CLINICAL SPECTRUM OF CERVICAL CANCER AND ITS CO-RELATION WITH HUMAN PAPILLOMA VIRUS IN JAMMU REGION

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DECLARATION

I, hereby, declare that the presented work in the thesis entitled "**Clinical Spectrum of Cervical Cancer and its Co-relation with Human Papilloma Virus in Jammu Region**" in fulfillment of the degree of **Doctor of Philosophy (Ph. D.)** is the outcome of research work carried out by me under the supervision of working as **Dr. Reena Singh**, Associate Professor, **School of Bioengineering and Biosciences**, Lovely Professional University Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgments have been made whenever the work described here has been based on the findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

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ABSTRACT

In recent years, the incidences of cervical cancer and mortality due to it have seen a declining trend signifying that women can successfully evade it. However, its global burden is highly skewed towards poor countries primarily due to a lack of awareness, poor screening, unavailability, and low uptake of prophylactic vaccines. In a developing country like India, the incidences of cervical cancer are among the highest among Asian countries. The epidemiological factors linked with cervical cancer in Jammu need due investigation so that the disease can be successfully averted. HPV is among the pivotal and foremost risk factors for the development of cervical cancer. Various other co-factors like smoking, early marriage, age at pregnancy, number of children delivered, use of oral contraceptives, socio-economic conditions, prior exposure to sexually transmitted infections, and vaginal bleeding, among others augment the probability of getting HPV infected and thereby progression into cervical cancer.

The present work was carried out to study the incidence of cervical cancer in the Jammu region. The epidemiological factors were also studied to assess their role and association with HPV in cervical cancer development. Patients were screened using cervical cytology and HPV tests. It was observed that the combination of cervical cytology and HPV testing yielded more accurate predictions compared to individual cervical cytology or HPV testing alone. Cervical cancer has regularly been linked to HPV as the primary cause. This virus, which is one of the risk factors for the development of cervical cancer, is spread through sexual contact. Certain high-risk HPV strains can cause cellular changes in the cervix after persistent infection, which raises the risk of cancer. Because of the close association of HPV with cervical cancer, it is crucial to take preventive steps like getting vaccinated against HPV, and getting frequent cervical screenings to prevent and treat the virus timely, and lower the chance of developing cervical cancer. The incidence of both HPV strains decreased from 2019 to 2020, again increasing in 2021 and then again decreasing in 2022. A similar trend was observed in the case of cervical cancer. The annual incidence of HPV16/31 in 2022 was 30 cases per 1000 females while the same for HPV 18/45 was 14 cases per 1000 females. The incidence rate of SCC in the year 2022 was 2 cases per 1000 females. The prevalence of the 16/31 strain in our study was higher (3. 88%), compared with the 18/45 strain which exhibited a lower prevalence of 1. 65%. The prevalence rate of Squamous cell carcinoma (SCC) was found to be 0. 18%, which means that a smaller subset of the population, roughly 18 out of every 10,000

people was diagnosed with SCC. The distribution of HPV infections by viral load categories (high, medium, low, and very low) showed the highest prevalence in the 36–45 age group, accounting for 36. 95% of cases. Conversely, the lowest prevalence of HPV infection (19. 21%) was observed in the 25–35 age groups. Among women aged 46–55 and 56–65, the prevalence of HPV infection was 22. 17% and 21. 67%, respectively. 18 % of the population had a high viral load (Ct<20), while 11% of patients had a low viral load (25≤Ct<30), indicating a low relative concentration of HPV viruses. 41% of patients were detected with medium viral load (20≤Ct<25) while 30% of the patients were detected with very low viral loads Ct ≥30, suggesting that HPV infections with moderate to low viral presence are prevalent which subdue gradually in due course of time. It was concluded that the risk of high-grade cervical lesions increased with an increase in high-risk HPV viral load. It is suggested that the combination of cervical cytology and PCR-based HPV testing can be adopted as a regimen in cervical cancer screening programs to reduce the burden of this disease in the Jammu region. We believe that cervical cytology alone is not efficient in the detection of cervical cancer as it can give false positives and negatives. It must be complemented with PCR-based HPV testing to improve the efficacy of diagnosing cervical lesions. In a low to medium-income country like India, it is not feasible to immediately shift from cytology-based screening to PCR-based HPV screening but gradually India should adopt PCR-based screening as a primary screening method for cervical cancer. It may be further complemented with immunohistochemistry (p16) -based markers or colposcopy for meticulous detection of cervical cancer and to reduce cervical cancer burden in India.

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Signature

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ABBREVIATIONS

ACOG = American College of Obstetricians and Gynecologists

ACR= American Cancer Society

ASC- Atypical squamous cell

ASC-H= Atypical squamous cells exclude high-grade lesions

ASCUS= Atypical Squamous Cells of Unknown Significance

ASR= Age-standardized mortality rates per 100, 000

CIN=Cervical intraepithelial neoplasia

DNA= Deoxyribonucleic Acid

HIV= Human Immunodeficiency virus

HPE= Histopathological examination

HPV= Human Papillomavirus

HR HPV= High-risk HPV

HSIL= High-Grade Squamous Intraepithelial Lesion

ICMR= Indian Council of Medical Research

LBC= Liquid Based Cytology

LSIL= Low-Grade Squamous Intraepithelial Lesion

OPD= Outpatient Department

PAP= Papanicolaou

PCR= Polymerase Chain Reaction

SCC=Squamous Cell Carcinoma

SCJ= Squamocolumnar Junction

STD= Sexually transmitted diseases

TZ= Transformation zone

VIA= Visual inspection of the cervix with acetic acid

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Chapter 1

INTRODUCTION

Cervical cancer was estimated to be the fourth most common cancer and the fourth leading cause of death for women in 2020, accounting for 3,42,000 deaths and 6,04,000 new cases globally (Cohen *et al*., 2019; Sung *et al*., 2021). There is a noticeable gap in mortality and prevalence between high-income countries (HIC) and low- and middle-income countries (LMIC), therefore, it is also referred to as a "disease of disparity" (Hull1 *et al*., 2020; LaVigne *et al*., 2017). The utmost propitious trait of cervix cancer is that it can be averted. Yet, it causes significant morbidity and mortality among women who are socially and economically deprived. According to WHO, cancer of the cervix comprises 12% of all cancers in women (Bray *et al*., 2018; Brisson *et al*., 2020). The overall incidence rate of cervical cancer is 14.1 and the mortality rate is 7. 1(Chen *et al*., 2023; Ferlay *et al*., 2024). The World Health Organization (WHO) reports that 70% of the instances of cervical cancer are linked to the human papillomavirus (HPV), which is considered a global burden (Williams, 2020). In LMICs, cervical cancer claims the lives of about nine out of ten women (Stelzle *et al., 2021*; Zhang *et al.,* 2021). The Indian scenario is even more horrendous with an incidence rate of 17.7 and a mortality of 11. 2. It is the second most frequent type of cancer among women in India and other low- and average-income nations (Ault, 2006). National Centre for Disease Informatics and Research (NCDIR) and National Cancer Registry Programme (NCRP) in India deal with systematic data collection from various population and hospital-based cancer registries. However, recent years have seen a slightly elevated incidence of breast cancer, while cervical cancer showed a slightly declining trend (Mathur *et al*., 2020; McLaughlin-Drubin ME, 2008). Out of the repertoire of causes, Human Papillomavirus (HPV) dissemination through sexual contact is the principal cause of this disease (Bontkes *et al.*, 2000). Most HPV infections are asymptomatic and only 1 in 10, 000 women advance to cervical cancer. Women in the age group 20 to 79 are at a lifetime risk of 79% of developing HPV infection (Asiaf *et al*., 2014; Kaku *et al*., 2008; Reid & Scalzi, 1985). Various studies have shown that 5% of sexually active women develop HPV infection at least once throughout their lifetime and thus have an increased likelihood of cervical cancer development (Stanley,2001)

The Cervix of the uterus is located at the junction of the uterine corpus and vagina and is a portion of the female reproductive system. It comprises fibro muscular tissue that includes columnar and stratified non-keratinizing squamous epithelia. The transformation zone of the cervix is particularly susceptible to HPV infection and in some cases initiates the progression of cervical neoplasia (Sanjose *et al.*, 2019). Cervical intraepithelial neoplasia (CIN) is a pre-cancerous condition that results in the uncontrolled growth or proliferation of cells on the epithelial surface of the cervix. The term Low-grade Squamous intraepithelial lesion is mild dysplasia (CIN 1). The term "high-grade Squamous intraepithelial lesion," or HSIL, refers to a group of abnormalities affecting Squamous cells in the cervix. This includes cervical intraepithelial neoplasia grades 2 and 3, together with moderate and severe dysplasia and malignancies in situ, which were formerly classified as CIN 2 and CIN 3. The presence of specific strains of the human papillomavirus (HPV), a common sexually transmitted infection, is closely associated with these problems (Al-Daraji & Smith, 2009; Alrajjal *et al*., 2021).

1.1 Factors responsible for Cervical Cancer development

Dissemination of the Human papillomavirus (HPV) through sexual intercourse is considered one of the chief and key risk factors for causing cancers of anogenital regions in both men and women (Braaten & Laufer, 2008). People with frail immune systems are at an elevated risk of getting cervical cancer (Fotra *et al.*, 2014). Sexual activeness at a younger age (before 18 years old) puts women at an elevated risk of developing cervical carcinoma (Schiller *et al.*, 2012). Numerous studies have indicated that women using oral contraceptives for a long time place themselves at a higher risk (Al-Daraji & Smith, 2009). Lack of early screening, Low socioeconomic background, Childbirth at an early age, early pregnancy, Chronic inflammations, Multiple pregnancies, Multiparous, and Smoking (Louie *et al.*, 2009;.

1.2 Genetics of HPV

Papillomaviruses are non-enveloped DNA viruses without an envelope consisting of 8000 bp long circular DNA. The genome includes early genes (E1 to E7), long control regions (LCR), and late genes (L1 and L2). The early genes form the proteins that are required for viral replication, proliferation, and oncogenesis while late genes produce the structural proteins of the HPV. E1 and E2 proteins are mainly liable for replication of viral DNA and transcriptional

regulation. E2 plays a vital role in gene amplification by producing two types of proteins one is the enhancer, and the other is the suppressor that regulates the transcription. The E4 protein manages the maturation and release of the virus by disrupting the cytoplasmic cytokeratin network in human keratinocytes (Doorbar, 2013). E5 proteins have a role in cell transformation and are also found to interact with several transmembrane proteins, like the epidermal growth factors. E6 and E7 proteins have a significant role to play in cell transformation and cell malignancy. E6 causes proteolysis of tumor suppressor protein p53. Decreased levels of p53 disrupt the normal cell cycle and its regulation in the infected cells. E7 protein binds with the tumor suppressor gene (one pRb causing the dissociation of the ERF-1 –pRb complex thereby realizing ERF-1. pRB and p53 are tumor suppressor proteins that suppress tumor growth via various downstream pathways such as PI3K/Akt pathway). Disruptions in the protein level of these proteins result in dysregulation of the normal cell cycle and uncontrolled cellular growth, (Jefferies & Foulkes, 2001; Morshed *et al*., 2014). E6 and E7 promote longevity of the cell cycle, leading to instability and eventually cervical cancer (Yim *et al.*, 2005) (Figure 1. 1).

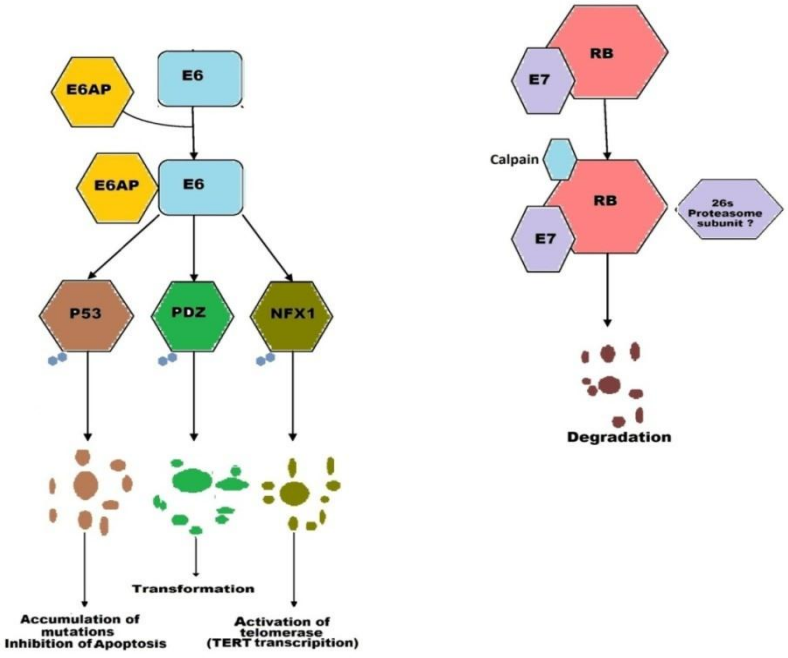

Figure 1.1: Malignancy by HPV with the combined action of the E6 and E7 oncoproteins.

1.3 Types of HPV

The human papillomavirus (HPV) represents a genus of the family papillomaviridae. Hundreds of papillomaviruses have been recognized and sequenced. The HPV is divided into two basics i.e., high-risk viruses, and low-risk viruses. This categorization of HPV is dependent upon its ability to cause cervical cancer. There are around 15 high-risk HPV types (15, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73). High-risk HPV causes various other categories of invasive cancer like anal cancer, oropharyngeal cancer, vaginal cancer, cancer of the vulva, and penile cancer as they are oncogenic. (Bontkes *et al.*, 2000, Srivastava *et al.*, 2012) **.** Low-risk HPV are non-oncogenic viruses. People infected with low-risk HPV are at a reduced risk of developing cancer. Various types of low-risk HPV include: 6, 11, 42, and 44. Low-risk types cause warts or lesions in the genital areas.

1.4 HPV Transmission

HPV infection is an ailment that is transmitted sexually. It is mainly transmitted through direct sexual contact, scratches, scars on genital areas and the mucous membrane of the infected partner, and indirect contact through hands.

1.5 Symptoms

HPV infections resulting in precancerous lesions are devoid of any significant detectable clinical symptoms. However persistent HPV infection may show symptoms like pain in the lower abdomen (PLA), irregular bleeding, post-menopausal bleeding, vaginal discharge smell (VD), bleeding between menses, post-coitus bleeding (PCB), etc (Mwaka *et al*., 2016; Skinner *et al*., 2016)**.**

1.6 Cervical Cancer Detection

The Papanicolaou test, also named the Pap smear test is the benchmark in cervical cancer screening (Ma *et al.*, 2012). Abnormal Pap results are indicative of HPV infection and can further be confirmed by an HPV test. Pap smear suffers from several shortcomings like high false-negatives, subjective interpretation, less sensitivity, and low predictive value, as about 33% of women who developed cervical cancer exhibited a normal Pap smear (Bobdey *et al.*, 2016; T. Y. K. Lim *et al*., 2019). As such HPV HPV-based detection by molecular methods represents a significant advancement in cervical cancer diagnosis. The correlation aimed HPV positivity and cervical cancer development can be an important marker in envisaging the prognosis and overall

survival of cervical cancer patients and thereby developing a personalized regimen for such patients. Several molecular biology techniques have been used for the identification of HPV. So far virus sequences have been cloned which are used as probes in HPV detection. Real-time PCR is also employed to identify the amount of HPV in cervical biopsies. Real-time PCR used in the analysis of cervical smears predicts the presence or development of high-grade cervical lesions based on the quantity of high-risk HPV in the cervical smear.

1.7 HPV treatment

Treatments like cone biopsy, loop electrosurgical excision technique (LEEP), or other surgical interventions may be suggested to remove aberrant cells and stop the development of cervical cancer, depending on the severity of the abnormalities (Khan & Smith-McCune, 2014; Ogilvie *et al*., 2018). The prophylactic vaccine is currently the finest tactic for the efficient management of cervical cancer however they are ineffective against previously established infections.

