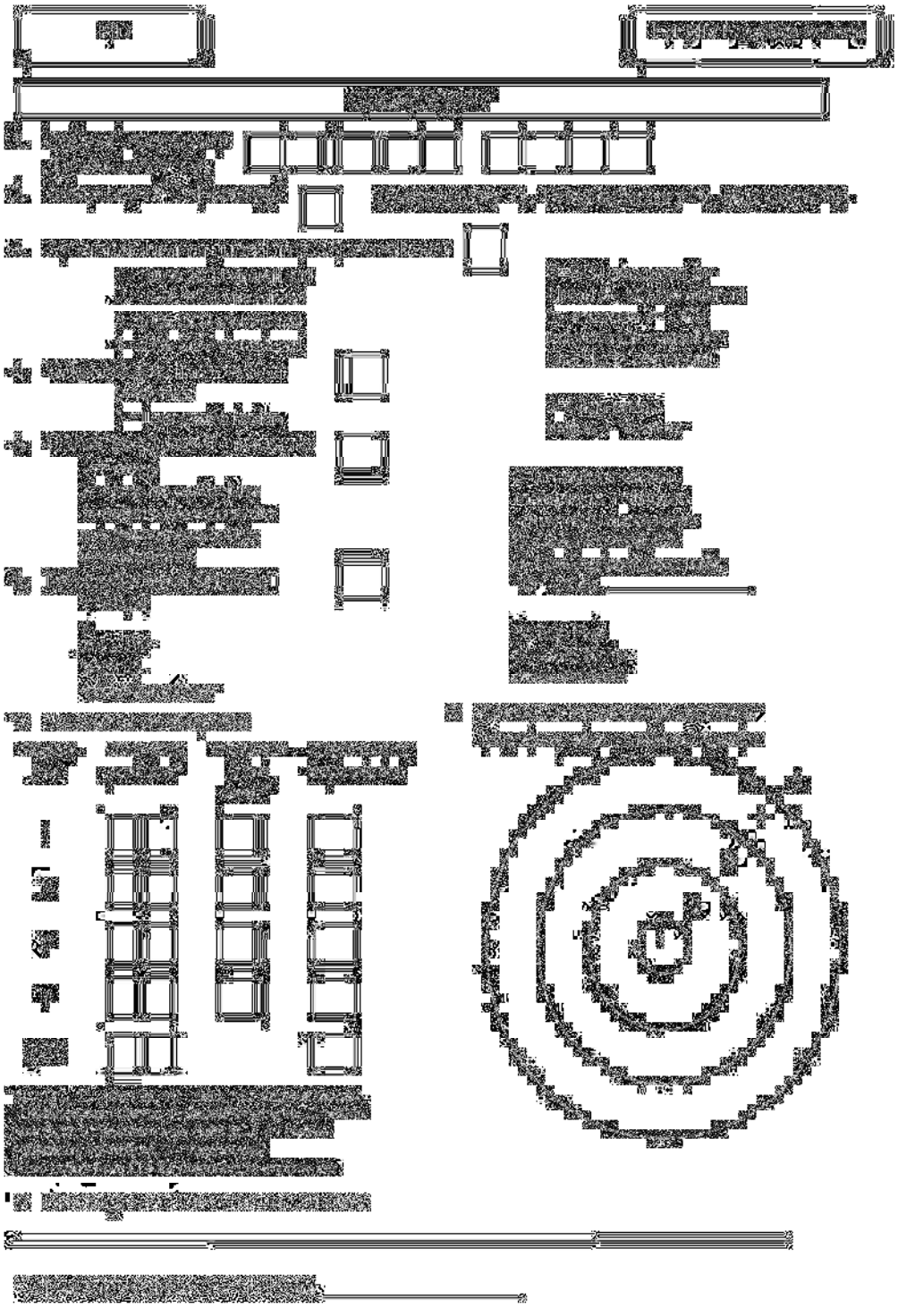


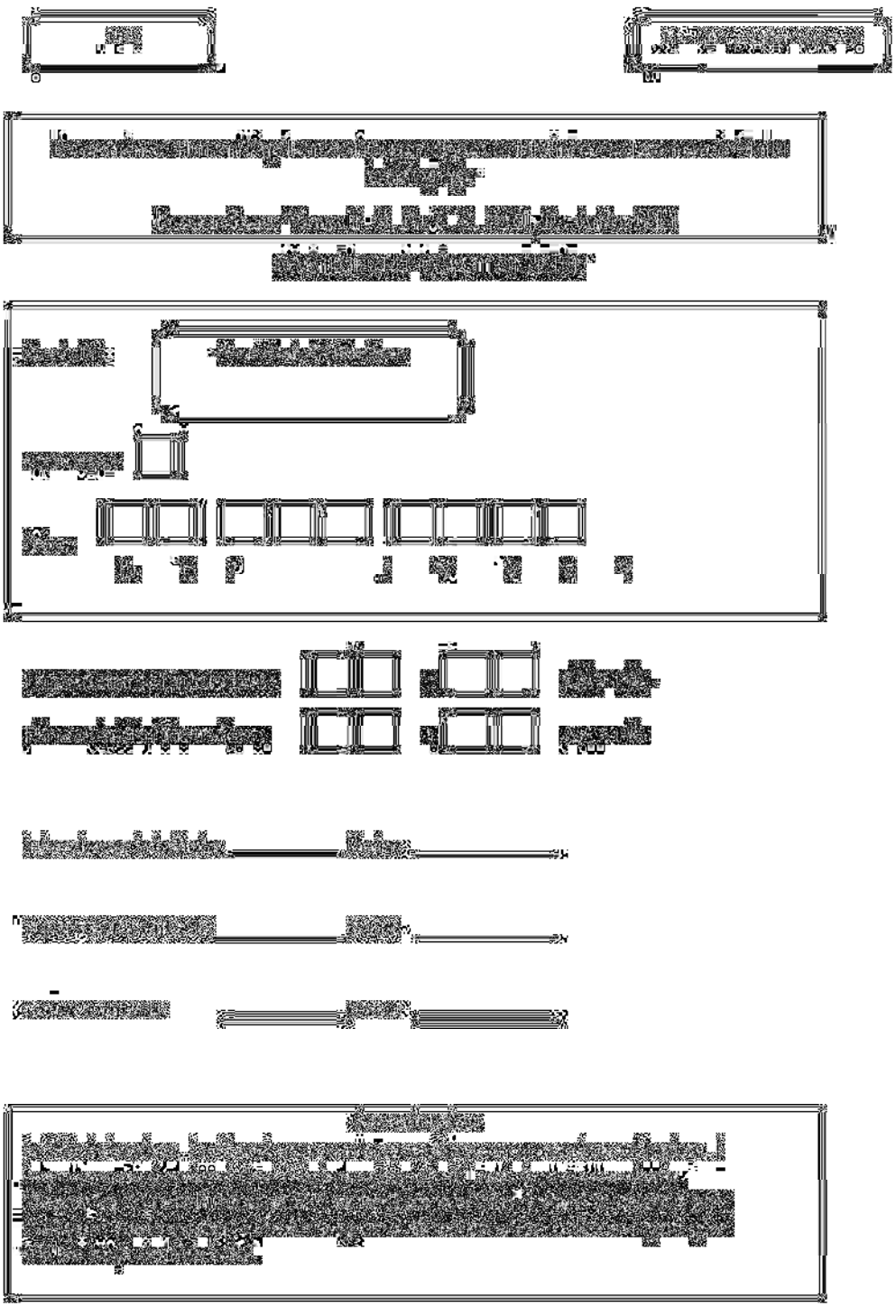


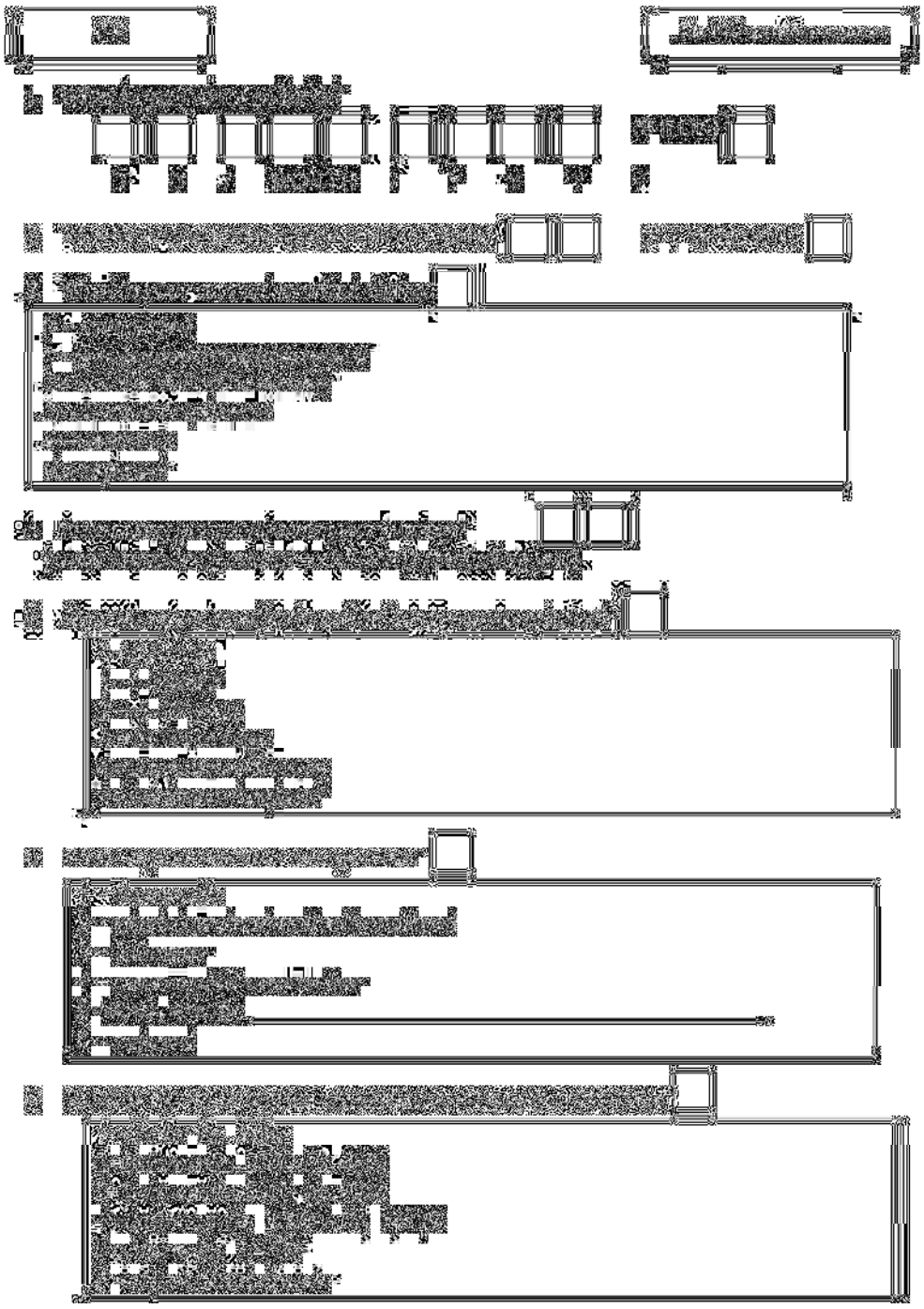


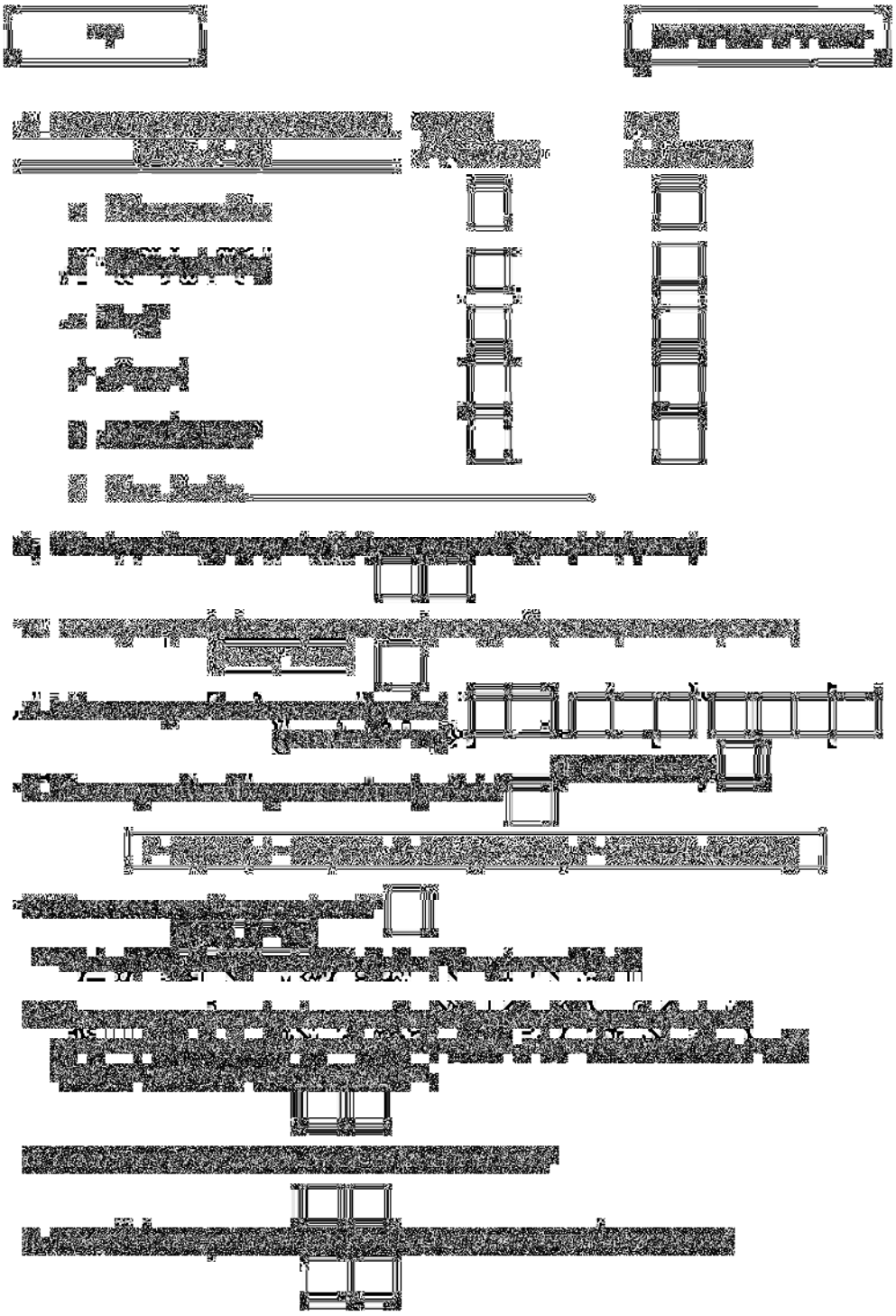


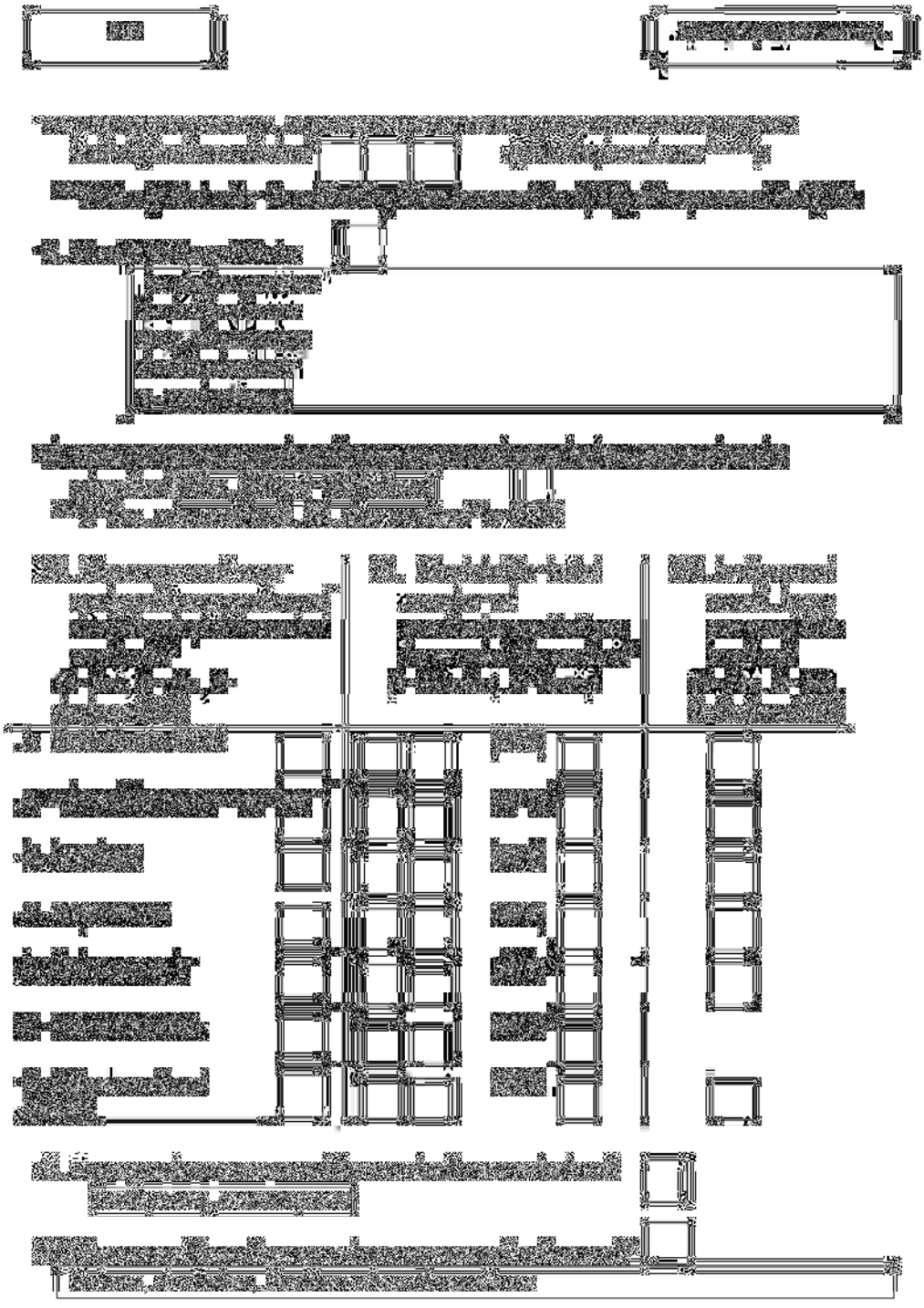


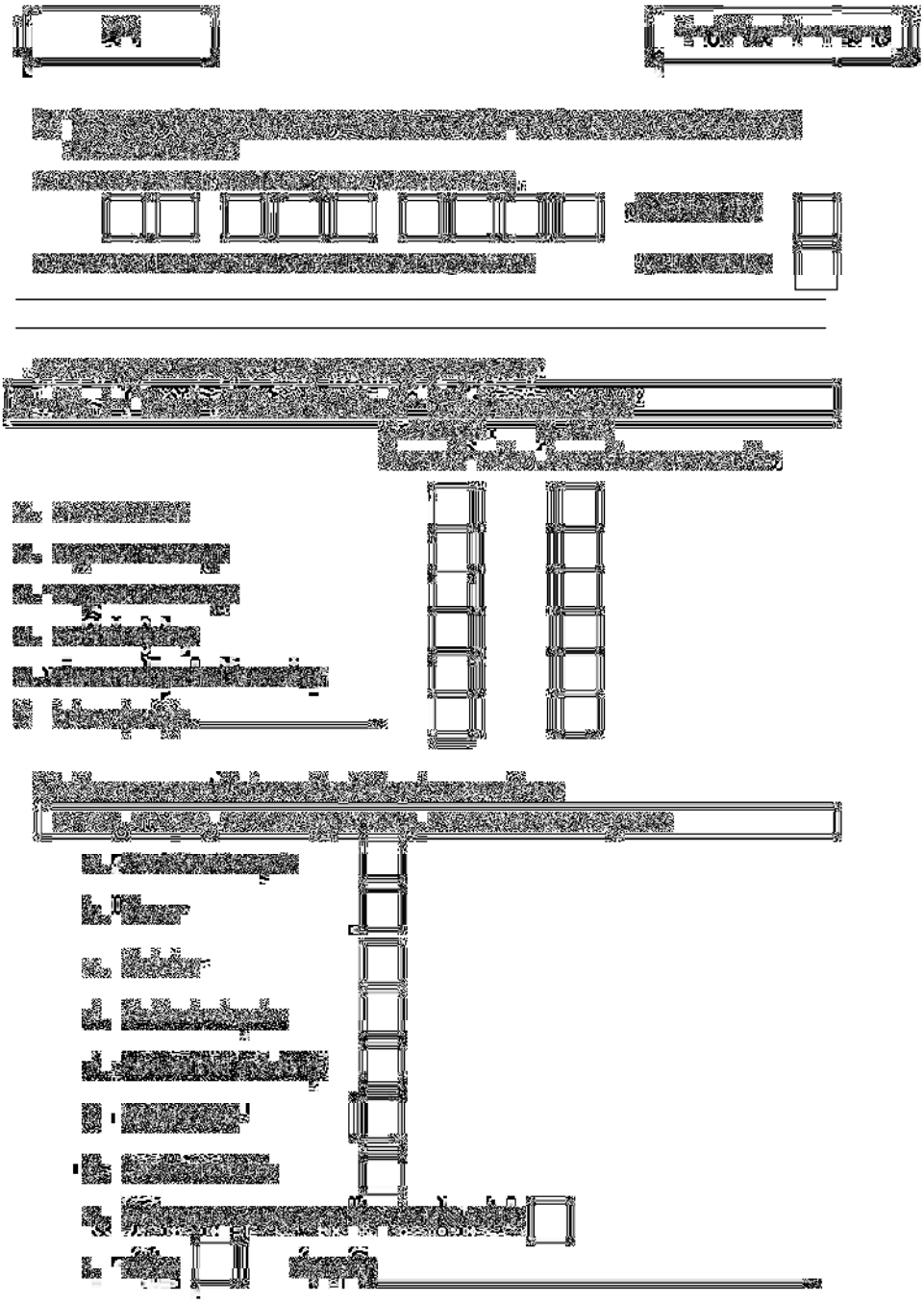


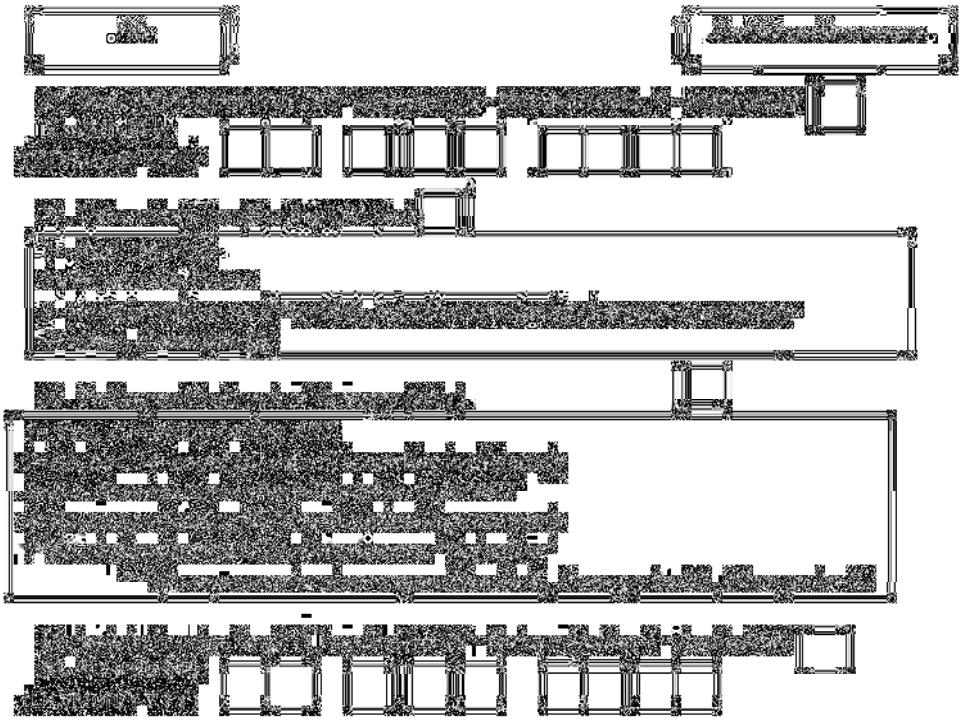


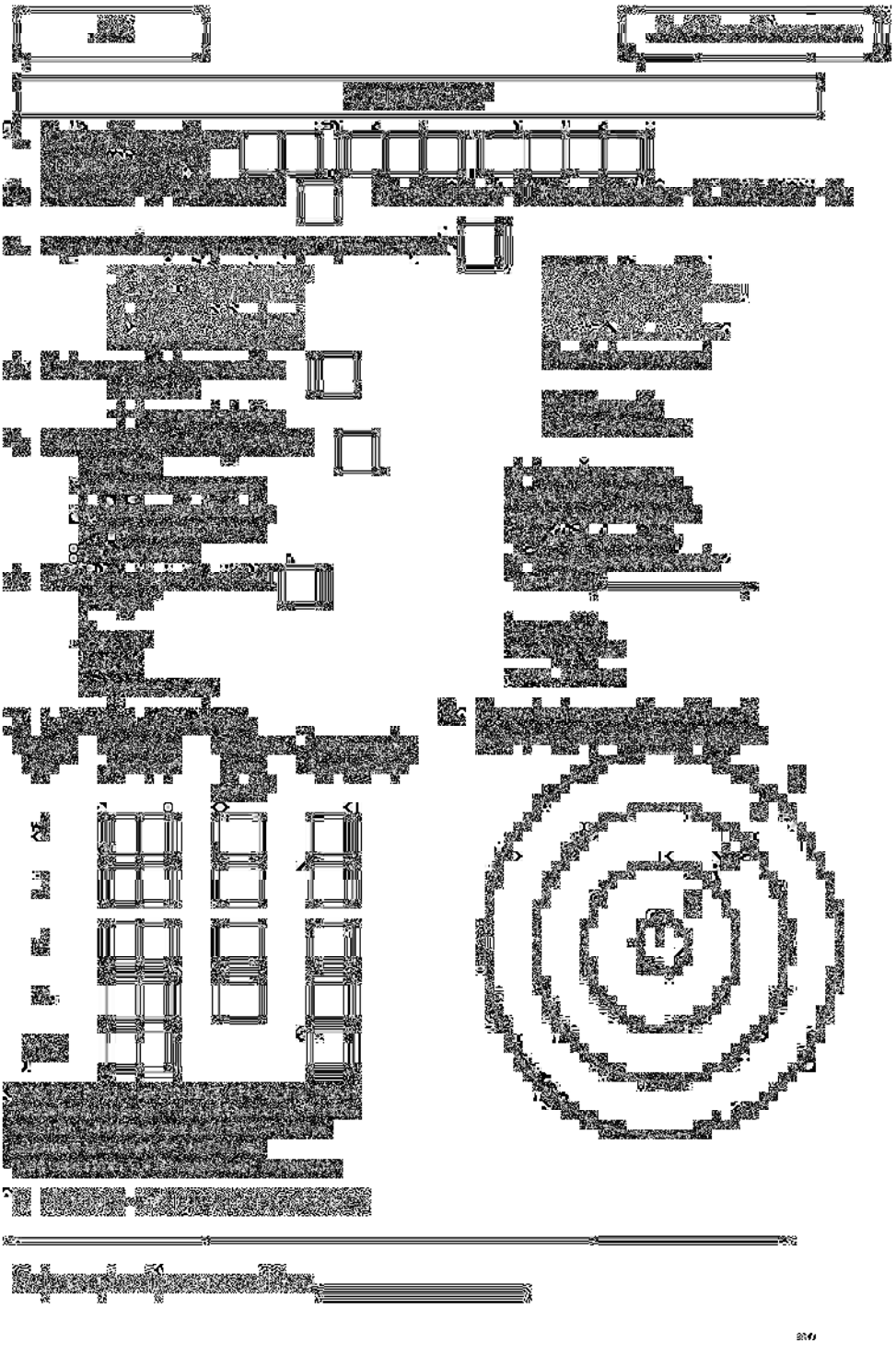












Annexure V: Colposcopy protocol

Colposcopy Protocol

The entire colposcopic procedure, including histologic sampling, will first be described to the patient. The clinician will inquire about potential pregnancy, hemorrhagic diathesis and allergies. Verbally recognize the patient's anxiety about the exam and suggest that you will describe your actions in advance. Ask her if she has any questions about the colposcopy procedure before you begin.

The process of colposcopy may be divided into four procedural steps; visualization, assessment, sampling and correlation.

1. **Visualization**

Assist the patient into the dorsal lithotomy position. Select an appropriate sized warm non-lubricated vaginal speculum to minimize discomfort but ensure proper visualization. Adequate visualization is absolutely necessary for a satisfactory colposcopic exam. Prolapsing vaginal walls, common in obese, pregnant or elderly women, frequently hinder proper visualization. Optimal visualization adjuncts (latex glove finger 'tube', condom or lateral side wall retractor) improve colposcopic viewing when necessary. Initially, the entire cervix will be viewed through the colposcope at low power magnification. Excessive vaginal or endocervical canal discharge will be noted and appropriate specimens obtained for culture, saline and KOH mount wet preparation and other relevant microbiologic tests. Colposcopy may be rescheduled until after treatment if severe infection is present.

Next, normal saline is applied to the cervix to remove obscuring debris and to moisten the epithelium. Two important findings may readily be observed. First, the presence of leukoplakia must be established prior to the cervical application of acetic acid. Second, atypical vessels are usually better noted prior to acetic acid application because of its mild vasoconstrictive properties.

Next, the green filter examination, designed to enhance angioarchitecture, may be performed. The green filter absorbs red light causing blood vessels to appear black and makes them somewhat easier to discern. Normal saline and acetic acid are then separately applied to the cervix to help discriminate normal from abnormal epithelium. First, 5% acetic acid is liberally and frequently applied to the cervix using cotton balls and ring forceps, large cotton swabs or by a spray technique. Gauze pads will not be used because of their abrasive properties. Cells with an increased nuclear-cytoplasmic ratio, either normal or abnormal, transiently appear white (acetowhite) against the normal pink epithelium following acetic acid application. The temporary white effect persists longer for more severe disease (thicker layer of abnormal cells).

The acetic acid will be gently applied twice for a minimum of 60 seconds. Thereafter, reapplication of acetic acid every three to five minutes or as often as necessary is essential to prolong and retain the acetowhite effect. Acetic acid will be reapplied to the cervix when columnar epithelium no longer blushes slightly acetowhite but returns to its normal red color. Sufficient acetic acid response is critically important prior to obtaining these digital images.

2. Assessment

Assessment of colposcopic findings permits identification of the squamocolumnar junction (SCJ) and transformation zone and, although its accuracy is not optimal, colposcopy permits estimation of suspected neoplastic lesions, their linear extent, size, and estimate of the degree of severity of each cervical lesion. Complete 360 degree delineation of the squamocolumnar junction, appearing transiently acetowhite, generally implies that the likely habitat for cervical neoplasia has been successfully surveyed. Squamous neoplasia is usually positioned along the squamocolumnar border and extends distally within the transformation zone. Glandular neoplasia adjoins the proximal squamocolumnar junction and extends towards the internal os.

The colposcopist indicate whether the entire SCJ and transformation zone were detected during the colposcopic exam. If detected, the location of the SCJ will be noted as ectocervical, at the os, or endocervical. Ectocervical means full visualization of the squamocolumnar junction with columnar and/or immature squamous metaplasia noted on the ectocervix. At the os equates to the squamocolumnar junction positioned at the external os and entirely identifiable. Endocervical is defined as the squamocolumnar junction position within the endocervical canal.

Morphologic correlates of vascular and epithelial alterations in the tramsformation zone can indicate neoplastic lesions. Leukoplakia and acetowhite epithelium are the two epithelial findings of the abnormal transformation zone. Punctuation, mosaic and atypical vessels are the vascular patterns found in the abnormal transformation zone. However, all these findings may be also present in normal epithelium. Therefore the colposcopist must critically appraise variations of these potentially abnormal characteristics. The colposcopist must also be aware of the warning signs of invasive cervical cancer.

The determination of the size and extent of the cervical lesions conveys information that directly impacts potential patient management options. For instance, very large lesions or lesions extending beyond colposcopic view within the endocervical canal that require treatment are best managed by excisional methods. The colposcopist will indicate on the computer-based colposcopy documentation system the presence of abnormal transformation zone findings and the size and characteristics of cervical lesions.

The colposcopist will also indicate whether the colposcopic examination was satisfactory or unsatisfactory. A satisfactory exam is defined as observing the entire SCJ, and the proximal and distal extent of all cervical lesions, when present. An unsatisfactory exam is defined as the inability to assess either entity. Colposcopists will appropriately document the explanation for unsatisfactory exams in the computer-based colposcopy documentation system.

Table: Summary of Assessment Terms and Definitions

Location of SCJ	
Ectocervical	Full visualization of the SCJ with columnar and/or immature squamous metaplasia noted on the ectocervix
At the os	SCJ positioned at the external os and entirely identifiable
Endocervical	SCJ positioned within the endocervical canal
Abnormal Transformation zone: Neoplastic lesions characterized by variations in:	
Epithelial morphologic characteristics	Leukoplakia Acetowhite Epithelium
Vascular morphologic characteristics	Punctuation Mosaic Atypical vascular patterns
Lesion size and extent: Number of cervical quadrants occupied	
Satisfactory Examination	Entire SCJ observed
	Proximal and distal extent of any lesions observed
Unsatisfactory Examination	Inability to observe entire SCJ
	Inability to observe proximal and distal extent of any lesions present

The critical assessment of cervical lesion severity enables the colposcopist to select a small amount of the abnormal tissue areas to sample although biopsy placement has been demonstrated to be inaccurate. Consideration of distinct features of the abnormal transformation zone findings permits some assessment of lesion specificity: Variation in the lesion margin; lesion color of acetowhitening or perceived density; caliber of vasculature, intercapillary spacing or vessel presence; the surface contour and presence of bizarre atypical blood vessels suggestive of invasive cancer help define the lesion morphology. Variability is cumulatively considered by several severity indices to predict lesion severity. Colposcopists may use a colposcopic index of their preference to predict the severity of disease. After documenting the overall impression of disease severity, colposcopists will select characteristics of single lesions in the Colposcopy Annotation Tool based on categories from the Reid Colposcopic Index (RCI).

Outline of Colposcopy Procedures

1. Describe colposcopy procedure to patient and answer questions
2. Obtain appropriate history (potential pregnancy, hemorrhagic diathesis, allergies)
3. Assist patient to dorsal lithotomy position