As per the 2011 census majority of the population of Jammu region are Hindus (67. 5%), Muslims comprise approximately 30% of the population while Sikhs and Christians are 2% and 0. 3% respectively (https://indianexpress.com/article/explained/share-of-muslims-and-hindus-in-jk-population-same-in-1961-2011-censuses/). Many studies have reported the incidence and prevalence of cervical cancer in the Kashmir region but no concrete report regarding the incidence and prevalence of cervical cancer in the Jammu region is available so far. Singh and his coworkers in 2022 showed that Jammu and Kashmir had the lowest incidence of cervical cancer all over India from 1990 to 2019. Jammu and Kashmir have also recorded the lowest mortality throughout India from 1990 to 2019. J&K also recorded the lowest percentage decrement from 1990 to 2019 indicating that there is no significant decrease in incidence and mortality rates during this period (Figure 1. 2). In a similar study on the epidemological factors associated with cervical cancer Fotra and his coworkers in 2014 showed that the incidence of cervical cancer was high in Hindu women (61. 8%) as in contrast to Muslim women in which incidence was found to be 26. 2% (Fotra *et al.*, 2014) .

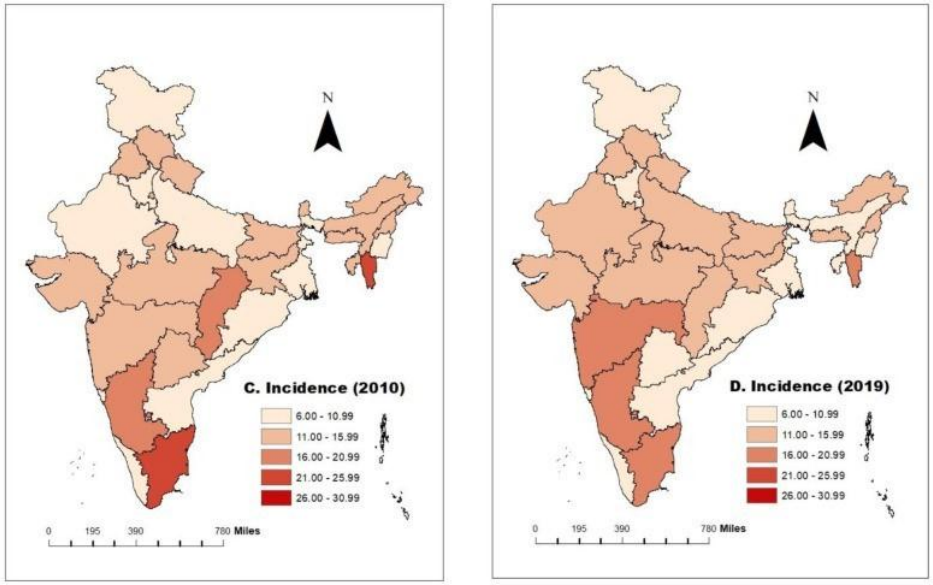

Figure 1.2: Cervical cancer incidence per 100000 women in India. (C). Incidence of cervical cancer per 100, 000 women in 2010. (D) Incidence of cervical cancer per 100, 000 women in 2019.

Khajuria and his team workers in the 2021 study assessed the knowledge and awareness regarding the cervical cancer risk factors among the educated youth of Jammu City. It was found that most of the students 81. 7% were aware of cervical cancer while 73. 1% thought they couldn't be affected by this disease as it affects only married and elderly women. Only 39. 2% of people were aware that cervical cancer is transmitted through sexual contact while about 63% of people knew the importance of regular screening for early and timely detection of cervical cancer. In light of the above studies, it is clear that there is no clear picture of cervical cancer in the Jammu region and there are few studies available assessing the role of various risk factors associated with cervical cancer in this region.

Thus, it is highly desirable to study the present status of incidence and prevalence of cervical cancer in the Jammu region and study various sociological factors associated with its incidence to design a robust approach that can be employed by the local administration to sensitize women on how the spread of cervical cancer can be averted by following the regular screening, maintaining personal hygiene, easy lifestyle changes and prophylactic vaccination**.** The area under study is a typical traditional society, strongly adhering to rudiment social norms with low hygiene and sanitation awareness among women.

Chapter 2

REVIEW OF LITERATURE

Cervical Cancer causes a substantial number of death and disease among women, just behind breast cancer. The worldwide load of cervical cancer is highest in lower-middle-income (LMI) and low-income countries, with about half of the emerging cervical cancer reports around the world occurring in women residing in these countries. These potential disparities in the global distribution of cancer of the cervix are further widening as recent interventions like cervical cancer screening programs and human papillomavirus vaccines are in vogue in developed countries. More than 85% of high-income countries have launched HPV vaccination while only 30% of LMI countries have such measures. A similar scenario has been observed for screening of women having cervical cancer as only 20% of women in LMI countries came for screening while in high-income countries more than 60% of women had cervical cancer screening**.** Despite their efficiency and cost-effectiveness in cancer therapeutics, these disparities in HPV vaccination uptake and screening persist in LMIC and high-income countries. Various randomized clinical trials established HPV vaccines to be safe and highly effective (vaccine efficacy ≥93%) against precancerous cervical lesions and persistent vaccine-type infections among women.

Various cancer registries throughout India were analyzed for trends, patterns, incidence, and mortality arising out of cancer from 2012-2016. It was observed that lung, mouth, esophagus, stomach, and nasopharynx cancers were prevalent in men, with lung cancer as the principal type in the southern region and metropolitan cities, while oral cancer was the prominent type in the Central and western areas. Males of the northeast region of India showed a higher incidence of stomach cancers, esophagus, and nasopharynx. Oral cancer and lung cancer are most frequent among Indian subcontinent males. Among Indian women, breast and cervical cancer were the most prevalent type. Metropolitan cities exhibited the highest incidence of breast cancer with an overall increasing trend whereas cervical cancer showed a slight decline. The maximum age-adjusted cancer incidence among males was observed in the Aizawl district (269. 4) while for females in the Papumpare district (219. 8). Both sexes exhibited an overall increase in cancer incidence rate with Kamrup urban exhibiting the highest annual percent change, (3. 8%). For the

year 2020 anticipated number of patients having cancer in India approximates 14 lakh, with lung, breast, cervix uteri, mouth, and tongue being the most prevalent sites of cancer development.

For more than a century, cervical cancer was believed to be linked with 'Sexual behavior', indicating the involvement of some kind of infectious agent transmitted sexually. Rigoni-Stern in 1842 was the first to observe that cervical cancer was generally seen in married women and virtually absent in catholic nuns. Subsequent epidemiologic studies reported that several first sexual intercourses at an early age, number of sexual partners, low socioeconomic background, and cigarette smoking enhance the threat of cervical neoplasia in women. For this reason, investigators focused on etiologic agents that might be passed by intimate sexual contact. Human papillomavirus has emerged as a primary sexually transmitted infectious agent responsible for cervical cancer development in women (Griffiths *et al*., 1991). The Papillomaviruses are known to cause squamous epithelial warts and lesions. Richard E. Shope was the first to isolate papillomavirus from warts present in the cottontail rabbit. Consequently, many papillomaviruses were isolated from different animals and humans. The group of Papillomavirus causing infection in humans is known as Human papillomaviruses. The human Papillomavirus taxon comprises several viruses belonging to the family of small DNA viruses, named, Papillomaviridae. Syrjanen in 2017 reported the existence of HPV in the nucleus of dysplastic squamous epithelial cells, particularly in those cases that had koilocytotic features. These observations were subsequently confirmed by other workers, and it was suggested by Zur-Hausen and Geissman, 1980 that the virus might be etiologically important in cervical cancer. Using electron microscopy and antibodies to the HPV capsid protein, several investigators identified the viral particles or HPV-related antigens in cervical intraepithelial neoplasia (CIN). These observations strongly implicated HPV as a possible etiologic agent in cervical neoplasia and led to a rush of molecular and clinical studies of HPV and its related lesions. For many years, it was believed that only one type of HPV was present but later it was proved by Villiers, 1989 who described sixty-five HPV DNA types associated with epithelial cancers of different organ sites in humans (Villiers, 1989). Papanicolaou-stained (Pap) smear still exists as the key method for detecting high-risk HPV. It scans for cellular changes in the cervix`s transformation zone, often caused by HPV. Cytological, HPV infection is typified by the presence of a "KOILOCYTE". A koilocyte is a cell of intermediate and parabasal layer, oval or rounded showing the perinuclear clearing of cytoplasm and thickened and folded cytoplasmic borders.

The current Bethesda System used for Pap smear reporting was first used in 1988**.** It presented an advanced insight into cervical neoplasia and a consistent diagnostic histologic terminology. Bethesda System categorizes abnormalities of the squamous cell into four groups: (i) Squamous cell Carcinoma (ii) ASC (atypical squamous cells), (iii) LSIL (low-grade squamous intraepithelial lesions), (iv) HSIL (high-grade squamous intraepithelial lesions). The ASC category has been further classified into two subcategories: the "atypical squamous cells of undetermined significance (ASC-US) " which consist of lesions having cellular irregularities indicative of SIL, and the atypical squamous cell. The existing LSIL and HSIL groups in the earlier classification of the Bethesda System were reserved. LSIL comprises mild cervical intraepithelial neoplasia and additional HPV-related lesions normally supposed to be a result of temporary HPV infection. HSIL comprised moderate to advanced cervical intraepithelial dysplasia and carcinoma in situ. Certain lesions of the HSIL group that were doubtful for invasion and inconclusive were reported as "HSIL, cellular features suspicious for invasion".

Regular screening and timely detection are bulwarks in cervical cancer prevention as early detection predicts a better prognosis. Cytology is established as a primary and traditional measure in the diagnosis of invasive and dysplastic uterine cervical lesions. It is very important to educate nursing staff about cervical cancer so that they are cognizant of the general public knowledge of cervical cancer and its prevention. Pap test has been established as a benchmark for cervical screening platforms. A Pap test when complemented with an HPV DNA testing augments the sensitivity and effectivity of cervical pathology. Pap smear testing is a simple, cost-effective, and efficient means for the identification of precancerous cervical epithelial lesions. Governments around the globe have recognized it as a primary screening method for reducing the occurrence, morbidity, and mortality owing to cervical cancer. A study by Sachan *and* his coworkers showed the efficiency of the Pap test in cervical cancer detection among multiparous women in the age range of 30–50 years. It was observed that vaginal discharge was the primary symptom among the women turning out positive for HPV infection occurring in 36. 96% of the women followed by abdominal pain in 25. 63% of women and irregular menses in 12. 78%, while 15. 15% of women were asymptomatic. 42. 66% of women had an infection or inflammation and 48. 84% exhibited a negative Pap smear test for malignancy. ASCUS identified in 2. 90% LSIL in 5. 09% and HSIL 0. 48% women, respectively. The study concluded that cervical cancer generally thrives in women aged between 40-50 years with its

precursor lesion generally appearing 5-10 years prior. Thus women must undergo no less than one Pap smear test before 45 years of age, which if abnormal should be complemented with colposcopy and guided biopsy. However, Pap test results are not very reproducible, further, these tests need to be frequently repeated as per the guidelines available to the pathologist. Therefore, the conventional Pap smear cannot alone fulfill the expectations of patients and clinicians. Several reports have shown that a significant number of false negatives appearing in Pap smear tests were later found to be positive in HPV tests.

Several second-generation tests for HPV DNA detection are used in epidemiological studies of carcinoma of the cervix in tandem with a Pap smear. PCR-based DNA detection employs the use of various DNA probes to assess the presence of HPV DNA in test samples. With the use of appropriate primers, PCR amplifies DNA up to a million fold and thus is capable of detecting small amounts of HPV DNA. The advantage is that it not only detects the HPV presence but HPV type can also be known to stratify the HPV infection into high or low-risk types based on the type detected. PCR offers the advantage of increased sensitivity and specificity however it requires greater skill than a Pap smear and also suffers from false positivity. However, if both the Pap and HPV tests appear negative, then the probability of developing cervical cancer is almost NEGLIGIBLE. HPV-plus-Pap testing combined helps pathologists with more sensitive reporting and bolsters women that "negative is negative" with greater certainty. Cytology is easily available throughout India, but in a country like India, it is not feasible to go for population screening as the patients do not comply with the pathologist's advice for frequent and repetitive screening at regular intervals. By using one of these methods, incidences, factors for HPV infection and correlation of Pap smear findings have been studied previously by many investigators. Villa and his coworkers 2006 described the PCR procedure using crude cell suspensions as an easy, sensitive, specific, and rapid detection method suitable for large HPV-screening programs as compared to Fluorescence in situ hybridization and Southern Blot analysis (Villa & Denny, 2006). Other screening methods and detecting HPV include cytology (Pap smear), immunohistochemistry, HC2 Cobas 4800, etc. The details of the detection methods are shown below (Table 2. 1).

Table 2.1: Various screening and detection methods of HPV.

Test	Probe	Target	Detects/ Advantages	Sensitivity (%)	References
Cytology	Whole cells		LSIL HSIL SCC	53. 3	(S. Zhang *et al.*, 2021; Wright *et al.*, 2015; Burd, 2003)
Immunohistochemistry	p16 Protein	Antigen	HR-HPV E7	85. 7	(Prigge *et al.*, 2017; Lewis *et al.*, 2018)
In-situ hybridization	cDNA, cRNA, synthetic oligonucleotide	RNA, DNA, Tissue	Specific target sequences	83	(Venuti & Paolini, 2012; Jensen, 2014)
PCR-based HPV detection					
HC2	RNA probes	DNA	13 HR-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68)	96. 3	(Venuti & Paolini, 2012)

Cervista	RNA probes	DNA	14 HR-HPV (13 of HC2 and HPV 66) Low sample requirement as compared to HC2	90-95	(Westra, 2014)
APTIMA	Primers	E6/E7 mRNA	14 HR-HPV	95. 3	(Heideman *et al*.,2013)
Cobas 4800	Fluorescent-labeled cleavage Primers	L1 gene of HPV 16 and 18, other 12 HR-HPV	14 HR-HPV, Distinguishes HR-HPV type 16 and 18	95. 2	(Laudadio, 2013)
OncoE6TM	Mouse Monoclonal antibodies	E6 oncoprotein	HR-HPV types	50-70	(Krings *et al.*, 2018; Westra, 2014)
CRISPR-Cas12a technology	RPA-Cas12a	L1 region	13 HR-HPV types	>70	(Gong, 2021)

One of the earliest attempts at establishing a connection between HPV infection and cervical cancer development was carried out in 1991 in a study comprising a cross-sectional study of 467 patients enrolled in university health care. An increasing number of sexual allies, oral contraceptive use, younger age, and people of black descent showed a strong correlation and independent association with HPV positivity. Age at initial sexual contact, erstwhile sexually transmitted disease, and smoking were related to HPV infection, but could not independently predict it. The study concluded the strong association between key risk factors of cervical carcinoma and genital HPV contagion establishing the role of HPV as an etiological factor in the cervical tumor.

A study by Rohan and his coworkers in 1991 analyzed 105 samples with the help of the PCR method to measure the relationship between risk factors for cervical cancer and HPV infection. Women having a past with multiple sexual allies, intercourse during menstrual periods and smoking cigarettes were more prone to HPV infection. The study reported an overall HPV infection incidence of 18%, for HPV6/11 it was 2. 9% and for HPV16/18 it was 10. In India, it was found that cervical cancer showed HPV positivity of 98% in invasive cancer cases and 20% in healthy controls. The most predominant type was HPV 16 while the occurrence of HPV 18 was very low. During the early years, only one type of HPV was believed to be the causal agent of cervical cancer, however, afterward about sixty-five different HPV DNA types were found to be linked with epithelial cancers of different organ sites.

The only type of HPV that decreases with increasing age is HPV type 16. Ninety percent of HPV types can be prevented by vaccination against common HPV types. Among squamous-cell carcinomas positive for HPV DNA, HPV 16, 18, 31, 33, 35 45, 52, and 58 accounted for more than 95%. Among various types of HPV, more than 30 have been reported to infect the genital tract. Although a strong correlation between oncogenic (high-risk) HPV strains and cervical cancer is well documented HPV only is not adequate for the alteration of cervical epithelial cells. An array of cofactors and molecular phenomena will determine the eventual transformation of HPV infection into cancer of the cervix. Thus early diagnosis and management of precancerous lesions can prevent HPV infection from advancing to cervical cancer (Chan *et al*., 2019).

The study examined the prevalence of various HPV types and their role in invasive cervical cancer development in 10058 cases in different 85 studies. HPV 16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66 and 70 were some of the predominant strains of HPV found in invasive cervical cancer (ICC) in decreasing order of prevalence. HPV 16 and HPV 18 types were predominantly found in squamous cell carcinoma (SCC) in all regions except Asia where HPV 52 and 58 were increasingly prevalent. HPV presence in ADC was lesser than SCC and HPV 18 followed by HPV 16 and 45 were predominantly found in all regions. HPV 16 and 18 were connected with most of the ICC cases in all regions in all regions however one of the other HPV types was also connected with one-fourth of ICC cases.

Data from eleven case studies from nine countries were used to assess cervical cancer development risk with various HPV types. It was found that in addition to HPV 16 and 18, types 33, 31, 35, 45, 39, 51, 56, 52, 59, 58, 73, 68, and 82 should also be categorized as high-risk or

carcinogenic types. Some HPV types are known to induce warts and lesions which could heal up with time, these types of HPV are called Low-risk (LR) HPV types. Continuous infection of some HPV types results in the growth of precancerous lesions and invasive cancers, and such types are named high-risk (HR) HPV types. High-risk risk-HPV infection is the chief causative agent responsible for the bulk of cases of cervical cancer (Franceschi *et al*., 2003). Syrjanen *and* his coworkers 2004 analyzed the time of the incidence of high-risk HPV infections, rates of acquisition, abnormalities in the pap test, and prognostic factors in 423 females taking part in a screening program in Russia formerly known as the Soviet Union. It was concluded that the gaining of HR HPV infection is considerably age-dependent. An elevated high-risk HPV load of more than 20 relative light units/control values was an independent analysis of the occurrence of atypical Pap smear. Patient category (a sexually transferred disease) and pregnancy could foretell incident HR HPV infection independently. HR HPV infection had a 3-month smaller acquisition time as compared to an atypical Pap smear. An increased load of high-risk HPV type was the lone predictor of pap smear abnormality while having a sexually transmitted disease in a young person also predicted incident HR HPV infection.

The factors responsible for invasive cervical cancer (ICC) were studied in American women subjected to oncogenic high-risk types of HPV in the early 1980s on 235 squamous cell carcinoma cases and 486 controls. They analyzed antibodies produced in response to HPV types 16, 18, 31, 45, and 52. The numbers of sexual companions, oral contraceptive use, and black descent were independent predictors of positivity among controls. The use of condoms was protective. Women exposed to HPV showed that Papanicolaou screening, yeast infection, and black race were considerably related to decreased cancer risk. Smoking exhibited a 2-fold increased cancer risk. History of nonspecific genital infection, low income, and education were some major predictors of increased HPV risk (Shields *et al.*, 2004). The role of abnormal cytologic test results and occurrence of risk factors for HPV infection in adolescent girls (mean age, 16. 1 years). It was observed that 64% of subjects showed cervical HPV. The number of sexual allies, their age, and douching were some of the independent risk factors of HPV infection. Cytologic abnormalities like ASCUS were observed in 20. 9% of subjects, 17. 0% exhibited low or advance-grade squamous intraepithelial lesions and these abnormalities were significantly related to HPV positivity. Although these abnormalities were notably related to HPV detection yet 51. 6% of HPV-positive females had normal cytologic reports. Sowjanya *and*

his coworkers 2005 worked on the distribution and frequency of high-risk HPV strains in normal women and in invasive squamous cell cervical carcinoma in Andhra Pradesh using Digene Hybrid Capture assay and the Roche PCR-based line blot assay. The majority of the types were HPV16, 18, 33, 35, 45, 52, 58, 59, and 73 in about 88% of the squamous cell carcinoma. HR-HPV infection had a prevalence of 10. 3 % in the sample collected by clinicians and 7. 0 % in the self-collected sample in a community-based screening program.

Healthy females with negative pap smears were screened for high-risk HPV strains to identify females with underlying cervical lesions of squamous intraepithelial type. Among females with negative pap results 10% exhibited inflammation and turned positive for carcinogenic. HPV types 16/18 and 155 harbored squamous intraepithelial lesions. It was proposed that high-risk HPV detection supplements routine cytology screening platforms in classifying high-risk women. Such females with negative pap smears may contain oncogenic HPV strains or the potential to develop CIN lesions. The first national assessment of the incidence of HPV infection among women of 14 to 59 years of age in the United States using PCR. An overall HPV incidence of 26. 8% was reported among US females aged 14 to 59 years (n= 1921). Females of 14 to 19 years showed an HPV prevalence of 24. 5%, 20 to 24 years with 44. 8% HPV incidence, 25 to 29 years with 27. 4% prevalence, 30 to 39 years with 27. 5% prevalence, 40 to 49 years with 25. 2% and 50 to 59 years with 19. 6% HPV prevalence. HPV prevalence increased with an increase in age from 14 to 24 years and a significant correlation was observed between them. High-risk HPV type 16/18 and low-risk HPV type 6/11 were detected. HPV-16 was detected in 1. 5%, HPV-18 in 0. 8%, HPV 6 in 1. 3, and HPV 11 in 0. 1% of female participants. The number of lifetime partners, age, marital status, and recent sex partners were some of the independent risk factors for HPV positivity.

HPV detection in cervical cancer and long-term prognostic value of cytology amid women negative for HPV showed that women who underwent routine screening every six years were well guarded and had a reduced chance of cervical cancer (Das *et al.*, 2000) (Figure 2. 1). The study analyzed the oncopotency of high-risk HPV 16 and 18 and their prevalence in cervical lesions in 130 study subjects in Chennai, India over three years. HPV 16 exhibited a higher prevalence of uterine cervical cancer in comparison to HPV 18 cases. However, HPV 18 infected cases showed an enhanced development of invasive cancer from precancerous lesions, which indicated the higher oncopotency of HPV type 18. In a study, Gupta and his co-workers in 2009

assessed the incidence of HR-HPV infection in the uterine cervix of females of the reproductive age group who had normal cytological pap smears. A total of 769 cytological negative cervical samples from women (age 18-45 years) in Delhi were analyzed for HPV DNA positivity. Further, these samples were subjected to stratification into high-risk HPV and low-risk HPV by polymerase chain reaction. 16. 6% of the normal cytological pap smears showed HPV prevalence when subjected to HPV-based molecular diagnosis. The majority of these HPV positives were the HR-HPV 16/18 subtype. As such in addition to traditional screening by pap smears, HPV-based detection of high-risk HPV types could envisage screening and prophylactic programs for cervical cancer cost-effectively.

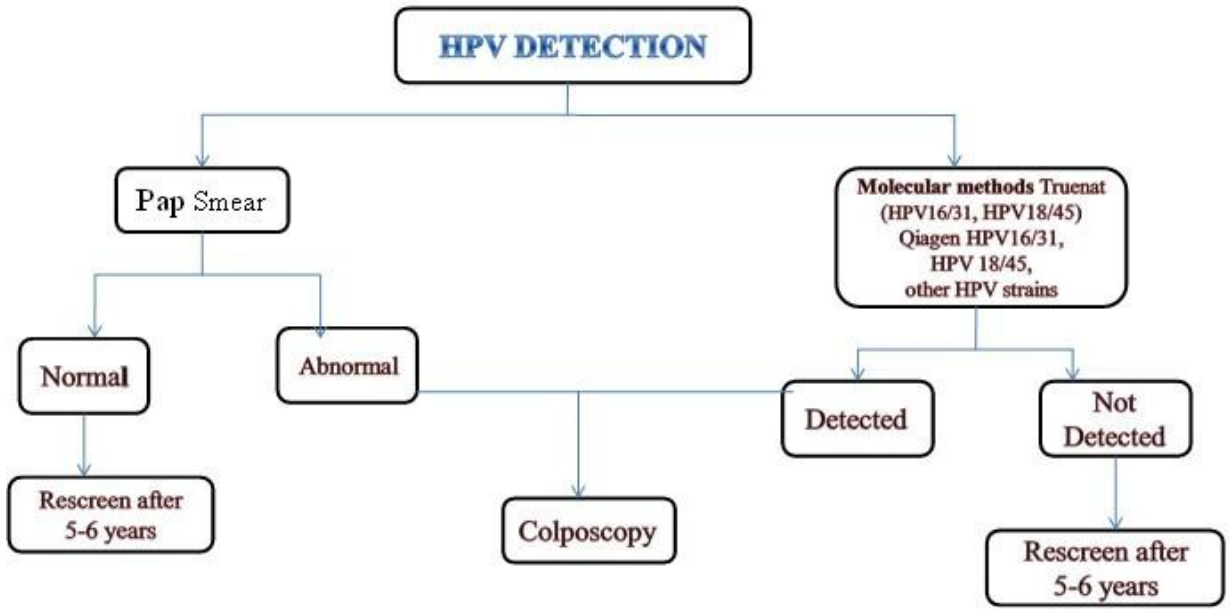

Figure2.1: Illustration depicting various HPV detection methods

The introduction of various hybridization techniques using cervical scrapes in the field of genetic engineering has created a new approach to studying HPV infection in the cervix. DNA hybridization can identify specific HPV types in lesions where they can't otherwise be detected. DNA hybridization is perhaps the best and the latest technique to qualitatively and quantitatively detect viral load. In DNA hybridization, a specific DNA strand is identified by using a known source DNA. The DNA is then cloned in a suitable vector, denatured to individual strands, radioactivity labeled, and mixed with the specimen denatured into single strands. Homologous strands of DNA anneal form radioactively labeled double-stranded DNA. A study on the role of

various risk factors and measures to control cervical cancer in India concluded that parity and increasing age are primary factors in cervical carcinogenesis. Sexually disseminated viruses such as HPV and Herpes simplex virus (HSV) were largely linked with Squamous Intraepithelial lesions. The occurrence of HPV infection among 225 Greek females visiting a gynecological outpatient clinic and observed that HPV infection was detected in 22. 7 % of women. HPV16 was the primary type detected. Alcohol consumption and several sexual partners showed a positive relation with HPV infection while age and monthly income exhibited a negative association (Stamataki *et al.,* 2010).

A study by Gheit and his coworkers in 2009 analyzed the distribution of HPV type in women positive for cervical cancer. Oncogenic HPV types were liable for greater than 90% of cervical cancer. Several other studies conducted worldwide established the high occurrence of HPV in cervical cancer patients. In particular, HPV 16 and 18 were recognized as high-risk oncogenic subtypes linked with HPV-related cervical cancer. HPV 16 has a higher frequency in high-risk HPV types in comparison to HPV 18.

The rapid rise of Human Papillomavirus infection shortly after the occurrence of HIV contamination in South African females. It was found that HPV infection enhanced rapidly during the initial years following HIV seroconversion, implicating dysfunction of the mucosal immune system at an initial stage of HIV infection and thus affecting HPV-related diseases.

It was observed that the incidence of HPV in Uttar Pradesh, India was 9. 9% in asymptomatic women. A total of 26 different strains of HPV were found in these women with HPV-16 in the majority. Non-vegetarian diet and rural residence showed a significant relation with HPV positivity ($p<0.01$). A similar study on the incidence and diffusion of HPV strains in cervical lesions of women from Varanasi and adjoining areas in India reported HPV-16 as the foremost high-risk HPV type in CIN (20%) and SCC (24%) groups while HPV 18 (40%) and HPV 31 (20%) were the most frequent types detected in adenocarcinoma and cervicitis, respectively (Garg *et al*., 2016).

Datta *and* his coworker in 2012 studied the persistence of HPV infection and type-specific incidence among young women from the urban slums in Delhi and found that high-risk HPV type infection was more dominant in young married women as compared to low-risk types. Also, among the high-risk HPV types, HPV16 exhibited the maximum incidence rate. The study

showed the prevalence of HPV and performed a genotype dispersal of HPV amongst females visiting a cervical cancer screening site in Lampang, Thailand. They reported high-risk HPV genotypes in 5. 4% of the women screened and found HPV-52 type as the most common followed by HPV-16, 58, 33, 51, and 56.

In 2014, a study concluded that the females of Jammu have inadequate knowledge regarding cervical cancer susceptibility, and thus, there is an immediate need to update the health standards and hygiene of women of the Jammu region. To reduce the risk factor, it is important to improve social awareness and enforce education strategies. Kantathavorn and his coworkers 2015 analyzed the HPV incidence and genotypic HPV distribution and found a 15. 1% overall HPV prevalence in Thai women. They also reported 6. 4% high-risk HPV, 3. 5% plausible high-risk, and 8. 5% low-risk HPV strains in the studied population (Kantathavorn *et al.,* 2015). In 2015, Bhardwaj and colleagues conducted a hospital-based study in the Jammu region of the J&K Union Territory, analyzing the prevalence of Human Papillomavirus (HPV) among symptomatic women. They discovered a notably high HPV prevalence of 40. 8% in this group (Bhardwaj *et al*., 2015). This finding underscores the significant burden of HPV infections among symptomatic women in the area, emphasizing the need for increased awareness, screening, and preventive measures in this region.

HPV DNA detection is the latest technique for detecting cervical cancer and is being conducted in India at various places. HPV DNA sensitivity of 45. 7% to 80. 9% was observed for identifying cervical intraepithelial neoplasia grade 2 or more. A peak age of 55-59 years was recorded for the occurrence of cervical cancer in India. A very high correlation of 88-96% was found between HPV infections with cervical cancer among women. More than two-thirds of cervical cancer patients in India existed at stages III and IV. Within the first year of diagnosis around 20% of females who develop cervical cancer die and the overall 5-year survival rate was 50%.

The study showed the liquid-based cytology results of 302 samples analyzed for cervical cancer. HPV 16 type was found in 30% of cases while 7% were found to be HPV 18 type. About 29 % of inflammatory samples appeared positive for HPV 16. Further squamous cervical abnormalities showed a higher HPV positivity (48. 6 % HPV 16 and 29. 7 % HPV 18) than glandular abnormalities (36. 5% HPV 16 and 18. 3 % HPV 18). Further, some of the cytological normal samples appeared positive upon HPV DNA testing viz. 25% HPV 16 and 4. 5 % HPV 18,

respectively. This implies the effectiveness and sensitivity of HPV DNA detection as compared to LBC.

A hospital-based study on HPV detection of women of the Jammu and Kashmir region showed that high-risk HPV-18 (7. 2%) and HPV-16 (2. 4%) were the foremost type next to HPV-33 (1%) and HPV-31 (0. 8%) types. 204 (40. 8%) cervical exfoliate samples out of 500 turned out to be positive cases of HPV infection. Females>65 years of age group showed the highest HPV prevalence of 71. 43% while women married before 18 years of age showed an enhanced HPV prevalence of 48. 44%). The highest HPV prevalence was seen in females having 4 or more children (50%) as compared to those with less than 4 children. A significant link was found between various demographic attributes like age, age at marriage, number of children, and HPV infection validated by a p-value <0. 05 (Fotra *et al.,* 2014) . Souho *and* his team 2015 reviewed the studies from 1994 to 2014 and found that HPV infection is significantly linked with many detrimental effects on the reproductive health of both men and women and also alters their fertility rate. Wilting and Steenbergen 2016 summarize how HPV oncogenes possess additional properties that regulate the DNA methylation machinery and mitotic checkpoints and lead to high-grade neoplasia or cancer. Current knowledge about the molecular variations happening in the host genome during HPV-induced tumor genesis helps us to decipher the biology of cervical cancer develop biomarkers for timely diagnosis and devise novel therapeutic targets for HPV-induced precancerous tissue.