4. Insert warmed, non-lubricated speculum of appropriate size and dimension into relaxed vaginal introitus
5. Visualize the entire cervix through the colposcope at low power

6. Use optional visualization adjuncts (i.e. condom or rubber glove finger 'tube' over speculum, lateral side wall retractor, etc) if necessary to enhance colposcopic visualization
7. Apply normal saline to the cervix to remove debris and moisten the epithelial surface
8. Examine the cervix through the colposcope paying particular attention to the vascular patterns
9. Activate the green filter of the colposcope and examine the vascular patterns, if desired
10. Liberally apply 5% acetic acid to the cervix using cotton balls and ring forceps, large cotton swabs or by spray technique. Avoid the use of 4 x 4 gauze pads
11. Re-apply acetic acid to the cervix for a minimum of 60 seconds. Thereafter reapply acetic acid every 3 to 5 minutes or when the columnar epithelium no longer is blanched white but is red.
12. Identify the entire squamocolumnar junction (360), if able
13. Identify acetowhite cervical lesions if present
14. Assess the severity of each cervical lesion
15. Take digital image
16. Obtain cervical biopsies and an endocervical curettage specimen per cervical biopsy protocol, if necessary
17. Inspect the vagina by colposcopic examination, if appropriate (discordance between cytology and colposcopic impression). Apply 5% acetic acid to the entire epithelium and then view by low power colposcopic magnification, noting acetowhite vaginal lesions. Apply one half strength Lugol's solution to the vagina and reinspect the vaginal epithelium. Vaginal lesions contain no glycogen and thus will appear yellow in contrast to the normal, transiently brown squamous epithelium.
18. Biopsy vaginal lesions when appropriate and place the specimen into a labeled bottle containing 10% buffered formalin.
19. Apply acetic acid to the vulva, perineum and perirectal areas for 3-5 minutes only if considered necessary. (Acetic acid soaked 4 x 4 gauze pads work well)
20. Inspect the vulvar, perineal and perirectal areas for acetowhite or non-acetowhite lesions
21. Biopsy vulvar, perineal and perirectal lesions when appropriate and place the specimen into a labeled bottle containing 10% formalin.
22. Document colposcopic findings using the digital system (colposcopic impression, annotated biopsy sites and cervical lesion location)

Annexure VI: CD4/CD8 result sheet

PID	Intramural-to-India study **Lab Report Form: CD4 (Immunophenotyping)**	BSI ID

Date of Blood Collection (DD/Month/YYYY) ___ ___ / ___ ___ ___ / ___ ___ ___ ___

Date of Immunophenotyping analysis (DD/Month/YYYY) ___ ___ / ___ ___ ___ / ___ ___ ___ ___

Parameters	Value	Unit
1. CD4 Absolute count	☐☐☐☐	cells/cu mm
2. CD4 Percent	☐☐	%
3. CD8 Absolute count	☐☐☐☐	cells/cu mm
4. CD8 Percent	☐☐	%
5. CD4:CD8 Ratio	☐☐☐	

_____
Staff initials/Date

Annexure VII: Letters and Certificates

KLE UNIVERSITY

(Formerly known as KLE Academy of Higher Education & Research, Belgaum)

[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956, vide Government of India Notification No. F.9-19/2000-U3(A)]

Placed in 'Category A' by MHRD, Govt. of India — Accredited 'A' Grade by NAAC

Nehru Nagar, Belgaum - 590 010, Karnataka State, India

Ph. : 0831-2444444 FAX : 0831-2493777 Web: http://www.kleuniversity.edu.in E-mail: info@kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF
EDUCATION FOR HEALTH PROFESSIONALS

KLE UNIVERSITY
EMPOWERING PROFESSIONALS

CERTIFICATE

This is to certify that

Dr./Mr./Mrs. Vinay. P.S.

has participated in the Workshop entitled

Biostatistics

on 7th & 8th Dec 2012 organised by KLEU Research Foundation

as a Delegate / ~~Resource Person~~.

Dr. JYOTI NAGMOTI
DIRECTOR, UDEHP

Dr. V. D. PATIL
REGISTRAR

KLE UNIVERSITY

(Formerly known as KLE Academy of Higher Education & Research, Belgaum)

[Declared as Deemed-to-be-University u/s 3 of the UGC Act, 1956 vide Government of India Notification No.F.9-19/2000-U.3(A)]

Accredited **'A' Grade** by NAAC Placed in Category **'A' by MHRD** (GoI)

Certificate

This is to certify that the Ph. D. Research Scholar ***Mr. Vinay P. S.***

has successfully completed the Certificate Course in Biostatistics conducted by Academic Affairs of KLE University during the academic year 2012-13.

Dr. N.K. Tyagi
Prof. Dept. Of Biostatistics

Dr. S.G. Karadesai
Director Academic Affairs

Prof. (Dr.) V.D. Patil
Registrar

[redacted]

[redacted]

[redacted]
Bethesda, Maryland 20892

August 21, 2015

Vinay P. S
Senior Lecturer, Microbiology
USM-KLE International Medical Programme (KLE University)
District stadium road
Nehru Nagar
JNMC Campus
Belgaum
India
Via email: vinay.ps.micro@gmail.com

Sub: Samples from the NIH-ICMR-funded 'Intramural-to-India' study

Dear Mr. Vinay,

Thank you for your efforts on the NIH-ICMR-funded 'Intramural-to-India' ('I-to-I') study being conducted at the J.N. Medical College, Belgaum.

I have no objection for you to use the HPV PCR results from the samples from HIV-positive women enrolled in the I-to-I study for your PhD work.

Please feel free to let me know if you have any questions or concerns.

Sincerely,

Vikrant V. Sahasrabuddhe, M.B.B.S., M.P.H., Dr.P.H.
Program Director
Breast and Gynecologic Cancer Research Group
Division of Cancer Prevention
National Cancer Institute
National Institutes of Health
United States Department of Health and Human Services

9609 Medical Center Drive
Rm 5E-338, MSC 9783
Bethesda, MD 20892, USA (for mail delivery)
Rockville, MD 20850, USA (for courier delivery)

Tel: (240) 276-7332
Fax: (240) 276-7828
vikrant.sahasrabuddhe@nih.gov
prevention.cancer.gov

ज्ञान-विज्ञान विमुक्तये

12th UGC - Networking Resource Centre
Workshop on Molecular Virology

भारतीय विज्ञान संस्थान

(March 20, 2015 - March 31, 2015)

Certificate

This is to certify that

Mr. Vinay P S

has attended lectures and carried out hands on practical sessions in

Molecular Virology

Prof. C. Durga Rao
Co-ordinator

Prof. Saumitra Das
Co-Organizer

UGC- Networking Resource Centre
Division of Biological Sciences
Indian Institute of Science
Bangalore - 560 012

Date : 31-03-2015

Prof. R. Manjunath
Co-Coordinator

Prof. S. Vijaya
Co-Organizer

राष्ट्रीय एड्स अनुसंधान संस्थान
NATIONAL AIDS RESEARCH INSTITUTE
(भारतीय आयुर्विज्ञान अनुसंधान परिषद)
(Indian Council of Medical Research)
(WHO Collaborating Centre for HIV Diagnosis and Monitoring of Antiretroviral Therapy)

Date: 4th September 2015

CERTIFICATE OF ATTENCANCE AND TRAINING AT NARI, PUNE

This is to certify that Mr. Vinay. P. S, Ph.D. scholar, KLE University, Belgaum has undergone training in Molecular methods for detection of *Human Papillomavirus* and *HPV* genotyping.

He has tested his Ph.D. samples under my supervision from 31st August 2015 to 4th September 2015.

Dr. Arati Mane
Scientist C

Plot No. 73, 'G' Block, M.I.D.C. Bhosari,
Pune - 411 026. Post Box No. : 1895
☎ : 27331200
FAX No. 0091-20-27121071
E-mail : nari@nariindia.org
Website : www.nari-icmr.res.in

राष्ट्रीय एड्स अनुसंधान संस्थान
NATIONAL AIDS RESEARCH INSTITUTE
(भारतीय आयुर्विज्ञान अनुसंधान परिषद)
(Indian Council of Medical Research)
(WHO Collaborating Centre for HIV Diagnosis and Monitoring of Antiretroviral Therapy)

Date: 26th November 2015

CERTIFICATE OF ATTENDANCE

To whomsoever it may concern

This is to certify that Mr. Vinay. P. S, Ph.D. scholar, KLE University, Belgaum has visited National Aids Research Institute (NARI), Pune for data analysis and compilation of his Ph.D. samples entitled **"Detection of Human Papillomavirus genotypes in HIV-infected women of child bearing age"** and has compiled and entered the sample testing results under my supervision on 26th November 2015.

This is for your kind information.

Dr. Arati Mane

Scientist C

Plot No. 73, 'G' Block, M.I.D.C. Bhosari,
Pune - 411 026. Post Box No. : 1895
☎ : 27331200
FAX No. 0091-20-27121071
E-mail : nari@nariindia.org
Website : www.nari-icmr.res.in

राष्ट्रीय एड्स अनुसंधान संस्थान
NATIONAL AIDS RESEARCH INSTITUTE
(भारतीय आयुर्विज्ञान अनुसंधान परिषद)
(Indian Council of Medical Research)
(WHO Collaborating Centre for HIV Diagnosis and Monitoring of Antiretroviral Therapy)

Date: 4th September 2015

CERTIFICATE OF ATTENDANCE AND TRAINING AT NARI, PUNE

This is to certify that Mr. Vinay. P. S, Ph. D. Scholar, KLE University, Belgaum has undergone training on Molecular methods of HPV genotype testing and also tested his Ph. D. samples entitled **"Detection of Human Papillomavirus genotypes in HIV infected women of child bearing age"** under my supervision from 12th August 2015 to 21st August 2015.

Plot No. 73, 'G' Block, M.I.D.C. Bhosari,
Pune - 411 026. Post Box No. : 1895
☎ : 27331200
FAX No. 0091-20-27121071
E-mail : nari@nariindia.org
Website : www.nari-icmr.res.in

राष्ट्रीय एड्स अनुसंधान संस्थान
NATIONAL AIDS RESEARCH INSTITUTE
(भारतीय आयुर्विज्ञान अनुसंधान परिषद)
(Indian Council of Medical Research)
(WHO Collaborating Centre for HIV Diagnosis and Monitoring of Antiretraviral Therapy)

Date: 12th August 2013

CERTIFICATE OF PARTICIPATION

This is to certify that Mr. Vinay P.S. attended the training and discussion regarding the implementation of HPV, I to I study, organized at NARI, Pune on the 12th and 13th August 2013.

12/8/13
Dr. Arati Mane
Scientist C

Plot No. 73, 'G' Block, M.I.D.C. Bhosari,
Pune - 411 026. Post Box No. : 1895
☎ : 27331200
FAX No. 0091-20-27121071
E-mail : nari@nariindia.org
Website : www.nari-icmr.res.in

KLE University

Accredited "A" Grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)

KLE Centenary IAMM (KC) CME on

"H1N1 Outbreak : Lessons learnt and Future directions"

Organised by : Department of Microbiology, J. N. Medical College, Belagavi

This to certify that

Mr. Vinay P.

bearing Reg. No. ..... Registered with ..... Medical Council

from Belagavi has Presented poster in the

KLE Centenary IAMM (KC) CME on "H1N1 Outbreak : Lessons learnt and Future directions" held on 28th May 2016.

Karnataka Medical Council has granted TWO (02) credit hours for Delegate.

Vide Letter No. K.M.C./C.M.E./468 /2016 dated :16-04-2016

Dr.(Mrs.) N. S. Mahantashetti
Principal,
J. N. Medical College, Belagavi

Dr. Uma B. R.
Zonal Chairman
KMC-CME Accreditation Committee

Dr. Sharada C. Metgud
Organising Secretary

Dr. Jyoti M. Nagmoti
Organising Chairperson

UNIVERSITI SAINS MALAYSIA

KLE SOCIETY INDIA

KLE CENTENARY

UNIVERSITI SAINS MALAYSIA

CERTIFICATE

This is to certify that

Dr. / MR. / MRS. Vinay P. S.

has Participated in the INTERNATIONAL CME On

'RESEARCH METHODOLOGY & COMMUNICATION'

On 19th November 2016,

Organized by Research Unit, USM-KLE, IMP Belagavi,

as a Delegate / Resource Person / Organizing Committee member.

Prof Datuk Dr Asma Ismail	Dr. H. B. Rajasekhar	Dr. Kamarudin Jaalam	Dr. Sadanand B. Patil
Vice-Chancellor	Director	Organizing Chairman	Organizing Secretary
USM Penang, Malaysia	USM-KLE-IMP, Belagavi	USM-KLE- IMP, Belagavi	USM-KLE-IMP, Belagavi

USM KLE
UNIVERSITI SAINS MALAYSIA KLE SOCIETY INDIA
SCHOOL OF MEDICAL SCIENCES
(USM-KLE INTERNATIONAL MEDICAL PROGRAMME)

INTERNATIONAL MEDICAL CONFERENCE - 2019

Certificate of Participation

This is to certify that ***Vinay P S*** *has presented* ***POSTER***

entitled as "Identification of Human Papillomavirus (HPV) types and infection in Women of child bearing age in Belagavi, Karnataka" in the International Medical Conference

"Winds of Change": The Practice of Medicine in the Era of Disruptive Technology

held on ***12th to 14th September 2019*** *at*

KLE Centenary Convention Centre, J. N. Medical College Campus, Belagavi

Organized by USM-KLE International Medical Programme,

Belagavi, Karnataka, India.

Dr. H. B. Rajasekhar Director USM-KLE IMP Belagavi	**Prof. Dr. Shaiful Bahari Ismail** Dean PPSP KK, Malaysia & USM-KLE IMP Belagavi	**Prof. Dr. Kamarudin Jaalam** Deputy Dean USM-KLE IMP Belagavi	**Dr. Ashok C. Pangi** Organizing Chairman USM-KLE IMP Belagavi	**Dr. Sadanand B. Patil** Organizing Secretary USM-KLE IMP Belagavi

Annexure VIII: Publications

International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article

[redacted]

ABSTRACT

[redacted]

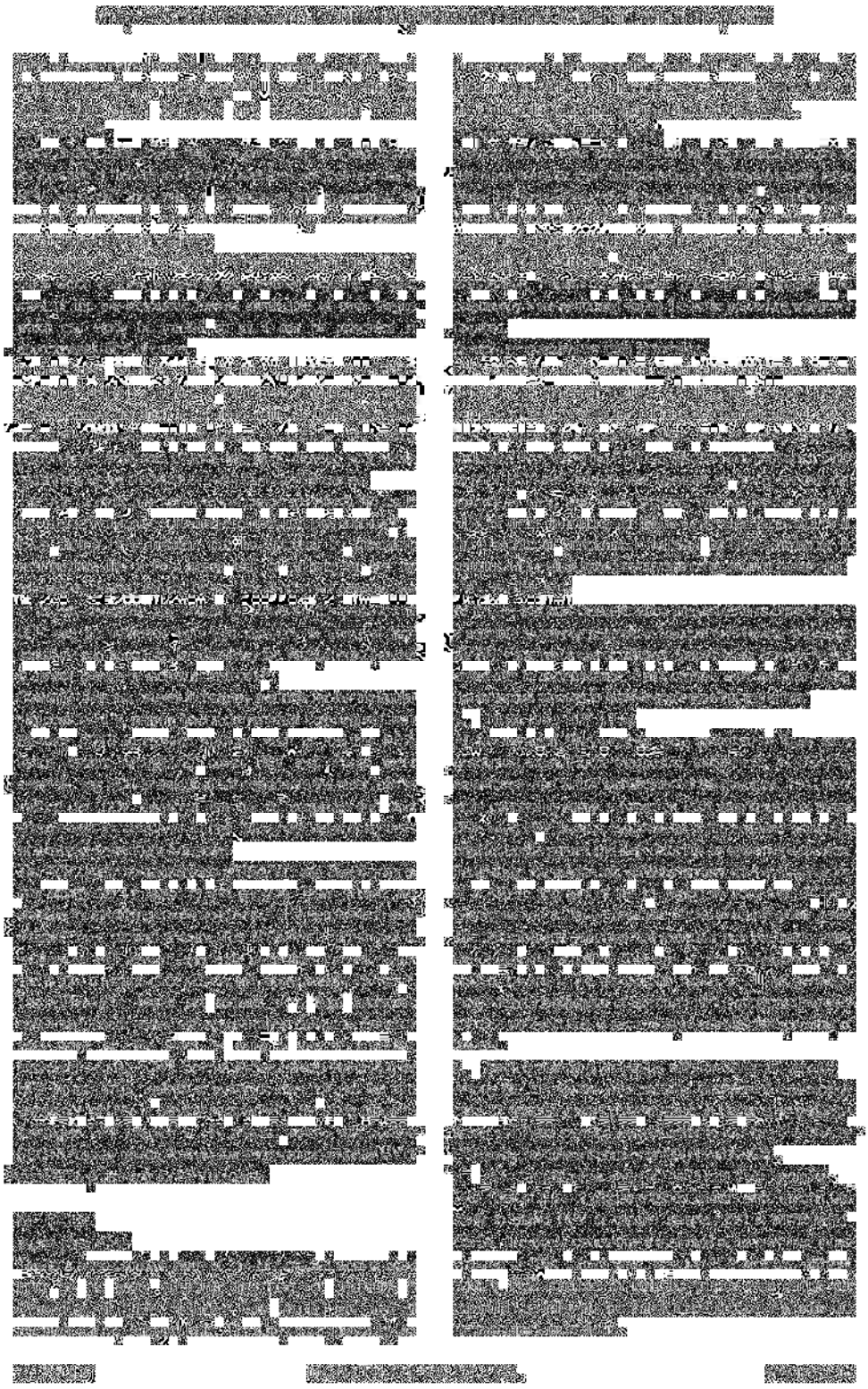
presence of HPV by Roche Linear array (RLA) genotype assay and thirty three samples which was positive for HPV in RLA were further tested for HPV DNA by using 2 different primers. **Results:** From the 33 samples which were positive in RLA, HPV DNA was detected in 63.7% (21 of 33) and 72.73% (24 of 33) of the samples by using the MY09/11 and the G5/G6 primer sets, respectively. Among the 24 HPV-positive samples,