A retrospective study on the HPV types in distribution among females having cervical cancer in Portugal from 1928 to 2005 showed that HPV16 (58. 2%) and HPV18 (9. 2%) were the most frequent types found in these women. In addition to these HPV33 (6%), HPV45 (4. 7%), and HPV31 (4. 5%) were also found. It was further concluded that almost 70% of cervical cancer reported were HPV 16 and 18 types. A similar study from the Amasya region in Turkey regarding the incidence and circulation of high-risk HPV showed that HPV 16 (23. 6%) and HPV 51 (9%) were quite frequent types found in women. Out of the total women sampled 2. 7 % were detected positive for HPV DNA and among these positive women 77. 8% were found infected by high-risk HPV strains. Women in the age group 30-39 years were particularly vulnerable to HPV infection. A wider study comprising women from all parts of Turkey showed somewhat similar results with an HPV positivity rate of 2. 79%. Out of these positive women majority, 63% were positive for a single HPV type while the rest showed infection by more than

one HPV type. HPV types 16 (11. 25%), HPV31 (7. 83%), HPV51 (6. 06), and HPV52 (3. 16%) were most frequent. A similar study on the distribution and incidence of HPV in vaginal and vulval abnormal cytological lesions and cancer tissue of Thai women showed HPV frequency of 47. 1% in vulval samples and 40% in abnormal cytology lesions. HPV16 was the primary type detected in abnormal cytology lesions and all cancers while HPV 18 was rare and existed as co-infection along with other strains of high-risk HPV. Additionally, low-risk HPV types viz. HPV 6, 11, and 70 were also identified in abnormal cytology lesions and vulva cancer samples, though all vaginal cancer samples were positive for only high-risk HPV strains.

A more universal study comprising women subjects from Europe, Asia, Latin America, North America, and Oceania was carried out to assess high-risk HPV types in cervical neoplasia. It was observed that HPV 16/51/52/56 was predominant in anogenital infection while HPV 16/39/51/52/56 in CIN1 and HPV 16/31/52/58 in CIN2/3. It was concluded that the 9vHPV (6/11/16/18/31/33/45/52/58) vaccine type could be a game changer in preventing the bulk of CIN1-3, regardless of geographic region. The incidence and genotyping of HPV and its relation with anogenital cancer found that the commonest oncogenic HPV types identified in the cervix and anus were HPV 68 and HPV 16. Maximum HPV infection was found in women having three or more sexual partners throughout life.

A study in 2012 analyzed the efficiency of a blend of Pap smear tests and HPV DNA tests in diagnosing the pre-invasive stage of cervical cancer. The results established a substantial link between HSIL and HPV-DNA positivity. A significant relationship was also found between abnormal cytology, HPV-DNA positivity, and colposcopic findings. Further, it was concluded that the Pap smear is more sensitive in the timely detection of pre-invasive stage cervical cancer than the HPV DNA test. An increased incidence of HPV-16 has also been found in comparison to HPV-18 in asymptomatic healthy subjects and cervical lesions in rural India. Similar reports were obtained in a study by Hussain *and* his coworkers in 2018 showed a high prevalence of HPV16 as compared to HPV-18 in South Indian subjects. A meta-analysis survey of prior studies from various geographical regions of India viz. North, East, Northeast, West, Central, and South indicated a higher prevalence of HPV (82%) in cervical cancer, 71. 42 % in HSIL, and 61. 30 % in LSIL. Among all the regions south and north India showed the highest HPV-16 prevalence amongst females.

Wolday and his coworkers in 2018 assessed a total of 233 women patients for cytology examination. Out of these 60. 5% exhibited normal cytology while 39. 5 exhibited abnormal cytology. HPV16 was a major determinant linked with cervical cancer in Ethiopian women. Furthermore, it was detected that Pap smear cytology evades an appreciable portion of HPV positives than those identified by PCR-based molecular methods. All females having abnormal cervical cytology were also positive for HPV DNA, whereas only 48. 9% of females having normal cytology had HPV DNA. Among these females, the incidence of high-risk oncogenic HPV was 83%. Besides high-risk HPV was more prevalent in females with abnormal cervical cytology as compared to females having normal cytology. Females belonging to rural areas were found to be the highest contributor to the incidence of cervical cancer (55%). Poor genital hygiene, reproductive behavior, insufficient resources, and lack of awareness result in the progression of the disease.

About 37. 3% of smears having inflammation and 57% with LSIL in cervical cytology turned HPV DNA positive whereas only one case of ASCUS turned out HPV DNA positive. The study concluded that educated women (25%) were less disposed to HPV infection as compared to educated women (51. 3%).

In 2018, Bhattacharya and his team conducted a study that examined the influence of socio-demographic factors on the distribution and prevalence of Human Papillomavirus (HPV) infections among women screened in a hospital setting in a town in West Bengal. Among the 36. 84% of women that turned out HPV-positive more than one-third were HPV16 and 18. Only 6. 4% of women were detected with abnormal cytological lesions, which is attributed to the lesser effectiveness of cytological detection compared to HPV-based DNA detection. 36% of the females with normal cytology exhibited HPV infections with more than one-third being HPV 16/18 and about 22% were other HPV strains. HPV prevalence decreased considerably with an increase in age. HPV 16/18 type was appreciably over-represented in all age groups in comparison to other HPV types. Severe cervical lesions showed increased HPV 16 prevalence and did not vary among Hindus and Muslims. Cytologically normal Muslim women showed a higher HPV 18 (24. 14%) in comparison to the Hindus (11. 91%), explicitly in females $\geq$ 30 years of age. Women using oral contraceptive pills showed a higher HPV16 prevalence irrespective of cytology.

Female sex workers (FSWs) in Chandigarh, India were analyzed for abnormalities in cervical smear and oncogenic HPV type prevalence. It was observed that liquid-based cytology samples exhibited better cytological details as compared to Pap smears. The number of unsatisfactory smears was also brought down to 1. 5% in LBC as compared to 11% in Pap. FSWs exhibited a higher number of inflammatory smears (51. 7%) as compared to healthy controls (34. 7%). The most dominant HPV types detected were 16/18. FSWs who had first sex at age ≤25 years, were frequent smokers, and had higher income exhibited a higher risk of acquiring hrHPV.

In 2009, Cohen and colleagues investigated the prevalence of HPV infections and their correlation with Cervical Intraepithelial Neoplasia (CIN) in a hospital-based study involving 100 women. It was found that 12 % of women exhibited high-risk HPV infection. Cytological examination revealed that 49% of women with inflammatory smear, 5% ASL VS, 16% LSIL, 5% HSIL, 4% koilocytosis, and 17% women had a normal smear. Eighty percent of women who showed HSIL in pap smear and 50% showing koilocytosis in Pap test were also HPV-positive. HSIL group comprised HPV-positive cases of more than 30 years old. 70% of patients underwent colposcopy, which showed that HPV positivity increased with increasing grades of colposcopic cases. Grade III cases showed an HPV positivity of 60% while 100% HPV positivity was observed in cases suggesting malignancy. The correlation between HPV DNA testing and histopathology recommended a strong relation between HPV positivity and advanced grades of CIN. HPV positivity of 33. 2% was observed in CIN 2 cases, 75% in CIN3 cases, and 100% in carcinoma in situ cases. The patients aged between 21-30 years exhibited the maximum number of high-risk HPV and this positivity decreased with increasing age.

In a comparable study conducted in 2019, a total of 2,478 cervical specimens from women in Middle Eastern countries were analyzed to determine HPV positivity and the presence of high-risk HPV (HR-HPV) DNA. Approximately 21% of these samples tested positive for HR-HPV, highlighting a significant prevalence of high-risk HPV types in this population. Cervical samples exhibiting abnormal cytology had an increased high-risk HPV positivity rate (50. 6%) in comparison to those with normal cytology (14. 7%). The high-risk HPV positivity rate increased as the severity of the cytological lesion increased.

A pan-India epidemiological report on cervical and breast cancer screening indicated that women of general caste, living in rural areas, marital status, and financially sound were more active in taking up screening across districts of India. The number of females taking cervical screening

(22%) was more compared to those who took up breast screening. Among those who took up cervical screening, Kerala was the top contributor followed by Maharashtra. The majority of the districts showed cervical cancer screening prevalence in the range of 10 to 20%. Various districts of Maharashtra, Assam, Kerala, West Bengal, Jammu-Kashmir, and Punjab showed a thick clustering of cervical screening. Districts in Uttar Pradesh and Madhya Pradesh also had a few clusters. At the district level, females having multiple sex partners and consuming oral contraceptives were negatively linked with cervical cancer screening uptake. A similar pattern was perceived among districts having a high percentage of Hindu women. A positive correlation was found between cervical screening and women who were insured. Such an all-India study highlights the core area and population group (rural or urban) that has little or very low screening prevalence rates and could thus be targeted for implementation of screening programs by the government to prevent the dissemination of cervical cancer.

A study regarding the prevalence of HPV in the Delhi NCR region indicated that HPV 16 was the primary type found in more than 95% of the cases followed by HPV 18. The subjects enrolled in the study were less than 35 years of age, belonged to rural households, and as such showed little awareness about cancer of the cervix and the significance of screening in its prevention. It was observed that HPV prevalence increased as age increased. Lack of education, early age marriage, first pregnancy at an early age, increased parity, poor hygiene, tobacco use, and poverty were important risk factors related to HPV infection. However, no association was found with oral contraceptive use which was in contradiction with many reports establishing a strong correlation between oral contraceptives with the development of cervical cancer. This could be probably due to rural participants and their shyness about disclosing information about oral contraceptive use.

The study examined approximately 1,500 unvaccinated, married Indian women to assess the prevalence, acquisition, and clearance of specific HPV-type infections among sexually active individuals. The most frequent HPV strains were HPV 16 (6. 5%), 31 (6. 1%), 58 (4. 9%), HPV 56 (4. 6%), HPV 68 (4. 3). HPV 35 (62. 5%), 52 (25%), 70 (25%), 16 (23. 6), 18 (22. 2%) exhibited higher persistence among the detected strains as compared to extremely low for HPV 6 (6. 7%) and HPV 11 (0%). The highest new HPV acquiring rate of 5. 6 per 1000 person-months of observation (PMO) was achieved by HPV 16. Strain-specific clearance rates of HPV varied between 2. 9-5. 5/100 PMO. Women with 2≤3 years amid marriage and initial cervical sample

collection had 41% lesser HPV 16 and/or 18 infections as compared to those with <2 years amid marriage and initial cervical sample collection. It was further observed that young Indian women exhibited lesser HPV occurrence and acquisition rates in contrast to their Western counterparts.

A study inspected the prevalence of human papillomavirus infection in patients in Japan and correlated HPV infection for uterine cervical lesions presence or absence. HPV detection was done by hybrid capture method and grades were analyzed by histological and cytological examination. Subjects with normal cytology reported an HPV-positive rate of 12. 3%. Subjects in their twenties showed a high detection and those in their forties a low detection rate. However, the subjects aged in their sixties did not report any HPV detection. Examination of cytological or histological grades for HPV-positive rates showed that subjects with abnormal cytology, cervical intraepithelial neoplasia, or SCC had high HPV positivity (P< 0. 01) as compared to subjects with normal cytology.

In 2022, Cadilla and colleagues investigated the effect of menopause on genital HPV infection. The study analyzed cervical samples from 330 women, including 75 menopausal and 255 non-menopausal participants. Results showed that non-menopausal women had higher overall HPV infection rates (77. 25%) compared to menopausal women (60%). However, the prevalence of high-risk HPV infections was similar between the two groups, at 43. 13% and 40%, respectively (Cadilla *et al*., 2022).

2. 1. Epidemiological factors and HPV

2. 1. 1. Sexual partners

Various studies have established the relationship between the increasing number of sexual allies and cervical cancer. The social class differences may be somewhat predictive of sexual behavior and thus were analyzed for the incidence of cervical cancer case studies in Spain. Upper social-class women exhibited a reduction of 60% in the prevalence of HPV as compared to lower social class.

Low socioeconomic status, sexual promiscuity of women, and poor genital hygiene were reported as three independent predictors of HPV infection. The study presented that age during first intercourse ≤20 years; a high number of sexual partners and prolonged oral contraceptive use are primary reasons for cervical cancer development (Cuzick *et al.*, 2000). A study on the factors associated with genital HPV infection in females showed that the acquisition of a new sex

partner was positively associated with HPV incidence and that non-penetrative sexual contact was a probable route of HPV infection in virgins. A similar study indicated that the chance of HPV infection varied with age, and the age group 20-29 years showed the greatest risk of infection, and thereafter it declined with age.

The most significant risk factor associated with HPV infection, which later developed into cervical cancer was the number of sexual partners in the past 2 years. Additionally, regularity of sexual contact and warts in a sexual partner was also recognized as independent risk factors for HPV infection. In a study, on sexually active women aged 18-85 years for 15 months in Hawaii, it was reported that cervical HPV acquisition decreased with increasing age and oral contraceptives use for longer duration. However cervical HPV acquisition increased with the number of sexual allies, condom use by a sexual partner, and use of hormonal creams (M. T. Goodman *et al*., 2009). It was further observed that low-risk HPV infection gets cleared more rapidly as compared to high-risk HPV with clearance times maximum among older women and women with multiple infections.

2. 1. 2. Age of Marriage

The association between the early age of marriage and cervical cancer risk development is an established norm. A study exhibited that about 86% of the females who were married before the age of 17 years of age had an enhanced likelihood years had an elevated risk of developing cervical cancer (Mayavati Mhaske1, Jawadekar S. J. 2, 2011). A study established that the early age of marriage is positively linked with cervical cancer, strengthening the findings of Capalash and Sobit who presented that early marriage age complemented with parity and low socioeconomic status were linked with cervical cancer development (. A study on the association of cervical cell morphology and early age of marriage showed that women who married at ≤18 years of age had significantly higher abnormal Pap smears, thus augmenting their chances of cervical cancer development.

2. 1. 3. Oral Contraceptives

Prolonged oral contraceptive use and cancer development is a generalized phenomenon observed in many types of cancer. Its association with cervical cancer showed that longer duration and frequency of hormonal contraceptive use escalate the chance of HPV infection resulting in dysplasia among women. The study showed that females with prolonged oral contraceptive use

had a greater danger of cervical cancer development in comparison to non-users (Khatun *et al.*, 2018). Various studies have shown that long-term pill use augments the possibility of cervical cancer development in a range of 50% to 200%. A study by the International Collaboration of Epidemiological Studies on cervical cancer analyzed about 16, 573 females having cervical cancer and 35, 509 females without cervical cancer and their association with oral contraceptive use. It was concluded that the cervical cancer risk increased with prolonged oral contraceptive use which decreased after oral contraceptive use was stopped and returned to that of the general population when no contraceptive was used for 10 years onwards.

2. 1. 4. Socio-Economic Factors

A plethora of studies have established the pivotal role of social and economic factors in the progression of cervical cancer. The study analyzed socio-demographic aspects connected with high-risk HPV infection in U. S. women and found that poor women were more prone to HPV infection. Prophylactic measures like pap test screening, HPV vaccines, and education should be accessible to all low/low-middle-income women to boost cervical cancer prevention efforts (Kahn, 2007). Geetha and her coworkers in 2013 while evaluating the sexual risk factors for cervical carcinogenesis reported that low socioeconomic status, place of residence, education, extended use of oral contraceptives use, and lack of knowledge about various screening and vaccination measures were appreciably allied with the possibility of developing cervical cancer (Geetha & Santhy, 2013). Restricted information about cervical cancer, the Papanicolaou smear test, and the necessity for timely diagnosis of cervical cancer resulted in an increased risk of cervical cancer development in Malaysian women aged between 21-56 years. A study highlighted that poor demographic factors, poverty, inadequate sanitary hygiene, limited access to basic sanitation facilities, lack of transportation, and financial challenges are significant risk factors for the development of cervical cancer (Kashyap *et al*., 2019b). Similar results were seen in a study conducted to assess the awareness and screening for carcinoma of the cervix among women in Mangalore city. Most of the women (>85%) had little understanding regarding cervical cancer and cervical cancer screening and less than 10% of subjects had undergone screening for cervical cancer in their lifetime.

2. 1. 5. Smoking

Smoking tobacco (including cigarettes, cigars, pipes, and hookah) along with high parity have been considered pivotal contributing factors, as they mediate HR HPV infection progression, and ultimately develop into cervical pre-cancer and cancer (Castellsagué X and Muñoz N 2003; Pista *et al.,* 2012). This study identified a substantial relationship between smoking and HPV DNA positivity. A higher odds ratio was displayed for smokers and ex-smokers as compared to that of non-smokers establishing an elevated risk of evolving Squamous cell carcinoma in the case of continuous cigarette smokers. Women who started smoking at a young age and those with higher smoking intensity were at a higher risk of developing SCC but not adenocarcinoma (International Collaboration of Epidemiological Studies of Cervical Cancer 2007).

2. 1. 6. High Parity

It has been observed that multiparity has also a positive effect on cervical cancer development. Studies have established that childbearing, irrespective of the number of children delivered, augments the probability of cervical cancer development while nulliparity exhibits a protective effect against cervical cancer. Females having insistent infection with high-risk HPV exhibited a strong effect of parity. It was observed that females infected with persistent high-risk HPV, parity considerably augmented the subsequent risk for immediate precursor lesion development into cervical cancer, (Jensen *et al.,* 2013).

2. 2. Cervical Cancer Management

The bulk of HPV infections are cleared in a short interval of time with only 10-20% of them progressing into cervical cancer. Despite a vaccine, presently there is no effective remedy for the management and prevention of cervical cancer. The vaccine too is a prophylactic one and not a therapeutic vaccine. HPV infection is mostly seen in regions where there is limited access to preventive vaccines. About 92 countries introduced HPV vaccination programmes by February 2019 (WHO, 2019). A decrease in the incidence of vaccine-based and non-vaccine strains has been seen in some preliminary studies. HPV vaccines can fight against lesions of the cervix and genital warts and this has been established by many studies (Cameron *et al.,* 2017; HPV vaccines activate the immune system to produce antibodies so that in future when the virus attacks it can prevent the body from infection. AS04 adjuvant attaches agonist of Toll-like receptor 4, MPL (3-O-desacyl-4′-monophosphoryl lipid A) with aluminum nut salt. These salts

are very safe to use in vaccines. It additionally strengthens the immune reaction to the vaccines. Lipid A is the major component of the cell surface of Gram-negative bacteria and is responsible for the end toxicity of Lipopolysaccharides. To stimulate the immunological responses, Lipid A binds to the TLR4. The primary cytokines participating in antiviral response and having direct immunomodulatory effects are Interferon (IFN-α, IFN-β, and IFN-γ) and the TNF-α and TNF-β). HPV nucleic acids integrate into the host cell genome, resulting in the obliteration of various early (E1 to E5) and L1 & L2 late genes. On integration, L1 & L2 are deleted due to which prophylactic vaccines failed to show their effect against HPV-associated cancers. Deletion of E2 during integration also results in an increment in the level of E6 and E7 leading to the carcinogenesis of HPV-related lesions. Oncoprotein E6 and E7 are prominent players in HPV-associated malignancies. These oncoproteins represent effective therapeutic targets of HPV vaccines as E6/E7 being foreign antigens overcome the problem of immune tolerance quite commonly associated with cancer vaccines (Ghittoni *et al*., 2010).

2. 2. 1. Prophylactic Vaccine

These vaccines stimulate neutralizing antibody response by targeting L1 and to a small extent L2. These vaccines are effective in individuals before infection. Three prophylactics HPV vaccines are currently accessible. The bivalent vaccine (Cervarix) licensed in 2007, protects against genotypes 16/18 (Monie *et al.,* 2008) while the Quadrivalent vaccine (Gardasil) shows protection against HPV 6, 11, 16, 18 genotypes. A Nonavalent vaccine (Gardasil 9) effective against strains 6/11/16/18/31/33/45/52/58 has been only licensed in the USA in 2016.

A DNA-free VLP (virus-like particle) of the L1epitope made by recombinant technology and combined with adjuvant is employed in the production of prophylactic vaccines. These are attenuated vaccines incapable of causing infection but effective enough to generate an immune reaction. Vaccine trials carried out in America and Europe showed that both bivalent and quadrivalent vaccines exhibited more than 90% efficacy against HPV infection among women receiving 3 doses and without any earlier exposure to HPV infection. VLP vaccines assemble identically to the HPV virion outer shell producing antibodies against it which react with and neutralize the original virus.

Amorphous aluminum hydroxyl phosphate sulfate (AAHS) behaves as an adjuvant in Quadrivalent and nonavalent vaccines (Gardasil and Gardasil 9). This vaccine is synthesized in

yeast. It was effective in averting cervical intraepithelial neoplasia and adenocarcinoma in situ among women. High-grade abrasions CIN 2/3 or adenocarcinoma in situ linked with HPV type 16 and 18 showed 100% efficacy. This vaccine showed an efficacy of 98. 9% opposed to genital warts associated with any of the four HPV types. The vaccine was also found to be effective for vulvar intraepithelial neoplasia grades 2/3, anal intraepithelial neoplasia grades 1/2/3, and vaginal intraepithelial grades 2/3. Both the vaccine and placebo recipients showed a similar occurrence of a systemic adverse event in clinical trials. After licensure, the HPV vaccine was temporarily linked to severe adverse events however exhaustive post-licensure studies did not report any serious adverse events. The bivalent vaccines show effectiveness against types 16/18 VLPs. Adjuvant ASO4 containing aluminum hydroxide and 3-deacylated monophosphoryl lipid A is employed in the bivalent vaccine. The studies conducted on bivalent vaccines suggest that the extent of defense of bivalent vaccines is long-lasting. ACIP recommends the administration of any one of these vaccines routinely for females between 11 to 26 years old via a three-dose manner, first dose at time zero after which subsequent doses should be given 1 to 2 months later. HPV prevalence declined from 11. 5% to 5. 1% in females between 14 to 19 years old.

Prophylactic vaccines show no therapeutic advantage and their mechanism involves the production of antibodies against capsid protein L1, which based on HPV-type, self-assembles into hollow shells called VLP. Protecting antibodies are generated by whole VLPs. Neutralizing antibody production requires conformational epitopes of L1. Clearance of infection of cells is reliant on the generation of a potent cell-mediated immune response, providing a strong explanation for the production of a VLP-based vaccine. In humans, several methods have been demonstrated that show that a robust type restricted serum antibody was generated in response to LI VLP in, almost 100% of vaccinated individuals. These methods are enzyme-linked immunosorbent assays (ELISA), competitive Luminex-based immunoassays, radioimmunoassays, and pseudo-virion-based neutralization assays. Prophylactic HPV vaccines are exceedingly successful against CIN2/3 and adenocarcinoma in high-risk HPV women. The nonvalent vaccines also show exceptional efficacy in avoiding CIN1 (relative risk reduced by 98. 9%), CIN2 (relative risk reduced by 97%), and CIN3 (relative risk reduction, 100%) neoplasia.

Women below 15 years old only needed dual doses of vaccine approved by the Advisory Committee on Immunization Practise. For those who start the vaccination from the age of 15 to 45 years, a three times vaccination schedule is approved at (0, 1-2, and 6 months). For immune-

compromised patients, a three-dose schedule is suggested regardless of sex and age. For public health benefits HPV, vaccines should be admirably used before starting sexual intercourse. Therefore adverse effects of HPV infection should be communicated through educational ways. The FUTURE I trial proves that the efficiency of the quadrivalent HPV vaccines against genital warts is 100% and 70% (vaginal warts) to 78% (vulvar warts) in the global population. The trials demonstrated that HPV vaccines show exceptional defense against cervical cancer, provided they are not previously infected by HPV types.

HPV vaccination for girls only has been anticipated to decrease the age standardized cervical cancer incidence in low middle-income countries from 19. 8 to 2·1 cases per 100 000 females-years over the following century representing an 89·4% efficiency and to avert 61·0 million reports during this period. HPV vaccination and at least one screening during a lifetime lessened the incidence to 95% while HPV vaccination and twice screening during a lifetime minimized the incidence to 96. 7%. This girls-only vaccination was expected to eliminate 60% (58–65) of cases in LMI countries with a threshold of four or lower incidences per 100,000 females per year. All LMI countries reached 100% removal for all three thresholds when twice lifetime screening was added. Following HPV vaccination, a 91% (range 88-93) reduction in cervical cancer was predicted in South Asia and 87% (range 84-88) in Saharan Africa. HPV vaccination in combination with twice screening during a lifetime resulted in a 97% reduction in HPV infection in South Asia to 90-96% in the sub-Saharan Africa region. It was concluded that HPV vaccination coverage for girls and twice-in-a-lifetime screening will result in cervical cancer eradication in low-income and LMI nations at the end of this century (Brisson *et al.,* 2020).

2. 2. 2. Therapeutic vaccine

A variety of therapeutic vaccines have been tried over the years primarily DNA, protein, and peptide-based vaccines (Figure 2. 2). These vaccines have exploited E6 and E7 DNA regions and peptides but still, no effective curative vaccine has been permitted for commercial purposes.

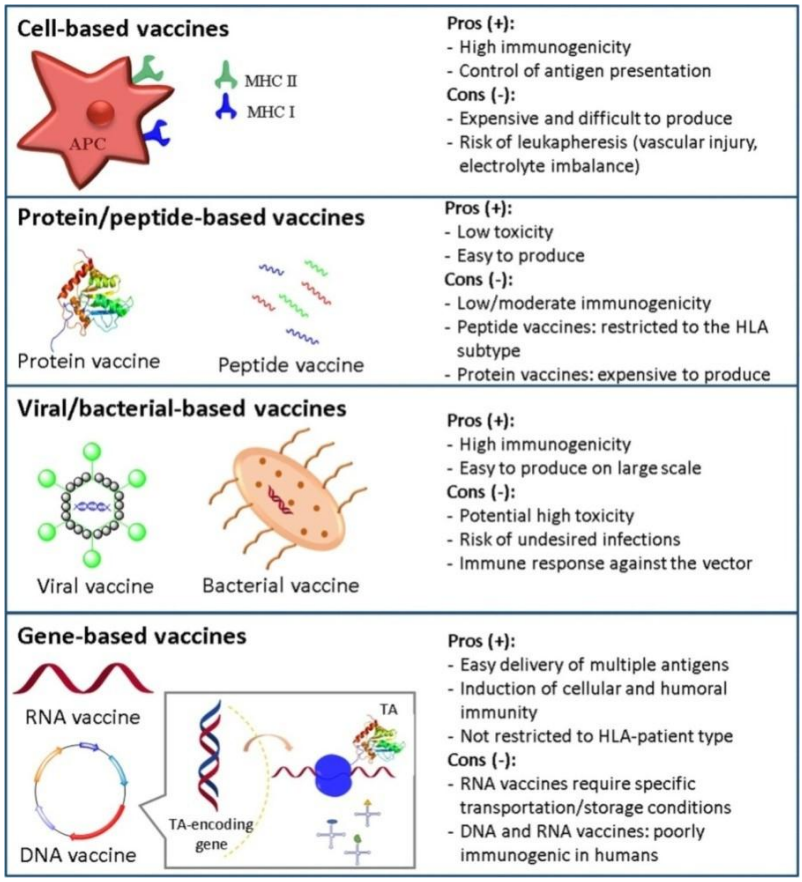

Figure 2.2: Different types of cancer vaccine. (Image courtesy: Lopes *et al.*, 2019)

According to the available data, 11% of females in the Jammu and Kashmir Union territory are affected by cervical cancer. This data also shows that there is little knowledge regarding cervical cancer and its prophylaxis among the females of Jammu. The proportion of the females who had taken primary education is (40. 7%). Thus, this is an urgent need to educate and aware women of health standards. Though emerging prophylactic measures and vaccines continue to emerge, the worldwide burden of this cancer is enormously high in low-income and low to middle-income nations. These countries lack awareness, expertise, hygiene, and funds that could minimize the risks linked with cervical cancer spread. India and low-middle-income countries account for significant morbidity and mortality. HPV vaccination strategies targeting adolescents before first sexual intercourse thus become pivotal in minimizing the incidence of cervical cancer. This further should be supplemented with regular and frequent screening, sexual education, female reproductive hygiene, and awareness programs warranted to lessen the problem of cervical cancer in poor countries. Several therapeutic vaccines targeting HPV have been experimented

with and are in various clinical phases, but not a single such vaccine has been approved so far. These strategies have been summarized in the following (Table 2. 2).

Table 2.2: Recent clinical trials of various types of therapeutic vaccines targeting cervical cancer

Vaccine Type	Vaccine	Antigen	Clinical Trials	Reference
Protein and peptide-based	TA-CIN	HPV16 L2/E6/E7	Phase I/NCT02405221	(Mo *et al.*, 2020)
	ISA101b	HPV16 E6/E7	Phase II/NCT04369937	
			Phase II/NCT04646005	
	INO-3112 (VGX-3100 + INO-9012)	HPV-16/18 E6/E7	Phase II/ NCT02501278	(Yang *et al.*, 2016)
Viral vector	TG4001	HPV16 E6/E7	Phase I/Phase II NCT03260023	(Mo *et al.*, 2020)
	PRGN-2009	HPV16/18 E6/E7	Phase I/Phase II NCT04432597	
Bacterial vector	ADXS11-001	HPV16 E7	Phase II/NCT02399813	(Yang *et al.*, 2016)
	Ad/MG1-E6E7	HPV16/18 E6/E7	Phase I/NCT03618953	(Mo *et al.*, 2020)
Liposome-based	PDS0101	HPV16 E6/E7	Phase II/NCT04260126	(Zhang *et al.*, 2021)
		HPV16 E6/E7	Phase II/NCT04580771	

Dendritic Cell-based	DC Vaccines targeting HPV E6/E7 Protein	HPV16/18 E6/E7	Phase I/NCT03870113	(Smalley Rumfield *et al.*, 2020)
DNA-based/Protein or Peptide-based	pNGVL4a-Sig/E7 (detox) /HSP70 with TA-CIN	HPV16 L2/E6/E7	Phase II/NCT03911076	(Mo *et al.*, 2020)
	pNGVL4aCRTE6E7L2 with TA-CIN		Phase I/NCT03913117	
DNA-based	pNGVL4aCRTE6E7L2	HPV16 L2/E6/E7	Phase I/NCT04131413	(Mo *et al.*, 2020)
	GX-188E	HPV E6/E7	Phase II/NCT02139267	(Choi *et al.*, 2020)
Drug based	Bintrafusp alfa	HPV E6/E7	Phase 1/ (NCT02517398 Phase II/ (NCT03427411	(Strauss *et al.*, 2020)
Combinational therapy	PDS0101 Bintrafusp alfa NHS-IL12	HPV16 E7	-	(Smalley Rumfield *et al.*, 2020)
Immunotherapy	ISA101, Nivolumab	HPV16 E6/E7	PhaseII/NCT02426892	(Massarelli *et al.*, 2019)
	TCR-engineered T cells	HPV-16 E7	Phase I/NCT02858310	(Nagarsheth *et al.*, 2021)
	TA-CIN+ GPI-0100	HPV-16 E6/E7/L2	PhaseI/NCT02405221	(Lee *et al.*, 2016)

2. 2. 3 Hypothesis of Research

In this study, we hypothesize a positive association between cervical cancer and Human Papillomavirus (HPV) infection. We further believe that specific risk factors, such as early marriage, early pregnancy, smoking, oral contraceptive pills, multiple childbirths, low socioeconomic status, and vaginal bleeding, contribute to the progression of cervical cancer. This hypothesis forms the foundation for our investigation, guiding the exploration of the complex relationships between HPV, identified risk factors, and the progression of cervical cancer in this Jammu region.