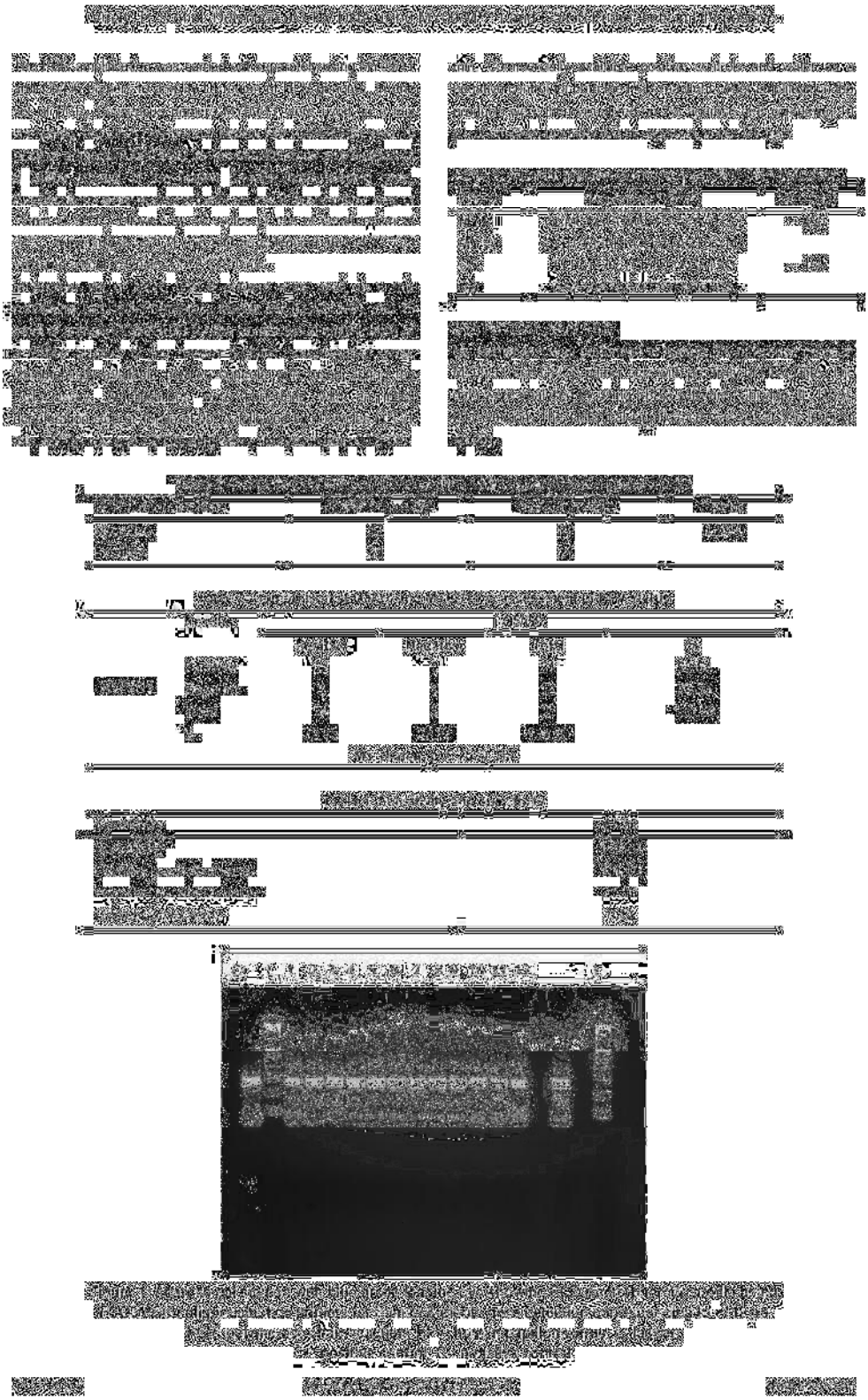
[redacted]

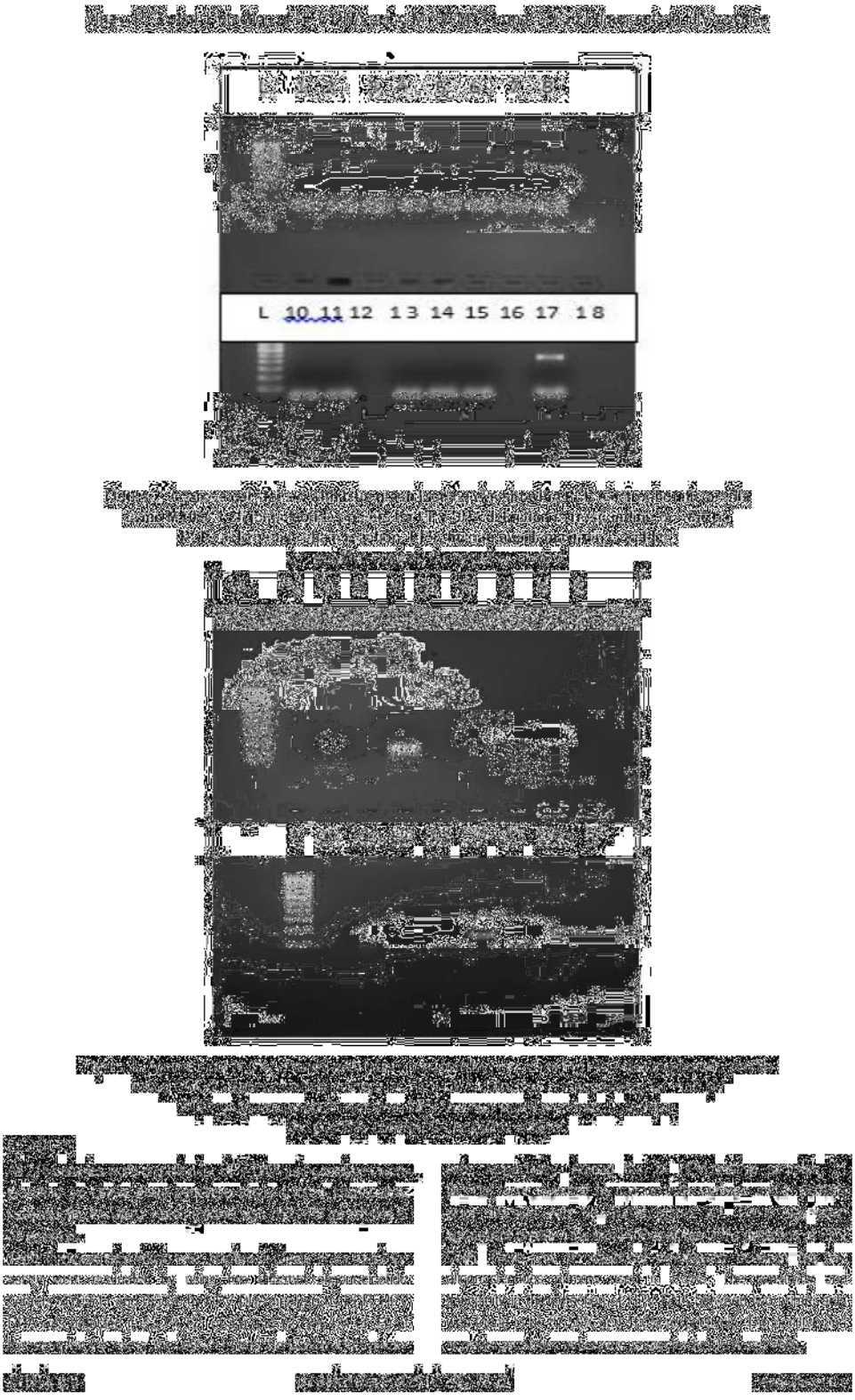
Received: 01-08-2016, **Revised:** 07-08-2016, **Accepted:** 27-08-2016

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2016.2.5.005	

[redacted]



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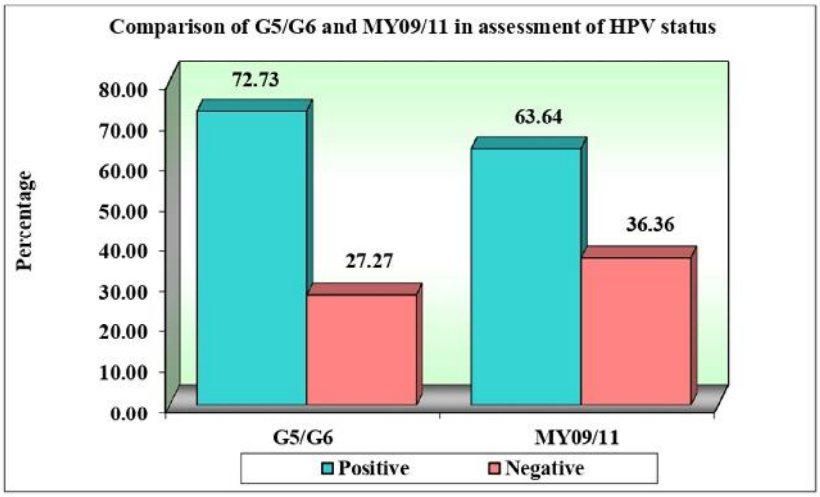
Comparison of G5/G6 and MY09/11 in assessment of HPV status
Percentage
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
72.73
27.27
63.64
36.36
G5/G6
MY09/11
Positive
Negative

Original Article

Detection and Genotyping of Human Papillomavirus among HIV-Infected Women from Belagavi: A District Place from the Southwest Indian State of Karnataka

Vinay Pala, Chidanand Patil[1], Mahantesh B. Nagmoti[2], Anita Dalal[3], Arati Mane[4]

Departement of Microbiology, KAHER, [1]Department of Microbiology, USM-KLE International Medical Programme, KAHER, Departments of [2]Microbiology and [3]Obstetrics and Gynecology, J N Medical College, KAHER, Belagavi, Karnataka, [4]Division of Microbiology, National AIDS Research Institute, Pune, Maharashtra, India

Abstract

Background and Aim: Human papillomavirus (HPV) infection is the established cause of cervical cancer. There is sparse literature with regard to HPV infection from the southern Belagavi region of India. This study was aimed to detect the HPV genotype distribution, the associated risk factors, and relation with cervical precancerous lesions among HIV-infected women from Belagavi, India. **Materials and Methods:** In this prospective observational study, a total of 214 HIV-infected women aged 18–45 years were recruited. Cervical samples were subjected to the Roche Linear Array assay for HPV detection and genotyping. Cervical status was determined by composite assessment of cytology, colposcopy, and histology. Data were analyzed using Software R version 3.6.0. **Results:** Of the 197/214 women with the adequate cervical sample, 86 (43.6%) were HPV positive, and 111 (56.3%) were HPV negative cases. A total of 132 (69.1%) women had normal cervical status, 26 (13.6%) had CIN1 lesions, 1 (0.5%) had CIN2 lesions, and 12 (6.3%) had CIN3 lesions. Single HPV infection was detected in 47 (54.6%) women and multiple (≥2) HPV genotypes were detected in 39 (45.3%). The HPV genotypes detected in descending order of frequency were HPV 16, HPV 33, HPV 35, HPV 52, and HPV 58. Ever pregnant (parous) women were 4.47 more likely to have HPV infection. **Conclusion:** A high prevalence of HPV infection, with a wide diversity of HPV genotypes and a greater prevalence of HPV 16 among HIV-positive women from Belagavi, India, was observed. Parity was the independent factor associated with HPV detection.

Keywords: Cervical intraepithelial neoplasia, colposcopy, genotype, human papillomavirus, pregnancy

Introduction

Human papillomavirus (HPV), a DNA virus, affects the basal epithelial cells, particularly of the skin and mucosal regions.[1] Among the identified HPV genotypes namely 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 are implicated in the causation of various types of cancer, including cervical, mucosal anogenital and head and neck cancers.[1] Furthermore, the chances of unidentified genotypes and possible chances of devastating ill effects exist.[2] Depending on their ability to cause cancers and lesions, the identified genotypes were broadly classified as high risk (HR) (genotype 16,18, 26,3 1,33,35,39,45,51,52,56,58,59, 66,68, 73,82) and low risk types (genotype 6,11,40,42,43,44,53,54,61,72,73,81).[3]

The transmission of HR HPV is directly attributed to several factors such as anatomical locations, HPV genotype, viral load, cofactors such as oral contraceptives, high parity, smoking, and sexually transmitted co-infections such as HIV and syphilis. In addition, being sexually transmitted infections, several studies have shown that HIV acts as an enhancer or co-factor of HPV infections.[4,5] Highly comprised host immune systems of the HIV-infected patients are highly susceptible to infections. Many investigations elucidated that HIV-positive individuals are more likely to have multiple HPV infections and a high HPV viral load than their HIV-negative counterparts.[6,7] In this regard, CDC has listed cervical cancer as one of the

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Quick Response Code:

Website: www.amhsjournal.org

DOI: 10.4103/amhs.amhs_4_21

Address for correspondence: Mr. Vinay Pala, Department of Microbiology, KAHER, Belagavi, Karnataka, India. E-mail: vinay.ps.micro@gmail.com

Submitted: 04-Jan-2021 **Revised:** 23-Aug-2021
Accepted: 26-Aug-2021 **Published:** 29-Dec-2021

How to cite this article: Pala V, Patil C, Nagmoti MB, Dalal A, Mane A. Detection and genotyping of human papillomavirus among HIV-infected women from belagavi: A district place from the southwest Indian state of Karnataka. Arch Med Health Sci 2021;9:209-15.

AIDS-defining illness. Meanwhile, several investigations have also reported HPV acts as a cofactor in HIV acquisition.[8]

In developed countries, the incidence of cervical cancer cases varies from 10 to 30 per 100,000 women.[9] The regional disparity in the incidence in India is due to a lack of necessary infrastructure and quality control of high-quality cytology screening that is not feasible for wide-scale implementation. It is reported that about 5% of the women in the general population in India harbor in the cervix.[10] Despite being the gold standard, Pap smear screening is not done routinely in Indian scenarios due to unawareness and casual attitude in women, lack of sensitivity and specificity, and regular follow-up visits.[11] Eventually, diagnosis of cancer is made only during advanced stages. Furthermore, the availability of HPV vaccines makes it essential to understand the geographical distribution of HPV genotypes.[2,9-12] Application HPV vaccines are effective in epidemiological studies in India will let public health to know about the vaccine used to protect Indian women. The older quadrivalent HPV vaccine covers five oncogenic types (31, 33, 45, 52, and 58). The new nine-valent vaccine added extra four genotype (6, 11, 16, and 18).[13] Currently three HPV vaccines–9-valent HPV vaccine (Gardasil® 9, 9 vHPV), quadrivalent HPV vaccine (Gardasil®, 4 vHPV), and bivalent HPV vaccine (Cervarix®, 2 vHPV) are available. All three HPV vaccines protect against HPV types 16 and 18 that cause most HPV cancers.

In another study from the Karnataka state, a comparative estimation of the virus infections of the cervix among women from the general and tribal population which revealed a different type-specific pattern of viral infection.[14] Studies showing HPV genotype distribution and their association with cervical disease status among HIV-infected women from the southern region of Belagavi, A distict place from southwest Indian state of Karnataka spare. Thus, this study was aimed to detect the HPV genotype distribution among HIV-infected women and to determine their association with cervical precancerous lesions.

Materials and Methods

The study was approved by the Ethical Committee of KLE University Belagavi, India (Ref No. KLEU/Ethic/2012-13/D-4573). This prospective observational study was carried out for 1 year from June 2013 to June 2014 in the Obstetrics and Gynecology Clinic of KLE's Prabhakar Kore Hospital and Medical Research Center, Belagavi, India. By convenient sampling technique, consecutive confirmed HIV-1 positive women, regardless of their current status of CD4+ count and antiretroviral treatment (ART) usage attending the clinic were recruited. After obtaining a written informed consent form, a total of 214 HIV-positive women aged 18–45 years, were enrolled in the study. Patients with a history of hysterectomy, abnormal bleeding who were pregnant and in active labor were excluded from the study. Other factors like hormonal contraceptives and tobacco smoking might influence the HPV infection, however, these factors are not included in the study.

Demographic data of all the participants were collected and recorded in a precoded pro forma. All patients underwent routine gynecological examination and were counseled on the risk of sexual behavior and provided with contraceptives if needed. A standardized noninvasive colposcopy examination was performed by a trained gynecologist and confirmatory procedures about histology were performed in participants with clinical evidence of cervical abnormalities. The colposcopy and histology results were reported as per Richart cervical intraepithelial neoplasia (CIN) system. Cervical smears were collected with the help of Ayer's spatula and cytobrush from the squamocolumnar junction. The glass slides were then fixed by using 95% ethyl alcohol. The smears were stained with Papanicolaou stain, and findings were reported according to the revised Bethesda system (2001). Cervical status for the participants was classified as "normal" based on normal cytology, "low grade" based on either low-grade squamous intraepithelial lesions on cytology or CIN1/2 on histology, and "high grade" based on high-grade squamous intraepithelial lesions on cytology or CIN3 and severe/invasive cancer on histology.[15]

Human papillomavirus genotyping

Cervical samples were collected from the transformation zone of the cervix immediately stored in the PreservCyt Solution at 4°C and transported to the laboratory at the Department of Microbiology where they were further stored at −20°C until DNA extraction. HPV detection and genotyping were done with the Linear array HPV genotyping test (Roche, Branchburg, NJ, USA). HPV and cellular DNA was released by lysing cervical cell specimens using lysis buffer under denaturing conditions at elevated temperatures in the presence of proteinase *K* followed by DNA purification in columns with a silica-based membrane using vacuum processing. All the pre- and post-DNA extractions were carried out in a separate room to avoid errors due to contamination. The polymerase chain reaction (PCR) amplicons were denatured and subjected to hybridization on linear array HPV genotyping strips coated with HPV type-specific and human beta-globin probes according to the manufacturer's instructions. The biotin-labeled amplicons hybridized to the probes only if the type-specific sequence matched those of the amplicons. The biotin-labeled amplicons were detected by colorimetric development, and the results were read visually by comparing the pattern of colored lines to the provided reference guide. Each run was performed with negative and positive controls provided by the manufacturer to monitor the quality and performance of the assay.

Statistical analysis

Statistical analysis was performed using software R version 3.6.0. please change to software R version 3.6.0 (R Core Team, Vienna, Austria). The normality of the data was determined using the Shapiro–Wilk test. Continuous variables with normal

Table 1: Prevalence of human papillomavirus genotypes stratified by cervical disease status, among HIV-infected women from Belagavi, Karnataka, India

	Overall ($n=86$), n (%) (95% CI)	Percentage (95% CI)					P*
		Normal ($n=53$)	Inadequate ($n=13$)	CIN 1 ($n=13$)	CIN 2 ($n=2$)	CIN 3 ($n=5$)	
Carcinogenic	51 (59.3) (48.92-69.69)	60.38 (47.21-73.55)	46.15 (19.05-73.25)	53.85 (26.75-80.95)	100 (100-100)	80 (44.94-100)	0.5757
Possibly carcinogenic	14 (16.28) (8.48-24.08)	15.09 (5.46-24.73)	0	30.77 (5.68-55.86)	0	40 (0-82.94)	0.1194
Non'un carcinogenic	47 (54.65) (44.13-65.17)	52.83 (39.39-66.27)	69.23 (44.14-94.32)	53.85 (26.75-80.95)	50 (0-100)	40 (0-82.94)	0.8511
Single	47 (54.65) (44.13-65.17)	58.49 (45.22-71.76)	61.54 (35.09-87.98)	38.46 (12.02-64.91)		60 (17.06-100)	0.2693
Multiple	39 (45.35) (34.83-55.87)	41.51 (28.24-54.77)	38.46 (12.02-64.91)	61.54 (35.09-87.98)	100 (100-100)	40 (0-82.94)	0.2683
Carcinogenic HPV genotypes							
HPV 16	17 (19.77) (11.35-28.18)	26.42 (14.54-38.28)	0	0	50 (0-100)	40 (0-82.94)	0.04
HPV 18	5 (5.81) (0.87-10.76)	3.77 (0-8.90)	0	15.38 (0-35)	0	20 (0-55.06)	0.2294
HPV 31	3 (3.49) (0-7.36)	5.66 (0-11.88)	0	0	0	0	0.6517
HPV 33	17 (19.77) (11.35-28.18)	13.21 (4.09-22.32)	23.08 (0.02-45.98)	38.46 (12.02-64.91)	50 (0-100)	20 (0-55.06)	0.2159
HPV 35	15 (17.44) (9.42-25.46)	11.32 (2.79-19.85)	23.08 (0.02-45.98)	30.77 (5.68-55.86)	50 (0-100)	20 (0-55.06)	0.2764
HPV 39	8 (9.3) (3.16-15.44)	11.32 (2.79-19.85)	7.69 (0-22.18)	7.69 (0-22.18)	0	0	0.959
HPV 51	6 (6.98) (1.59-12.36)	7.55 (0.04-14.66)	15.38 (0-35)	0	0	0	0.5287
HPV 52	15 (17.44) (9.42-25.46)	11.32 (2.79-19.85)	23.08 (0.02-45.98)	30.77	50 (0-100)	20 (0-55.06)	0.2924
HPV 56	4 (4.65) (0.02-9.10)	7.55 (0.04-14.66)	0	0	0	0	0.6107
HPV 58	17 (19.77) (11.35-28.18)	13.21 (4.09-22.32)	30.77 (5.68-55.86)	30.77 (5.68–55.86)	50 (0-100)	20 (0-55.06)	0.3173
HPV 59	6 (6.98) (1.59-12.36)	5.66 (0-11.88)	0	15.38 (0–35)	50 (0-100)	0	0.093
HPV 68	2 (2.33) (0-5.51)	3.77 (0-8.90)	0	0	0	0	>0.99
Possibly carcinogenic HPV genotypes							
HPV 53	6 (6.98) (1.59-12.36)	7.55 (0.04-14.66)	0	7.69 (0-22.18)	0	20 (0-55.06)	0.5982
HPV 66	2 (2.33) (0-5.51)	3.77 (0-8.90)	0	0	0	0	>0.99
HPV 67	2 (2.33) (0-5.51)	0	0	7.69 (0-22.18)	0	20 (0-55.06)	0.1209
HPV 70	2 (2.33) (0-5.51)	1.89 (0-5.54)	0	7.69 (0-22.18)	0	0	0.6282
HPV 73	1 (1.16) (0-3.43)	1.89 (0-5.54)	0	0	0	0	>0.99
HPV 82	4 (4.65) (0.02-9.10)	3.77 (0-8.90)	0	15.38 (0-35)	0	0	0.2899
Individual "noncarcinogenic" or "unknown carcinogenic" HPV genotype							
HPV 6	1 (1.16) (0-3.43)	1.89 (0-5.54)	0	0	0	0	>0.99
HPV 11	1 (1.16) (0-3.43)	0	7.69 (0-22.18)	0	0	0	0.3918
HPV 40	1 (1.16) (0-3.43)	0	7.69 (0-22.18)	0	0	0	0.3983
HPV 42	10 (11.63) (4.85-18.40)	9.43 (1.56-17.30)	23.08 (0.02-45.98)	15.38 (0-35)	0	0	0.5917
HPV 54	2 (2.33) (0-5.51)	3.77 (0-8.90)	0	0	0	0	>0.99
HPV 55	5 (5.81) (0.87-10.76)	7.55 (0.04-14.66)	7.69 (0-22.18)	0	0	0	0.8721
HPV 61	8 (9.3) (3.16-15.44)	13.21 (4.09-22.32)	0	7.69 (0-22.18)	0	0	0.4993
HPV 62	6 (6.98) (1.59-12.36)	5.66 (0-11.88)	7.69 (0-22.18)	7.69 (0-22.18)	0	20 (0-55.06)	0.8901
HPV 71	3 (3.49) (0-7.36)	3.77 (0-8.90)	7.69 (0-22.18)	0	0	0	>0.99
HPV 72	6 (6.98) (1.59-12.36)	3.77 (0-8.90)	0	15.38 (0-35)	50 (0-100)	20 (0-55.06)	0.0495
HPV 81	1 (1.16) (0-3.43)	1.89 (0-5.54)	0	0	0	0	>0.99

Contd...

Table 1: Contd...

	Overall (n=86), n (%) (95% CI)	Percentage (95% CI)					P*
		Normal (n=53)	Inadequate (n=13)	CIN 1 (n=13)	CIN 2 (n=2)	CIN 3 (n=5)	
HPV 83	2 (2.33) (0-5.51)	1.89 (0-5.54)	0	7.69 (0-22.18)	0	0	0.6277
HPV 84	8 (9.3) (3.16-15.44)	9.43 (1.56-17.30)	7.69 (0-22.18)	15.38 (0-35)	0	0	0.8921
HPV CP6108	2 (2.33) (0-5.51)	1.89 (0-5.54)	0	7.69 (0-22.18)	0	0	0.6172
HPV IS39	2 (2.33) (0-5.51)	1.89 (0-5.54)	7.69 (0-22.18)	0	0	0	0.5952

*Chi-square test. HPV: Human papillomavirus, CI: Confidence interval, CIN: Cervical intraepithelial neoplasia

distribution were presented as mean ± standard deviation and compared using paired t-test, whereas Chi-square test was employed for dichotomous data. Mann–Whitney U test was performed for variables without normal distribution. Categorical variables were presented as frequencies and percentages. Factors influencing the HPV were analyzed logistic regression test. Risk was also evaluated by the prevalence odds ratio and their 95% confidence interval (CI). A $P < 0.05$ was considered statistically significant at 95% CI.

Results

Out of 214, 197 samples were considered for further analysis, as the remaining 17 samples were contaminated. The mean age of the women was 33.93 ± 5.7 years and the median CD4 + count was 468 cells/mm^3 (interquartile range: 344–629). Majority of the women were receiving ART (96.26%). Sixty-nine (32.2%) patients are married and cohabiting with their husbands, 59 (27.6%) were illiterate, 28 (59.8%) reported total family income < 2500 INR monthly. A total of 20.09% of the study participants reported age at first sexual intercourse as 18 years. Twenty-five (11.7%) patients are reported to have ≥2 lifetime sexual partners.