Chapter 3

AIMS and OBJECTIVES

1. **To find the incidence and Epidemiology of cervical cancer in the Jammu region.**

2. To detect the types of HPV (16, 18, 31, 45) prevalence or any new type in the Jammu region

3. To establish a correlation between HPV and Cervical cancer in the Jammu region.

Chapter 4

MATERIAL and METHODS

The study was conducted from 2018 to 2022 in the Swastik diagnostic laboratory and Department of Gynecology Govt. Medical College, Jammu. The study was carried out after obtaining permission from the institutional ethical committee of the Department of Gynecology, Jammu (IEC/GMC/2020/85). This permission made sure that the research followed ethical norms and rules and that the participant's rights and welfare were maintained at all times.

4. 1 Patient enrolment

The females were informed about the aims and objectives of the study and written approval duly signed by each female was collected. Relevant information regarding age, early marriage, early pregnancy, parity, multiple sexual partners, women with sexually transmitted diseases, chief complaints, irregular vaginal bleeding, etc. was recorded on a pre-designed questionnaire approved by HOD of Swastik Diagnostic Laboratory, Jammu.

4. 1. 1 Inclusion criteria for women enrollment in the study

1. *Both pre-and post-menopausal women presenting with abnormal vaginal bleeding:* Women who had pre-menopausal (having not reached menopause) or post-menopausal (having gone through menopause) may experience irregular vaginal bleeding.

2. *Post-menopausal vaginal bleeding:* Vaginal bleeding that happens after a woman has gone through menopause is known as post-menopausal vaginal bleeding.

3. *History of sexually transmitted diseases (STDs):* Women's reproductive health may be affected if they have a history of STDs such as gonorrhea, HPV, or Chlamydia.

4. *Women who got married young and were pregnant for the first time:* Women whose reproductive health may be affected by being married or getting pregnant while they were young, Early married and early pregnant women were those who were married before the age of 22.

5. *Multiparous women:* Women who have undergone several pregnancies, regardless of the outcome (live birth, stillbirth, or miscarriage), are referred to as multiparous women.

6. *Women belonging to low socioeconomic backgrounds:* People with low socioeconomic backgrounds are those who don't have sufficient funds to meet their basic needs, like housing,

healthcare, and education. People with an annual income less than >200000 were considered in low socioeconomic status.

7. *Women using contraceptives particularly, oral contraceptives:* Women who utilize birth control methods, including oral contraceptives.

8. *Smoker or non-smoker women:* A smoker is a person who smokes tobacco products regularly, like cigars or cigarettes. A person who does not smoke tobacco products is referred to as a non-smoker.

4. 1. 2 Exclusion criteria for women enrollment in the study

1. N*ulliparous and infertile women:* Nulliparous women are those who have never given birth, while infertile women are those who have been unable to conceive.

2. *Women with abdominal hysterectomy:* The women who have had their uterus surgically removed through an abdominal incision.

3. *Established cases of cervical cancer:* Women who have received a cervical cancer diagnosis or treatment were considered established cases of the disease.

4. *Pregnancy and puerperium:* Pregnancy refers to the period from conception to childbirth. Puerperium, also known as the postpartum period, refers to the time immediately after childbirth when the body is returning to its pre-pregnancy state.

5. *Unmarried women:* Women who are not legally married.

4. 2 Sample collection

In the present study, 4000 cervical smear samples were taken from patients with some gynecological complaints attending the Outpatient Department (OPD) of Gynecology and Obstetrics, Govt. Medical College (GMC), Jammu, and Swastik Diagnostic Laboratory, Jammu. Cervical tissue (Exfoliates) samples collected from the posterior vaginal pool by endo-cervical brushes were rinsed in a centrifuge tube containing 1% Phosphate Buffer Saline (PBS), pH 7. 4 and stored at -20ºC for further analysis. These samples were sent to the laboratory for testing. Liquid Based Cytology (LBC) and HPV testing was done for cervical smear samples.

Protocol of study: Flow chart

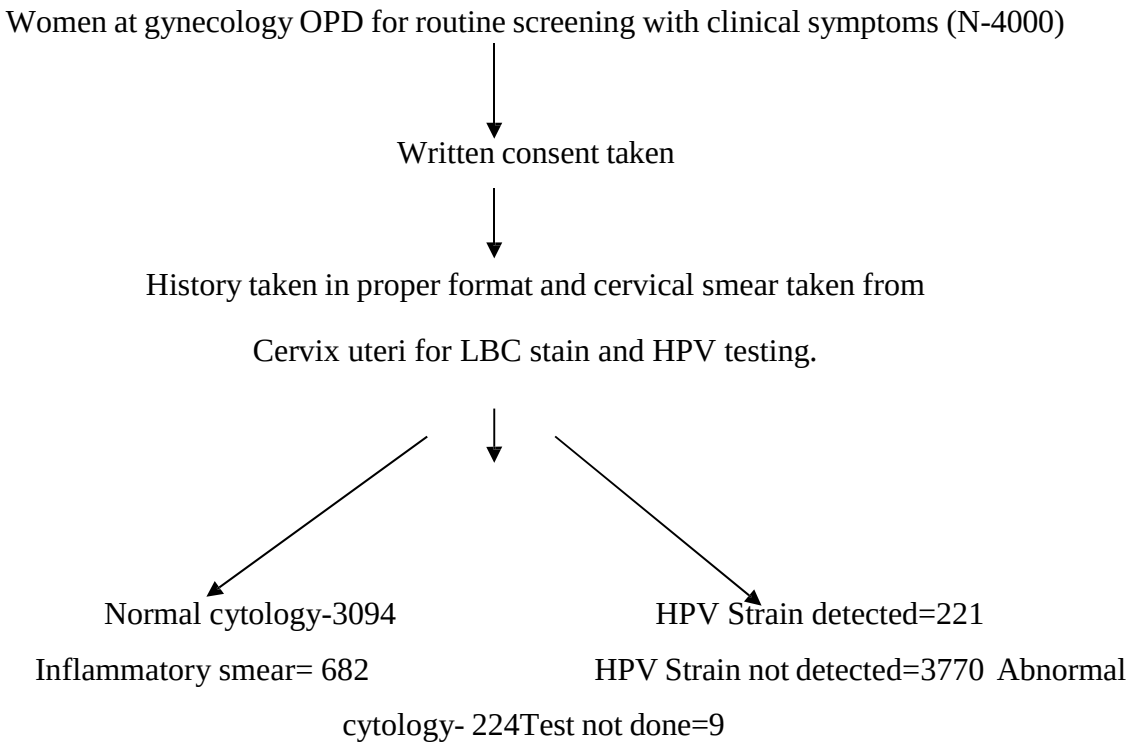

Figure 4.1: Illustration depicting the protocol for sample collection and testing.

4. 3 Liquid-Based Cytology procedure (BD Sure path vial)

1. *Vortexing and labeling:* The vaginal swab sample was vortexed for 1 to 2 minutes to ensure thorough mixing. This step allowed the cells to get free from one another, and also, the cell clusters from the specimen collection device were set free. The slide was then labeled with a pencil to track the sample.

2. *Insertion into cassette:* The labeled slide was inserted into a cassette and held in place with a cup. In the chamber of the cassette, 1 ml of BD density reagent was added along with 1 ml of the sample.

3. *Centrifugation:* The cassette was centrifuged for 5 minutes at 230 rpm. During centrifugation, non-diagnostic debris and excess inflammatory cells were separated from the diagnostic cervical cells. The non-diagnostic debris and excess cells were discarded in the supernatant, while the diagnostic cervical cells settled in the sediment at the bottom of the cassette.

4. *Washing and drying:* The sediment containing the diagnostic cervical cells was washed twice with blended alcohol to remove any remaining debris. The slide was then dried on a hot plate to prepare it for staining.

5. *Staining:* Finally, the smear on the slide was stained using an LBC staining method to visualize the cervical cells and any abnormalities present. This method ensures that the cervical cells are properly prepared and stained for examination, allowing for accurate diagnosis and detection of any abnormalities.

4. 3. 1 Staining (BD Cytology stain kit, REF 491458)

The slide was Dip and out in Tris Buffer(800 milliliters of distilled water with 8. 76 grams of NaCl and 6. 05 grams of Tris buffer solution (pH 7. 6)). The volume was adjusted to 1L.

The sample was stained for four minutes with hematoxylin. To enable nuclear staining, the slide was stained in hematoxylin for 5 minutes.

The slide was again dipped and out in Tris buffer to get rid of the extra stain.

The slide was then dipped and out in blend alcohol (propanol 60. 1% and ethanol 99. 9%) to dehydrate the tissue.

The slide was again dipped in Blend alcohol (propanol 60. 1% and ethanol 99. 9%)

Next, The slide was stained in Eosin for 4 minutes. Eosin is likely a counter-stain or a differentiation solution.

Lastly, The slide was again dipped in alcohol and then it was rinsed in acetone.

The slide was rinsed in xylene.

Using the mounting medium DPX, mounted the slide to preserve the stained tissue and observed under a microscope at 20X magnification

4.3.2. Reporting of LBC

After the slide was prepared, microscopy analysis was performed with the assistance of a pathologist, following the Bethesda System. A standardized format for reporting results from vaginal and cervical cytology is called the Bethesda System. It provides specific criteria for the classification of cells. The stained slide was examined to find out the morphology and identify any abnormalities. The nucleus cytoplasmic ratio plays an important role in reporting cervical cytology. The nuclear-cytoplasmic ratio also referred to as the nucleus-to-cytoplasm ratio, N: C ratio, or N/C, is a metric utilized in the field of cell biology. This measurement quantifies the relative sizes, often in terms of volume, of a cell's nucleus compared to its cytoplasm. It indicates the proportion of cellular space occupied by the nucleus relative to the cytoplasm. The pattern of Bethesda is as follows (Nayar & Wilbur, 2017) Table 4.1.

Table 4.1: Bethesda System of Reporting Cervical Cytology

Acronym	**Description**	**Reference**
NILM	No cellular evidence of neoplasia. This term should be stated in the general categorization section or the interpretation/ result section of the report, whether or not there are organisms or other non-neoplastic findings.	(Nayar & Wilbur, 2017)
ASCof Undetermined Significance (ASC-US)	Abnormal cells where the alterations may not necessarily point to a precancerous lesion.	(Bajaj *et al*., 2022)
Low grade Squamous Intraepithelial Lesion (LSIL)	Cells exhibiting mild abnormalities, commonly linked to mild dysplasia (Cervical Intraepithelial Neoplasia, or CIN) or Human Papillomavirus (HPV) infection, are referred to as LSILs.	(Chakravarthy *et al*., 2022 ; Bamanikar *et al.*, 2016)

HighGrade Squamous Intraepithelial Lesion (HSIL)	Cells with moderate to severe abnormalities, indicating moderate or severe dysplasia (CIN1 and CIN2), are classified as HSIL.	(Sinchana *et al.*, 2022 ;Elsheikh *et al.*, 2006)
Squamous Cell Carcinoma (SCC)	Cells exhibiting characteristics of invasive malignancy are called squamous cell carcinoma.	Martín-Vallejo *et al*., 2022

4.4 HPV DNA Testing

HPV DNA was performed on a Truenat RTPC analyzer. It is the semi-quantitative detection of HPV types 16, 18, 31 and 45. It helps in the detection of symptomatic or asymptomatic infection in female cervical specimens. It works on the principle of Real-time PCR. Molecular Reagents Used: Template DNA, nucleotides, primers, and thermostable DNA polymerase.

4.4.1 Procedure of HPV Testing (TruenatReal Time PCR)

Truenat Real-Time PCR Test consists of:

- Universal Kit sample Pre Treatment Pack (Molbio Trueprep Auto)
- Universal cartridge-based Sample Prep Kit. (Molbio True Prep Auto).
- Chip-based Real-Time PCR. (Molbio Truenat HPV-HR)

4.4.2 Sample Pre-Treatment Pack (Decontamination)

The swab specimen was taken using a standard nylon-flocked swab. Specimen along with swab was inserted into transport medium and mixed well. Excess liquid was squeezed from the swab. Transport medium decontaminated the specimen and prepared it for further storage and extraction. The specimen was stable for up to 3 days at 40ºC / 7 days at 30ºC. Take 500 µl of sample in True prep AUTO Universal Sample Pre-treatment (USPT) Lysis buffer bottle using 1 ml disposable pipette. Vortex it well and keep it for incubation for 10 minutes at room temperature or 37ºCfor further procedure of DNA extraction. For effective cell lysis and nucleic acid release, the specimen had to be uniformly dispersed throughout the lysis solution, which required careful mixing of the tube's contents. To process the specimen further, the whole contents of the Universal Sample Pre-treatment Pack (USPT)

were used. The materials or methods in this pack were designed to clean the sample and get it ready for nucleic acid extraction. These chips are designed to detect HPV using purified nucleic acids extracted from patient samples. Since patient samples may contain high levels of PCR inhibitors, it's crucial to perform pre-treatment to remove these inhibitors and ensure accurate results.

4.4.2.1 Material used for Trueprep Auto Universal sample Pre-treatment Pack

1. Lysis buffer
2. Disposable pipette

4.4.2.2 Storage and Stability

The Universal Sample Pre-treatment Pack was stored between 2 and 40°C and remains stable for two years after it is manufactured. At 45°C and above, it remained steady for a month as well.

4.5 Universal cartridge-based sample preparation kit. (Extraction)

After the pretreatment process open the Trueprep AUTO UNIVERSAL CARTRIDGE-based sample prep kit pack and place the cartridge on cartridge on stand. Open the black cap of the cartridge; transfer all the content of USPT using 3 ml of disposable pipette and close the cap. The process gets started. Nucleic acids extracted from cervical sample biological specimens were purified using the Universal Cartridge Sample Prep Device. Nucleic acids from cervical samples were purified using the Truprep AUTO Universal cartridge Bases Sample Kit procedure, which uses a proprietary matrix contained in a cartridge. Additionally, the cartridges have Internal Positive Control (IPC) that was already loaded. An analysis from sample to result was validated by the IPC, a comprehensive process control that goes through every step of the specimen's processing, from extraction to amplification.

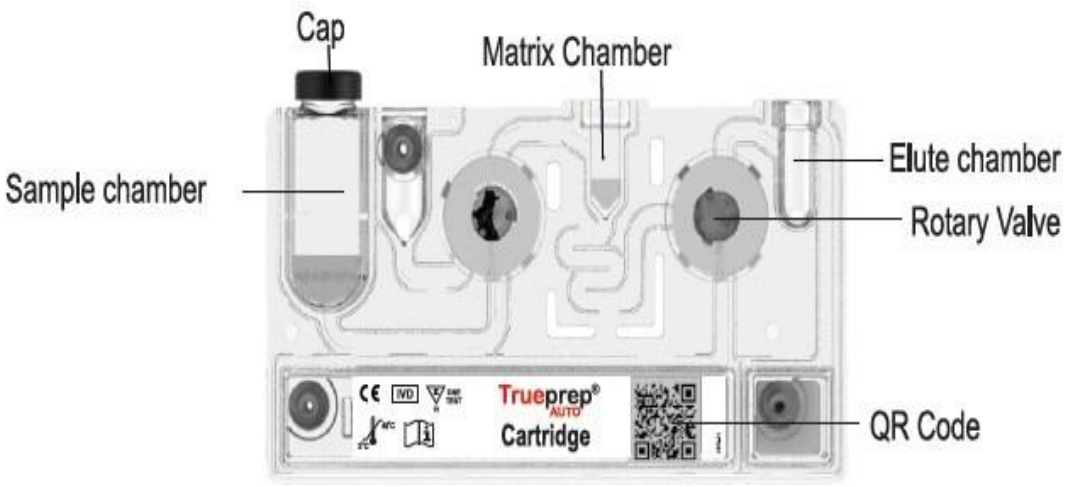

Figure 4.2: Illustration depicting the Universal Cartridge for DNA extraction and purification of samples (Image courtesy: Hariprasad *et al*., 2020)

The bound DNA/RNA was eluted and collected in an elution chamber upon completion of processing. The cartridge's dump area holds all of the waste produced during the operation. The elute was moved into the tube used for elute collecting (ECT). After that, six microliters of the elute were transferred to Truenat chips so that the True Lab Real-Time PCR analyzer could continue to examine it.

4.5.1 Materials Used

A. The Reagent Pack comprised of:

1. Wash Buffer A and B - To wash inhibitors from the sample

2. Elution Buffer- To elute nucleic acids

3. Priming Waste -To purge residual liquid from tubing (Sherman *et al.*, 1994).

Table 4.2. Contents in extraction kits

Contents	**Purpose**
1. Cartridges	Cartridges with immobilized internal control.
2. Elute collection tube	for collection and storage of extracted nucleic acids

3. Disposable Transfer	for piercing the seal of the elute chamber and transferring pipette extracted nucleic acids from the elute chamber of the cartridge. 3 ml (Remmink *et al.*, 1995).

4.5.2 Storage and Stability

Trueprep AUTO Universal Cartridge Based Sample Prep Kits were stored between 2°C and 40°C that can remain stable for two years after the date of manufacture. Additionally, they remained stable for a full month at temperatures as high as 45°C.

4.6 Real-time PCR-based test

Real-time quantitative polymerase chain reaction is a PCR approach that enables real-time monitoring of the PCR's progress. Through numerous orders of magnitude, a chosen HPV DNA was amplified in vitro using PCR, an enzymatic process that produces thousands to millions of copies of a particular DNA segment. Real-time PCR is a technology that offers a dependable means of quantifying the relationship between the number of target sequences that were started before amplification by PCR and the total amount of amplicon that was accumulated during a certain PCR cycle. The E6 and E7 genes of high-risk HPV types 16, 18, 31, and 45, contained the target sequences for this test.

The required components are

1. Primers (Sequences by the manufacturer)
2. Thermostable DNA polymerase,
3. Nucleotides (dNTPs), and template DNA.
4. Probe
5. Dye (SYBR Green I)
6. Thermocycler attached with spectrophotometer.
7. DNA Chip

4.6.1 Quantification

The quantity of amplicon produced after a predetermined number of PCR cycles depends on the number of DNA molecules present in the starting mixture. Relatively little amplicon will be produced if the PCR process begins with few DNA molecules present. Conversely, a higher amount of product will result from a larger amount of beginning molecules. Because of this relationship, the amount of product created by PCR can be used to determine the number of DNA molecules present in samples.

A typical real-time PCR amplification curve was created by plotting the amount of PCR product (amplicon) against the number of reaction cycles. The amplification curve had three major phases: the linear (beginning), exponential (logarithmic-linear), and plateau phases. The levels of the product's fluorescence emission during the first few cycles of the PCR process indicated the linear ground phase and did not go above the baseline. PCR reached its ideal amplification phase during the exponential phase, doubling the result after each cycle. During this phase, all the reaction components were not limiting, and the perfect reaction conditions were reached.

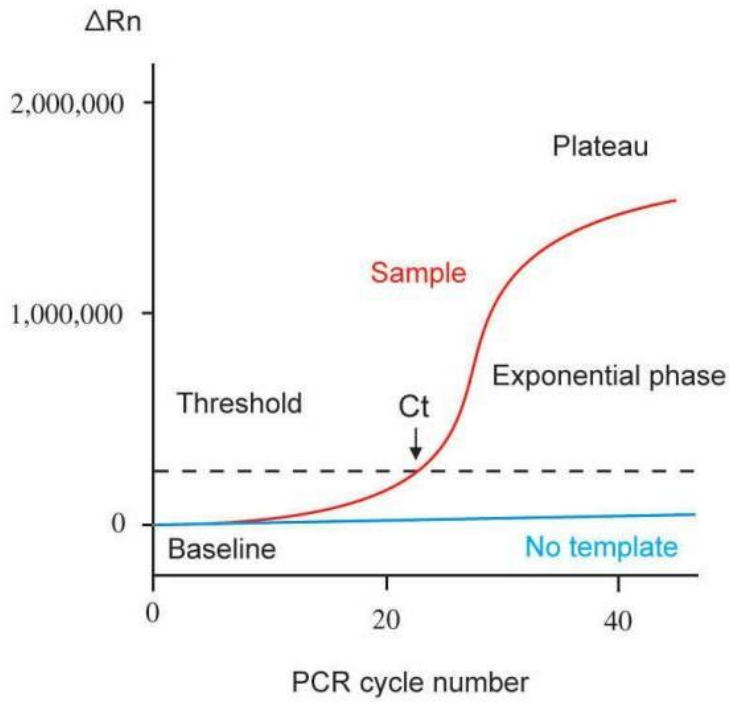

Figure 4.3: Graph displaying a single quantitative PCR amplification curve in real-time.

The amplicon's fluorescence emission at each time point less the baseline's fluorescence emission is the formula for ΔRn. Ct represents the threshold cycle. A baseline is the accumulation of the fluorescent signal of a reporter over PCR cycles. However, it is less than the detection limits of the device. The number of amplification cycles needed for the fluorescent signal to exceed the threshold is identified as the Cycle threshold (Ct) (Molijn *et al.*, 2005).

4.6.2 Dyes used in PCR fluorescence

SYBR Green I was the primary dye molecule used for the fluorescence process. It is attached to the double-stranded DNA minor groove. Although it barely showed any fluorescence, free SYBR Green I bound to double-stranded DNA. Its fluorescence signal could then be amplified hundreds of times. The amount of dye that bound to the PCR product increased along with it, and the amount of double-stranded DNA molecules was indicated by the intensity of the fluorescence signal.

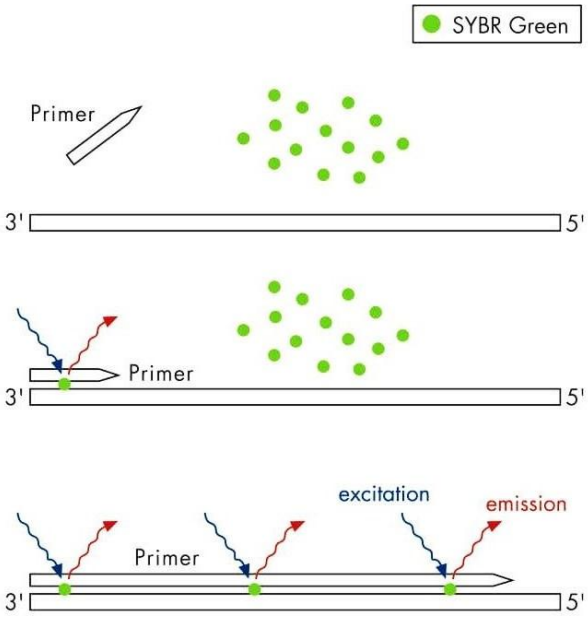

Figure 4.4: SYBR Green I dye functions as an intercalating agent for double-stranded DNA. Image courtesy: (Overbergh *et al.*, 2009)

The fluorescence is negligible when it is free in solution. SYBR Green I molecules, however, intercalate into the double-stranded amplicons during primer elongation and polymerization, increasing detectable fluorescence through this process.

4.6.3 Probes for PCR Fluorescence

TaqMan Probe was used as a hydrolysis probe, which binds to a particular target DNA sequence. Its mode of action is dependent on Taq polymerase's 5′–3′ exonuclease activity, which hydrolyzes the attached probe during PCR amplification. A fluorescent reporter dye was present at the 5′ end of the TaqMan probe, while a quencher dye was present at the 3′ end. Fluorescence was quenched when the reporter and quencher were in close proximity when they were intact. The reporter dye was released during PCR upon binding to the target sequence and cleavage by the polymerase, increasing fluorescence. Fluorophores were captured by the optoelectronic sensor and displayed an amplification curve.

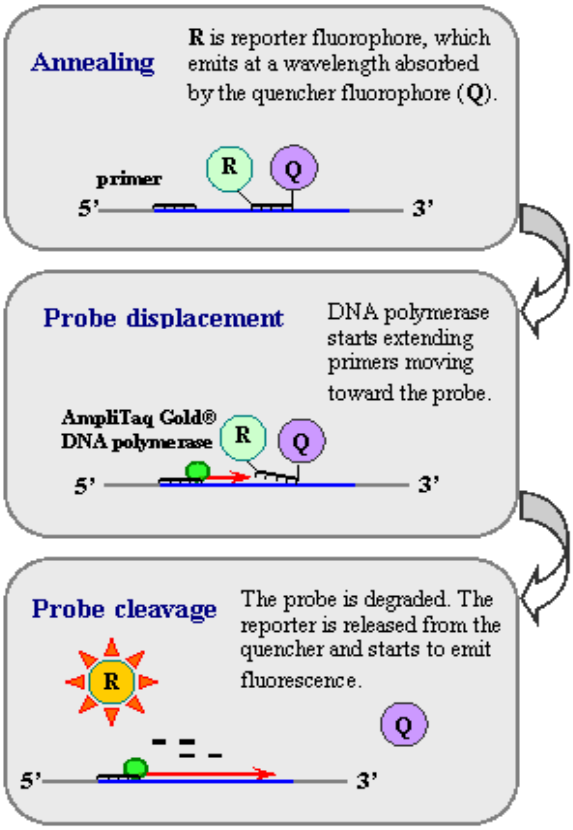

Figure 4.5: Illustration depicting the TaqMan probe assay. (Image courtesy: https://www.ncbi.nlm.nih.gov/probe/docs/techqpcr/)

The Taqman utilizes the 5' endonuclease activity of Taq DNA polymerase to cleave an oligonucleotide probe during PCR, producing a detectable signal. The probes are fluorescently labeled at their 5' end and are non-extendable at their 3' end due to chemical modification.

Specificity is achieved at three levels: through two PCR primers and the probe. The findings on the screen showed "DETECTED" for a positive result or "NOT DETECTED" for a negative result at the end of the test run. For positive specimens, the viral load was shown on the result screen as "HIGH (Ct<20), " "MEDIUM (20≤Ct<25) ", "LOW (25≤Ct<30) " or "VERY LOW (Ct ≥ 30) ".

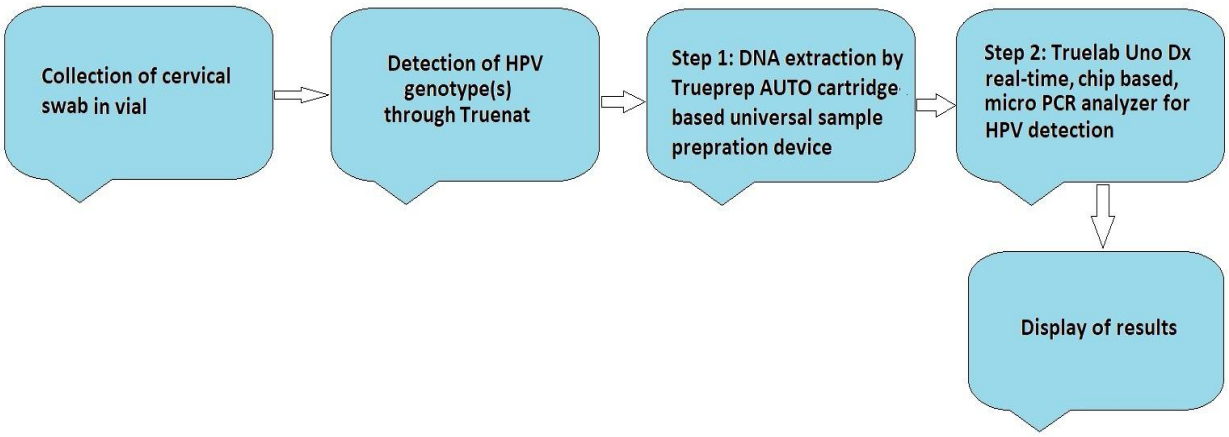

Figure 4.6: Truenat Real-Time PCR study design for the detection of HPV genotypes.

4.7 Detection of HPV genotype samples by QIAGEN multistrain Analyzer

In the case of abnormal cytology samples that did not test positive for high-risk HPV strains such as HPV 16, 18, 31, and 45, individuals might still be infected with other HPV strains. Further testing with the QIAGEN multistrain detection system was performed on these samples to find out the potential risk.

4.7.1 QIA screen HPV PCR Test

The QIAscreen HPV PCR Test employs fluorescent probes to detect one or more accumulating PCR products. It is a multiplex, real-time PCR-based assay targeted against the E7 gene of 15 (possibly) hrHPV strains. An amplification curve is produced after the fluorescent signal grows logarithmically throughout each PCR cycle. The sample is regarded as positive for that target after the target's amplification curve crosses its threshold. Four distinct fluorescent dyes, each representing a different target, can be detected simultaneously in a reaction. 1). HPV 16, 2). HPV 18, 3). The pool of 13 other hrHPV strains, and 4. The human β-globin gene is the four distinct targets. HPV 16, HPV 18, and the pool of 13 additional hrHPV genotypes are all individually

detected by the QIAscreen HPV PCR Test. The human β-globin gene serves as a sample control to assess the DNA quality of the sample and identify any possible inhibiting agents.

4.7.1.2 Kit contents

The components of the QIAscreen PCR-based kit for HPV detection are listed in Table 4.3.

Table 4.3: Kit Contents

Name of Component	Description
Master Mix (1 tube)	Transparent color 1080 µl
Positive Control (1 tube)	Transparent color 100 µl
Negative Control (1 tube)	Transparent color 100 µl

4.7.1.3 Consumables and Equipment

Hologic PreservCytSolution, Standard DNA extraction kits, such as QIAampMinElute Media kits (QIAGEN, cat. no.937036), 0.1 ml strip tubes and caps.

4.7.1.4 Equipment

Pipette 1-10ul and 10-100ul, Sterile DNAse-free pipette-tips, Disposable gloves, Vortex mixer, Benchtop centrifuge.

4.7.1.5 Equipment for real-time PCR

Rotor-Gene Q 5plex HRM System or Rotor-Gene Q MDx 5plex HRM instrument having Rotor-Gene Q software 2.3.1 or higher. QIAscreen run template and QIAscreen channel analysis templates for channels green (HPV 16), red (HPV 18), yellow (HPV Other), and orange (β-globin).

4.7.2 Sample Preparation

4.7.2.1 DNA extraction

The assay was compatible with DNA extraction kits such as QIAamp MinElute Media. Cervical samples were suspended in Surepath, CellSolution, and PreservCyt. The QIAscreen HPV PCR Detection Kit was used to target the HPV genome's E7 gene. A single examination of the PCR test could identify 15 high-risk HPV (HR-HPV) types. Individual findings for the HR genotypes,

HPV-16 and HPV-18, and pooled data for the remaining HR genotypes such as 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68, were provided.

4.7.2.2 Sample processing

4.7.2.3 PCR on Rotor-Gene Q 5 plex

The percentage of DNA utilized as PCR input for cervical specimens (scrapes) was suspended in Surepath containing 0.25% of the 10 ml Surepath sample. This equated to 25 µl of each type of sample. DNA extraction procedures were carried out so that 5 µl DNA extract corresponded with a 25 µl cervical specimen (scrape) sample since at most 5 µl of extracted DNA could be used as input in the PCR. This ensured that the right fraction of the cervical sample was used in the PCR alongside a positive and negative control.

4.7.2.3 a QIAscreen HPV PCRSet up:

4.7.2.3b Preparation of the Rotor-Gene Q 5 plex

The bench area, pipettes, and tube rack were cleaned using a DNA-degrading solution before use to prevent template or nuclease contamination. The solution was mixed gently by inverting 10 times, then briefly centrifuged before use to collect the solution at the bottom of the tube.

⇩

15 µL of the QIAscreen Master Mix was dispensed into the appropriate tube strips. The reaction setup was carried out at room temperature. Once dispensed, return the QIAscreen Master Mix to the freezer to avoid any material degradation.

⇩

The tubes were transported to a separate area to dispense the QIAscreen positive control and DNA sample.

⇩

Added 5 µl of the negative control to tube position 2. Mixed it by pipetting up and down or by flicking the tube, and then closed the tube by pressing the cap.

⇩

Added 5 µl of the QIAscreen positive control to tube position 1. Mixed it by pipetting up and down or by flicking the tube, and then closed the tube.

⇩

Added 5 µl of DNA sample to the appropriate tubes containing the QIAscreen Master Mix. Mixed it by pipetting up and down or by flicking the tubes, and then closed the tubes by pressing the caps.