A total of 132 (69.1%) women had normal cervical status, 26 (13.6%) had CIN1 lesions, 1 (0.5%) had CIN2 lesions, 12 (6.3%) had CIN3 lesions, while the rest had inadequate colposcopy. None of the women had invasive cervical cancer.

Out of 197 participants, 86 (43.6%) cases were detected with any HPV genotype; single HPV genotypes were found in 47 (54.6%) and multiple (≥2) HPV genotypes were found in 39 (45.3%) cases. 111 women were negative for HPV infection. The HPV genotypes detected in descending order of frequency were HPV 16, HPV 33, HPV 35, HPV 52, and HPV 58. The distribution of HPV genotypes by cervical status, among HIV-infected women is presented in Table 1. HPV 16 and HPV 72 were the significant genotypes detected in women with CIN lesions as compared to those with normal cervical status [Table 1].

The association of participant characteristics with HPV positivity is depicted in Table 2. By logistic regression model, ever pregnant women or parity was found to be at the significant risk factor of getting any HPV infection. By multivariable logistic regression, the presence of non/un carcinogenic, possibly carcinogenic, carcinogenic, and single and multiple HPV showed no significant association with CIN2+ lesions [Table 3].

Discussion

A high percentage of research indicated that HIV-infected women are at higher risk of developing cervical cancer due to the persistence of immunosuppression. Furthermore, higher prevalence of multiple opportunistic infections and a broad range of HPV genotypes were reported in HIV-infected women.[12] To the best of our knowledge, this cross-sectional study is the largest one to date to determine the prevalence and

Table 2: Risk factors associated with human papillomavirus infection

Factor	Any HPV	Single HPV	Multiple HPV	HPV 16	Non-HPV 16
Age (years)	0.99 (0.94-1.05)	0.97 (0.91-1.03)	1.02 (0.96-1.09)	0.99 (0.90-1.09)	1.01 (0.92-1.11)
Education					
Literate	0.50 (0.02-5.66)	1.57 (0.07-18.25)	-	-	-
Illiterate			Reference		
Occupation					
Salaried	0.90 (0.48-1.72)	0.63 (0.31-1.30)	1.59 (0.71-3.86)	3.30 (0.87-21.69)	0.30 (0.05-1.15)
Housewife			Reference		
Ever pregnancy					
Yes	4.47 (1.01-31.58)	-	1.01 (0.21-7.37)	-	-
No			Reference		
Number of pregnancies					
>3	0.86 (0.39-1.87)	1.14 (0.45-2.73)	0.73 (0.25-1.91)	0.88 (0.18-3.19)	1.13 (0.31-5.43)
3 or less			Reference		
Number of sex partners					
2 or more	1.80 (0.73-4.59)	1.34 (0.47-3.51)	1.41 (0.47-3.83)	2.12 (0.53-7.11)	0.47 (0.14-1.90)
1			Reference		
Use of contraception					
Yes	0.73 (0.37-1.42)	0.89 (0.43-1.90)	0.90 (0.40-2.10)	1.42 (0.45-5.41)	0.71 (0.18-2.22)
No			Reference		
Screened for CC					
Yes	0.96 (0.16-5.53)	1.06 (0.85-1.28)	0.95 (0.68-1.18)	1.11 (0.78-1.35)	0.93 (0.74-1.28)
No			Reference		
STI symptom					
Yes	1.10 (0.52-2.34)	1.30 (0.5-2.93)	0.95 (0.35-2.37)	0.92 (0.20-3.20)	1.08 (0.31-5.11)
No			Reference		
Hist of opportunistic infection					
Yes	1.64 (0.55-5.57)	1.45 (0.42-6.71)	1.83 (0.45-12.39)	-	-
No			Reference		
CD4 (%)	1 (0.97-1.02)	0.99 (0.96-1.01)	1.01 (0.99-1.04)	1.01 (0.97-1.05)	0.98 (0.95-1.03)

*Absence of outcome like any HPV, single HPV is a taken as internal standard, 197 subjects considered, -: Indicates that variables are excluded as there are zero counts. HPV: Human papillomavirus, STI: Sexually transmitted infections, CC: Cervical cancer

Table 3: Relationship of prevalent carcinogenic human papillomavirus genotypes with risk of cervical intraepithelial neoplasia 2+ and cervical intraepithelial neoplasia 3 in HIV positive subjects

Variable	OR (95% CI)	
	CIN 2+ and 3 (versus N)	CIN 2+ and 3 (versus ≤CIN1)
Carcinogenic	4.31 (0.67-84.07)	2.37 (0.29-49.12)
Possibly carcinogenic	2.22 (0.26-14.56)	2.60 (0.27-20.92)
Non/un carcinogenic	0.63 (0.11-3.33)	0.76 (009-5.80)
Single	0.54 (0.09-2.71)	1.22 (0.18-10.49)
Multiple	1.86 (0.37-10.62)	0.82 (0.10-5.69)

*OR are adjusted for age, Number of pregnancy, use of contraception, and CD4. Normal and inadequate CIN is considered as "≤CIN1." OR: Odds ratio, CI: Confidence interval, CIN: Cervical intraepithelial neoplasia

distribution of specific HPV genotypes among HIV-infected women in Belagavi, A distict place from the southwest Indian state of Karnataka.

The present study documented an overall higher prevalence of HPV infection (43.6%) with multiple genotypes indicating that HIV-positive women are indeed at greater risk for the development of cervical cancer. Recent research has demonstrated different findings for HPV prevalence among HIV-infected women; furthermore, the tendency to a higher prevalence among HIV-infected cohort is invariably identified.[6,8] Globally, the estimates of the prevalence of HPV among these cohorts varied by the level of the HIV epidemic and region.[5] This variation of HPV prevalence in various geographical locales could be to differing immunological and behavioral status of the participants, as well as the differences in sensitivity of the assays and primers used for PCR.[14]

In the present study, diverse HPV genotypes were detected in women with both normal services as well as with cervical precancerous disease. This diversified HPV genotypes in HIV-infected women are more frequently associated with the activation or persistence of pre-existing infections, due to reduced immunity.[15] Minkoff *et al*. and Sun *et al*. reported HPV DNA prevalence in their studies as 73% and 60% of HIV-infected women, respectively. Studies conducted in Sao Paulo and Brazil also showed 98% and 100% of HPV among HIV-infected women, respectively.[16-19]

In the present study, HPV 16 was the commonest genotype found among HIV-infected women, followed by HPV 33, HPV 52, and HPV 58. Similar to our study findings, Mane *et al*.[15] also reported that HPV 16, HPV 52, and HPV 58 as the most commonly encountered genotypes in their study conducted in HIV-infected women in the Western part of the country.[14] In contrast, Luque *et al*. and Badial *et al*. reported a greater prevalence of other genotypes among HIV-positive women.[20,21] Generally, HPV 16 has good evolutionary ability to escape the immune surveillance effects, while genotypes other than HPV 16 are often controlled by immunity.[22,23] Research also indicated that increased HPV prevalence are in direct proportion with the severity of immunosuppression.[14] We found that parity as the only factor is significantly associated with any HPV infection. This can be attributed to the changed hormonal milieu due to pregnancy and immune response might favor the presence or persistence of HPV infection.[24,25] According to Liu *et al*., 2018 HIV-positive women have higher risk of acquiring HPV, with risk inversely associated with CD4 cell count.[26] According to Levi *et al*., 2002 HIV-positive women have higher risk of acquiring HPV, with risk inversely associated with CD4 cell count.[19]

Our study had its limitations, and first we did not consider the ART duration for further evaluation of differences between the immunocompetent and immune-replete status of women on ART. Second, we did not follow-up the patients after hospital discharge. Also, we acknowledge that we will not be able to generalize the findings due to the small sample size and single-centered nature of the study, which emphasizes the need for larger and prospective cohort studies further to elucidate the association of immunosuppression with HPV risk. Our study definitely provide incident data, however, it requires further screening at primary health centers to have the effective study of incidence and/or prevalence

Conclusion

We observed a high prevalence of HPV infection among HIV-positive women from Belagavi, India, with a wide diversity of HPV genotypes and a greater prevalence of HPV 16. Being pregnant (parity) was the only independent factor associated with HPV detection, warranting further studies to determine the correlation with pregnancy outcomes. In Cosmo Politian cities/3 tier cities there is a gap in the knowledge which might be essential for the policymakers, and also present publication might help treating clinicians towards incidence data. The present finding is also having relevance to cancer prevention programs early screening of the HPV can definitely help in early diagnosis and treatment, which in turn might reduce mortality. However, our study might not directly associated with HVP vaccine.

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Conflicts of interest

There are no conflicts of interest.

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Detection of human papillomavirus genotypes in human immunodeficiency virus-negative women in Belagavi, Karnataka

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Abstract:

BACKGROUND: Women are known to be at high risk of human papillomavirus (HPV) infection and its associated cervical pathology. However, limited data are available on circulating genotypes, especially among human immunodeficiency virus (HIV)-negative women. Therefore, the present study was designed to gauge the prevalence of HPV genotypes in HIV-negative women.

MATERIALS AND METHODS: This cross-sectional study was conducted on a total of 96 HIV-negative women in the age range of 18–45 years. Cervical samples were collected from the transformation zone of the cervix using the deoxyribonucleic acid collection device (cytobrush), which was rinsed in 20 ml of PreservCyt® vial (Hologic, Inc.). HPV genotyping was done with the linear array HPV genotyping test (Roche, Branchburg, NJ, USA) at National Aids Research Institute, Pune.

RESULTS: A significant linear increasing trend in proportion of carcinogenic and noncarcinogenic genotypes over grade was observed ($P = 0.039$ and $P = 0.0024$, respectively). HPV 59 was reported to be the most common genotype followed by 16, 53, 62, and 72 but without any statistical significance.

CONCLUSION: Screening strategies incorporating HPV genotyping and vaccination should be effective in preventing cervical cancer in HIV-negative women.

Keywords:

Carcinogens, genotype, *Papillomaviridae*, prevalence, vaccination

Introduction

Human papillomavirus (HPV) is nonenveloped deoxyribonucleic acid (DNA) virus with 7900 bp that infects keratinocytes of the stratified cutaneous and mucosal epidermis. HPV is classified into more than 200 genotypes, out of which more than 50 genotypes are associated with anogenital infections, and these genotypes are further subdivided into two broad groups as high-risk (HR) and low-risk (LR) genotypes based on its oncogenic potential[1,2] as well as cutaneous and mucocutaneous based on tissue tropism.[3]

HPV infection is identified as one of the most common sexually transmitted infections worldwide along with human immunodeficiency virus (HIV) and syphilis. Certain genotypes of HPV are also identified as the leading cause of cervical cancer, which is the second most common cancer in middle-aged women. HPV genotypes have been associated with the development of several types of squamous epithelial cell tumors such as cervical, vulvar, anal, penile, and oropharyngeal cancer representing 6% of cases worldwide.[4,5] The most common HR-HPV is 16 and 18, and approximately 70% of cervical cancer cases are due to these genotypes. LR-HPV, especially 6 and 11, are

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predominantly involved in the development of genital warts.[6]

Cervical cancer prevention programs consist mainly of cervical cancer screening by Papanicolaou (Pap) smear for early detection of precancerous lesions.[7] Although screening programs are in place and effective, cervical cancer remains a burden for any nation due to a large proportion of women who remain unscreened or unaware, as well as false-negative Pap test results due to poor sensitivity of the test.[8-10]

With the recent advancement in HPV vaccine introduction, there is a need for knowing the genotypes prevalent in the community.[11] Since the sensitivity and specificity of Pap smear are poor, cervical cancer screening programs are switching from cytology-based screening to HR-HPV testing. Therefore, PCR testing is a sensitive and noninvasive method for determining the presence of a cervical infection since HPV genotyping is important to determine the presence of HR types. However, limited data are available on circulating genotypes, especially among HIV-negative women.[12]

In light of the above knowledge, the present study was designed to gauge the prevalence of HPV genotypes in HIV-negative women in Belagavi, Karnataka, and investigate the types of HPV most frequently found in these patients.

Materials and Methods

Study design

This cross-sectional study was approved by the Human Research Ethics Committee of the KLE University, Belagavi, India, in June 2013. The study participants included all HIV-negative women aged from 18 to 45 years (reproductive age), attending department of obstetrics and gynecology. Women were excluded if they had never been pregnant, had a hysterectomy, were in active labor, had abnormal bleeding, had history of sexually transmitted disease, or were unwilling to participate in the study. The study was explained to patients in their local language or English. The informed consent was obtained from all the study participants after providing the study fact sheet. A total of 96 HIV-negative women of age ≥19 years were included in the study.

Data and sample collection

Demographic data of all the participants were collected and recorded in a structured questionnaire, which was approved by the ethics committee through interview by a gynecologist. After collecting data and informed consent, participants were subjected to routine gynecological examination which included general, pelvic, and speculum examination and sample collection. Based on the data provided by the participants, they were counseled on risk of sexual behavior and provided with contraceptives if needed. Return appointments and referrals were made as required by the gynecologist.

Samples for Papanicolaou test

The smears were collected with the help of Ayer's spatula and cytobrush from the squamocolumnar junction by following the American Society of Cytopathology guidelines. The cellular material obtained was quickly smeared on a clean grease-free glass slide. Two smears were prepared for each case. The glass slides were then fixed by using 95% ethyl alcohol. The smears were stained with Pap stain. Slides were examined under light microscope and were reported by a pathologist according to the 2001 Bethesda System.[13-15]

Samples for human papillomavirus genotyping

Cervical samples were collected from the transformation zone of the cervix using the DNA collection device (cytobrush) which is rinsed in 20 ml of PreservCyt® vial (Hologic, Inc.). Samples were immediately stored at 4°C and transported to the laboratory at the department of microbiology where they were further stored at −20°C until DNA extraction.

Human papillomavirus genotyping

HPV genotyping was done with the linear array HPV genotyping test (Roche, Branchburg, NJ, USA) at National Aids Research Institute (NARI), Pune.

Total DNA was extracted from cervical samples collected in PreservCyt medium solution and purified using the AmpliLute Liquid Media Extraction Amplicor kit, following the manufacturer instructions (Roche Molecular Diagnostics, Branchburg, NJ, USA). All samples were genotyped by Linear Array HPV Genotyping Test as previously described.[15,16]

Statistical analysis

Data analysis was done using R i386.3.5.1. R Foundation for Statistical Computing Vienna, Austria statistical software z. Continuous data were represented in the form of mean ± standard deviation, and the categorical variables were represented by the frequency table. Trend analysis was done using Cochran Armitage trend test. Crude odds ratio was used to study the factors influencing the HPV. A $P \leq 0.05$ was considered statistically significant.

Ethical clearance

Institutional Ethical Committee with Ref no KLEU/Ethic/2012-13/D-4573 dated 18.03.2013.

Results

A total of 96 participants were enrolled in the study of mean age 32.07 ± 6.79 years, consisting of 13 HPV-positive and 83 HPV-negative cases.

Almost 84.38% of women were married of which 14.8% had HPV. Among the participants 74.47% used the contraceptives. The current study reported that age was significantly associated with non HPV 16 genotypes which is contradictory to the study by Teixeira et al. that stated higher HR HPV prevalence in older age groups than in younger age groups (<26 years: 7.3%; 26–30 years: 13.2%; 31–35 years: 11.9%). Condom followed

Table 1: Baseline characteristics

Factor	Subcategory	Overall, *n* (%)	HPV (based on genotype result)	
			Yes (*n*=13), *n* (%)	No[a], *n* (%)
Age group	19-24	14 (14.74)	3 (21.43)	11 (78.57)
	25-29	14 (14.74)	2 (14.29)	12 (85.71)
	30-34	29 (30.53)	4 (13.79)	25 (86.21)
	35-39	20 (21.05)	1 (5)	19 (95)
	≥40	18 (18.95)	2 (11.11)	16 (88.89)
Marital status	Married	81 (84.38)	12 (14.81)	69 (85.19)
	Divorced	7 (7.29)	0	7 (100)
	Widow	3 (3.13)	0	3 (100)
	Not disclosed	5 (5.21)	1 (20)	4 (80)
Number of family members	1-2	5 (5.21)	1 (20)	4 (80)
	3-4	45 (46.88)	7 (15.56)	38 (84.44)
	5-6	32 (33.33)	3 (9.38)	29 (90.63)
	≥7	14 (14.58)	2 (14.29)	12 (85.71)
Education	Literate	88 (91.67)	11 (12.5)	77 (87.5)
	Illiterate	8 (8.33)	2 (25)	6 (75)
Addiction	Yes	12 (12.5)	2 (16.67)	10 (83.33)
	No	84 (87.5)	11 (13.1)	73 (86.9)
Ever pregnant	Yes	87 (90.63)	12 (13.79)	75 (86.21)
	No	9 (9.38)	1 (11.11)	8 (88.89)
Number of pregnancies (among ever pregnant women)	1	26 (29.89)	6 (23.08)	20 (76.92)
	2	38 (43.68)	4 (10.53)	34 (89.47)
	3	17 (19.54)	2 (11.76)	15 (88.24)
	4	6 (6.9)	0	6 (100)
Number of live births (among ever pregnant women)	0	2 (2.3)	0	2 (100)
	1	40 (45.98)	8 (20)	32 (80)
	2	30 (34.48)	2 (6.67)	28 (93.33)
	3	14 (16.09)	2 (14.29)	12 (85.71)
	4	1 (1.15)	0	1 (100)
Age of 1st vaginal intercourse	Minor	7 (7.29)	3 (42.86)	4 (57.14)
	18-22	65 (67.71)	10 (15.38)	55 (84.62)
	23-27	18 (18.75)	0	18 (100)
	28 and above	4 (4.17)	0	4 (100)
	Never	2 (2.08)	0	2 (100)
Number of sexual partners (excluded 2 subjects)	1	89 (92.71)	12 (13.48)	77 (86.52)
	2	5 (5.21)	1 (20)	4 (80)
Use of contraception	Yes	70 (74.47)	10 (14.29)	60 (85.71)
	No	24 (25.53)	3 (12.5)	21 (87.5)
Type of contraceptive (among subjects who used contraception)	Birth control pill	14 (20)	0	14 (100)
	Injectable contraceptive	6 (8.57)	2 (33.33)	4 (66.67)
	Condom	51 (72.86)	9 (17.65)	42 (82.35)
	Diaphragm	0	-	-
	Copper-T	39 (55.71)	6 (15.38)	33 (84.62)
	Others	1 (1.43)	0	1 (100)
Period of using contraception	Month	14 (20)	3 (21.43)	11 (78.57)
	Year	56 (80)	7 (12.5)	49 (87.5)
Use of contra. In last month	Yes	31 (44.29)	2 (6.45)	29 (93.55)
	No	39 (55.71)	8 (20.51)	31 (79.49)
Screened for cervical cancer			-	-
How many times screened			-	-

[a]Genotype result "No" is considered as HPV absent. HPV: Human papillomavirus

by Copper-T was reported as the common type of contraceptive used [Table 1].

It has been observed that out of 13 HPV-positive cases, 7 had Grade 1+, 5 had Grade 2+, 9 had Grade 3+, and 2 had Grade 4+. A significant linear increasing trend in proportion of carcinogenic and noncarcinogenic genotypes over grade was observed ($P = 0.039$; $P = 0.0024$, respectively). HPV 59 was reported to be the most common genotype followed by 16, 53, 62, and 72 but without any statistical significance [Table 2].

Using univariate logistic regression, it was concluded that age was significantly associated with non-HPV 16 genotypes. Using odds ratio, it has been concluded that, for 1-year increase in age, odds of absence of non-HPV 16 genotype increased by a factor 1.14 [Table 3].

Discussion

During the last few years, Pap smear is being replaced with HPV genotyping due to high sensitivity and specificity.

Table 2: Prevalence of human papillomavirus genotypes, overall and stratified by grading among human papillomavirus

Genotype	Overall, *n* (%) (95% CI)	Grading				*P*
		1+ (*n*=7)	2+ (*n*=5)	3+ (*n*=9)	4+ (*n*=2)	
Carcinogenic	12 (52.17) (32.52-71.31)	28.57 (6.47-64.77)	20 (2.25-62.86)	88.89 (58.55-98.77)	50 (6.08-93.92)	0.0396*
Possibly' carcinogenic	3 (13.04) (3.81-30.87)	0	20 (2.25-62.86)	11.11 (1.23-41.45)	50 (6.08-93.92)	0.1485
Noncarcinogenic	8 (34.78) (18.02-55.11)	71.43 (35.23-93.52)	60 (20.94-90.56)	0	0	0.0024*
Carcinogenic HPV genotypes						
HPV 16	2 (8.7) (1.85-25.09)	0	0	22.22 (4.93-54.38)	0	0.268
HPV 59	10 (43.48) (24.99-63.50)	28.57 (6.47-64.77)	20 (2.25-62.86)	66.67 (34.78-89.58)	50 (6.08-93.92)	0.1487
Possibly' carcinogenic HPV genotypes						
HPV 53	2 (8.7) (1.85-25.09)	0	20 (2.25-62.86)	0	50 (6.08-93.92)	0.268
HPV 70	1 (4.35) (0.47-18.58)	0	0	11.11 (1.23-41.45)	0	0.4442
Individual "noncarcinogenic" or "unknown carcinogenic" HPV genotype						
HPV 42	1 (4.35) (0.47-18.58)	14.29 (1.59-50.08)	0	0	0	0.1918
HPV 54	1 (4.35) (0.47-18.58)	14.29 (1.59-50.08)	0	0	0	0.1918
HPV 61	1 (4.35) (0.47-18.58)	14.29 (1.59-50.08)	0	0	0	0.1918
HPV 62	2 (8.7) (1.85-25.09)	14.29 (1.59-50.08)	20 (2.25-62.86)	0	0	0.2542
HPV 72	2 (8.7) (1.85-25.09)	14.29 (1.59-50.08)	20 (2.25-62.86)	0	0	0.2542
CP6108	1 (4.35) (0.47-18.58)	0	20 (2.25-62.86)	0	0	0.7871

*Significant. HPV: Human papillomavirus, CI: Confidence interval

Table 3: Logistic regression analysis

Factor	Any HPV	Single HPV	Multiple HPV	HPV 16	Non-HPV 16
Age (years)	0.92 (0.83-1.01)	0.93 (084-1.03)	0.85 (0.64-1.05)	1.16 (0.95-1.55)	0.88 (077-0.98)
Education					
Literate	0.43 (0.08-3.17)	0.34 (0.06-2.63)	0.49 (0.02-11.09)	0.49 (0.02-11.09)	0.34 (0.06-2.63)
Illiterate			Reference		
Addiction					
Yes	1.33 (0.26-6.88)	1.67 (0.23-7.84)	1.32 (0.06-29.12)	7.55 (0.27-209)	0.67 (0.03-4.13)
No					
Ever pregnancy					
Yes	1.28 (0.15-11.17)	2.86 (0.16-52.46)	0.09 (0.01-1.63)	0.56 (0.02-12.45)	1.04 (0.16-21.21)
No			Reference		
Number of sex partners					
2	1.65 (0.08-12.61)	2.03 (0.21-19.95)		3.25 (0.14-76.41)	2.03 (0.09-15.81)
1 or less			Reference		
Use of contraception					
Yes	1.28 (0.35-6.19)	1.77 (0.35-8.80)	0.36 (0.01-9.81)	1.93 (0.09-41.64)	0.99 (0.24-4.05)
No			Reference		

HPV: Human papillomavirus

Linear array genotyping and PCR testing with several primers were commonly used in many laboratories for HPV detection.[17,18] Linear array test, launched in 2006, is a PCR-based strategy with pooled primer sets, coupled to hybridization to specific probes for 37 anogenital HPV genotypes immobilized on a nylon strip.[19-24]

The prevalence of HPV DNA in the present study was observed to be 13/96, which was in slight contrast with the study conducted by Sun *et al*.[25] with HPV DNA being detected in 36% of negative women, and Minkoff *et al*. found a prevalence of 43%.[26] A study by Veldhuijzen *et al*. showed the prevalence of HR-HPV as 31.8% and LR-HPV as 32.4% in HIV-negative women.[27] A meta-analysis conducted by Women's Interagency HIV Study, 1999 showed prevalence of 28% in HIV-negative women, respectively. Brazilian studies conducted by Campos *et al*.[28] found HPV DNA prevalence in 23.7% of cases. Nevertheless, Levi *et al*.[29] showed 100% of HIV-negative women positive to HPV DNA. In this study, control group women were selected in a cervical pathology clinic, and a high positivity was expected to HPV DNA.

Among the patients in our study who were positive, HPV DNA type 59 and 16 were most common in 10 cases, whereas in a study conducted by Queiroz *et al*., the most common genotypes found were HPV 16 and HPV 52.[22] In our study, most of the women had infection with single genotype, and this was in concordance with several studies carried out among HIV-negative women. Findings of several studies resonated with the findings of the present study, thus concluding that HIV-negative women were less prone to getting HPV infection.[22-24]

A study by Clifford *et al*. and Sahasrabuddhe *et al*. found that the prevalence of HPV 16 was lower in the general population and that other high-risk types (e.g., HPV 18, 31, 33, 51, 52, and 58) were prevalent.[30,31] Many others have also demonstrated that other HPV subtypes, except 16 and 18, are clearly playing a role in HPV prevalence.[32-34] In a study conducted by Rodolfo *et al*., the most common HPV genotype in HIV-negative women was identified as HPV 16.[35]

The current study reported that age was significantly associated with non-HPV 16 genotypes which was not in harmony with the findings of the study by Teixeira *et al*. that stated higher HR-HPV prevalence in older age groups than in younger age groups (<26 years: 7.3%; 26–30 years: 13.2%; 31–35 years: 11.9%) but no trend with age was found ($P = 0.10$). These contrasting results could be due to variation in the study design.[36]

To our knowledge, our study is the first of its kind in our geographical location to know the genotypes circulating in HIV-negative women as very few prospective studies on HPV epidemiology in HIV-negative women have been conducted.

The study, however, had few potential limitations as well. First, the FDA has approved vaccines, which were not effective against the most common genotypes found in our study. Second, further studies with larger population are required to identify the most common genotypes prevalent in our geographic location so as to use vaccine in prevention of HPV diseases.

Conclusion

Newer vaccines such as the nonavalent HPV vaccine present the possibility of better coverage for women and will need to be evaluated. Strengthening preventive efforts is necessary to improve early detection through increasing accessibility to screening programs, adherence to follow-up among those with lesions, and intensifying health education for women living with HIV.

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Conflicts of interest

There are no conflicts of interest.

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