⇩

Once a set of 4 tubes had been filled, the tubes were capped.

4.7.3 Interpretation

QIAscreen positive control samples gave Ct values <29 for β-globin, <30 for HPV 16 and HPV 18, and <32 for other HPV. In case there was any discrepancy, though the analysis settings were corrected, the experiment was repeated. QIAscreen negative control samples should give a signal above the threshold till the end of the PCR run. In case of a signal before cycle 40, the experiment was repeated.

4.7.4 Interpretation of the sample results

1. If the Ct value of HPV 16 or HPV 18 is < 36, another HPV is <33.5, and the Ct value of β-globin is any then HPV results were positive.
2. If the Ct value of HPV 16 or HPV 18 is ≥36 or not defined other HPV is ≥33.5 or not defined and the Ct value of β-globin is ≤30 HPV results were negative.
3. If the Ct value of HPV 16 or HPV 18 is ≥36 or not defined other HPV is ≥33.5 or not defined and the Ct value of β-globin is >30 then the HPV result is Invalid.

4.8 Incidence

The number of new instances or incidents for the population at risk of the event during a given time period is known as the incidence. In medicine, the term "incidence" refers to the number of newly discovered cases of a disease or condition per population that is considered at risk within a given time period.

4.9 Prevalence

The percentage of people in a population that has a specific disease or condition at a given time or during a given period of time is known as its prevalence. It is often expressed as a proportion or a percentage.

4.10 Statistical Analysis

Data from all 4000 patients was subjected to statistical analysis. The P-value at 95% CI was calculated by regression and chi-square test for measuring the significant association of different epidemiological factors with HPV infection. The primary objective of the statistical analysis was to identify correlations between eight different factors and the HPV strain. Regression analysis was selected as the method to explore the relationship between each factor and the HPV strain. Our analytical and statistical tasks were conducted using the 'Analysis Pack' in Microsoft Excel in conjunction with Power BI. The 'Analysis Pack' offers robust analytical capabilities, enabling a comprehensive analysis leading to data-driven conclusions. Power BI was specifically chosen for its advanced data visualization and business intelligence features, facilitating the creation of interactive charts based on regression analysis results.
In our regression analysis, the "HPV Strain" served as the independent variable (Y variable), while each factor was considered a dependent variable (X variable). Through the regression analysis performed using the 'Analysis Pack, ' we assessed the p-values obtained. With p-values below 0.05, we rejected the null hypothesis, confirming the relationships between the variables. This process was repeated for each factor individually and collectively to determine the factor contributing the most to the HPV strain.

Chapter 5

RESULTS

5.1 Patient enrollment:

4000 patients were enrolled in the study after obtaining their consent. A detailed history regarding symptoms and risk factors was taken and filled in the questionnaire.

5.2 Sample collection:

In this study, 4000 women's cervical tissue samples (exfoliates) were collected from the Out Patient Department (OPD) of Gynecology and Obstetrics, Govt. Medical College (GMC), Jammu. In the Swastik Diagnostic Laboratory and GMC Jammu, Pap tests were conducted and HPV testing was done at Jammu's Swastik Diagnostic Laboratory,

5.3 Demography

A total of 4000 female subjects were analyzed for cervical cytology. Table 5.1 shows the age-wise distribution of patients screened for cervical cancer screening. In our study out of the total 4000 patients enrolled 2471 patients (61.77%) were Hindus while 1394 patients belonged to Islam comprising 34.85% of the sample size. 107 patients comprising 2.7% of the population were Sikhs while 28 patients constituting 0.7% of the population belonged to Christianity. Among these women 3356 were married, 361 were divorced and 283 females were widowed. Patients were categorized into four age groups 25-35, 36-45, 46-55, and 56-65 years. The highest number of patients, 40.4% (1615) was seen in the age group 36-45 years, followed by 38.8% (1553) in the age group 25-35 years, 14.9 % (597) in the age group 46-55 years and 5.9% (235) in the age group 56-65 respectively. Out of 4000 women, 45.10% were married early before 22 years of age, 24.67% between 22-25 years, 21.40% between 25-30 years, and 8.8% were married after 30 years. In terms of pregnancy majority of the women enrolled 43.62% were pregnant before 22 years of age, 25.57% of women were pregnant between 22 and 25 years, and 23.5% of women were pregnant between 25 and 30 years while only 7.3% of women were pregnant after 30 years of age. In terms of parity majority of the women, 2210 (55.25%) were uniparous, 16.97% of women had 1-2 children, 724 (18.10%) women had 2-3 children, 217 women (5.42%) had 3-4 children while 170 females (4.25%) had 4 or more children. The majority of the patients enrolled in the study (61.57%), belonged to the rural background while 38.42% of women were

residents of urban areas. The most common gynecological symptoms reported by the females enrolled in the study included lower abdominal pain in 27.4% of patients, vaginal discharge in 25.5% of patients, menstrual disorder in 22.6% of patients, and itching and vaginal burning in 22.4% of patients. The educational status of the females was also taken into consideration the majority of the females (46.85%) had access to primary education, 30.77% of females were educated up to high school, 12.05% of females had less than primary education while 10.32% females had education higher than high school. The economic status of the women enrolled was also recorded. Out of the total women enrolled in the study majority (42.17%) belonged to low economic status having an average income less than 200000 per annum, 30.90% of women had an average family income between 200000-and 400000, 18.57 women had an average family income of 400000-600000 and 8.35 % women had an average family income more than 600000.

Table 5.1: Distribution of various epidemiological factors among the patients enrolled for the study.

Category Factor	**No of patients**	**Percentage**
Age in Years	**N=4000**	**%**
25-35	1553	38.8
36-45	1615	40.4
46-55	597	14.9
56-65	235	5.9
Religion		
Hindu	2471	61.77
Islam	1394	34.85
Sikh	107	2.67
Christianity	28	0.7
Area of residence		
Rural	2463	61.57

Urban	1237	38.42
Marital status		
Married	3356	83.9
Divorced	361	9.02
Widowed	283	7.07
Early Marriage		
<22	1804	45.10
22-25	987	24.67
25-30	856	21.40
>30	353	8.8
Early Pregnancy		
<22	1745	43.62
22-25	1023	25.57
25-30	940	23.5
>30	292	7.3
Parity		
0-1	2210	55.25
1-2	679	16.97
2-3	724	18.10
3-4	217	5.42
>4	170	4.25
Education status		
Less than Primary	482	12.05
Primary	1874	46.85
High School	1231	30.77

>High School	413	10.32
Gynecological symptoms		
Pain in the Lower Abdomen	1136	28.40
Vaginal discharge	1049	26.22
Menstrual Disorder	974	24.35
Itching and Vaginal Bleeding	841	21.05
Economic Status Income/annum		
>200000	1687	42.17
200000-400000	1236	30.9
400000-600000	743	18.57
>600000	334	8.35
Status of Smoking		
Non-Smoker	3308	82.7
1-5 cigarettes/day	507	12.67
5-10 cigarette/day	153	3.83
>10 cigarettes/day	32	0.8
Use of Oral Contraceptives		
No Pill	2796	69.9
Taking contraceptives for up to 1 year	806	20.15
Taking contraceptives for more than 1 year	398	9.95
History of STD		
Yes	1211	30.27
No	2789	69.72

In terms of smoking status, 82.7% of females enrolled were non-smokers, while 12.67% of females smoked 1-5 cigarettes per day, 3.83% of females smoked 5-10 cigarettes per day and 0.8% of females smoked more than 10 cigarettes per day. History of Sexually transmitted diseases was also one of the epidemiological factors studied in our research. It was observed that 69.72% of the females enrolled in the study had no prior exposure to sexually transmitted diseases while 30.27% of females had encountered sexually transmitted diseases earlier in their life. Another epidemiological factor studied in the research was the use of oral contraceptives. It was observed that 69.9% of females did not use oral contraceptives in their lifetime while 20.15% of females have used such pills for a duration of up to 1 year and 9.95% of females have been exposed to oral contraceptive pills for more than one year. The study showed that Muslim women (Gujjar, Bakarwal) were observed to be more susceptible to HPV infection. This susceptibility to HPV infection among this subset was due to their association with low socioeconomic status and a higher rate of multiparity.

5.4 Primary Screening:

4000 females were subjected to primary screening by PAP smear and HPV testing. Out of the total females screened by pap smear testing, a maximum of 3094 (77.4%) were normal. 682 (17.0%) females had inflammatory cytology. Rest 224 (5.5%) females were PAP smear positive i.e.cervicitis or another benign lesion. Among these PAP smear-positive female samples, 135 (3.4%) female samples were typed (ASCUS), 49 (1.2%) female samples were LSIL type, 33 (0.8%) female samples were HSIL type and 7 (0.2%) female samples were SCC type.

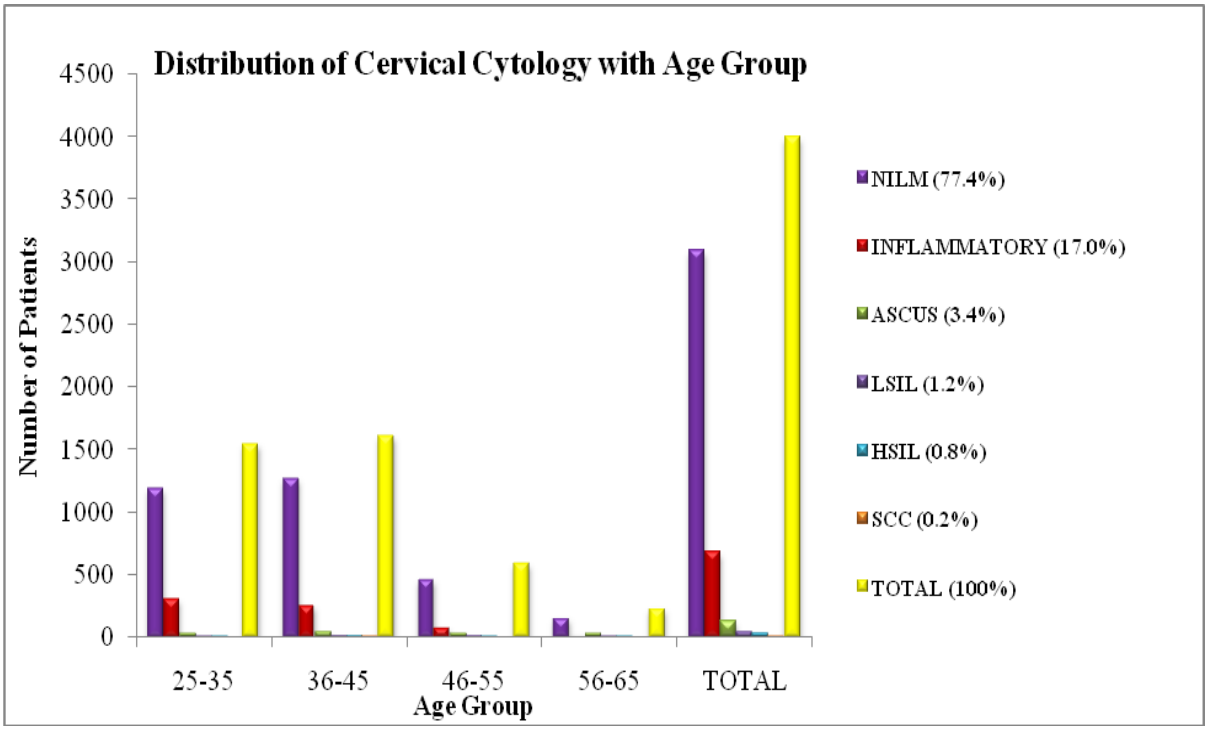

Figure 5.1 Bar graph of age group-wise distribution of women enrolled for cervical cytology (LBC) in the study.

In the present study, women were categorized by the cytology results NILM, Inflammatory, ASCUS, LSIL, HSIL, and SCC. NILM (Negative for intraepithelial lesion or malignancy), Inflammatory (Indicated inflammation which may or may not be cancerous, ASCUS (Atypical cells of undetermined significance), LSIL (Grade Squamous intraepithelial lesion), HSIL (High grade Squamous intraepithelial lesion), SCC (Squamous cell carcinoma). It was observed that the testing rates for Liquid-Based Cytology (LBC) varied across different age groups. Specifically, 38.8% of individuals in the 25-35years age group, 40.4% in the 36-45 years age group, 14.9% in the 46-55 years age group, and 5.9% in the 56-65 years age group underwent LBC testing. After analyzing the test results, it was found that 77.4% of women had a normal result (NILM), 17.1% showed signs of inflammation, 3.4% had Atypical Squamous Cells of Undetermined Significance (ASCUS), 1.2% presented with Low-grade Squamous Intraepithelial Lesion (LSIL), 0.8% reported. High-grade Squamous Intraepithelial Lesions (HSIL) and 0.2% were diagnosed with Squamous Cell Carcinoma (SCC) (Figure 5.1).

5.5 Micrograph of Cervical Cytology

PAP smear cytology of routine smear showed superficial (pink) and few intermediate blue cells. In contrast, the inflammatory smear showed superficial & intermediate cells obscured by a dense acute inflammatory infiltrate (Figure 5.2).

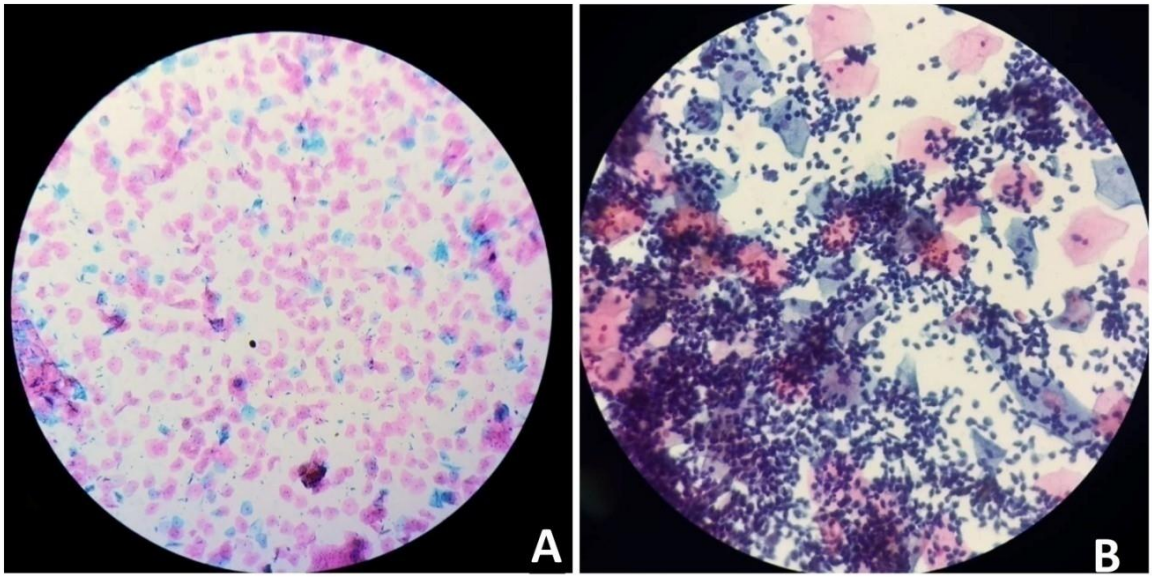

Figure 5.2: Micrograph of LBC of (A) Normal Pap smear with superficial (pink) & few intermediate (blue) cells (B) LBC inflammatory smears with superficial & intermediate cells obscured by a dense acute inflammatory infiltrate.

LBC of ASCUS showed cells with an enlarged nucleus as compared to normal. LSIL showed a cluster of intermediate cells having enlarged nuclei with irregular outlines (red arrow) (Figure 5.3). In contrast, HSIL showed parabasal cells with enlarged hyperchromatic nuclei, with the background showing dense inflammation. Compared to LSIL, HSIL exhibited smaller squamous cells that were present singly or in syncytial groups with higher nuclear-to-cytoplasmic ratios and more pronounced nuclear abnormalities.

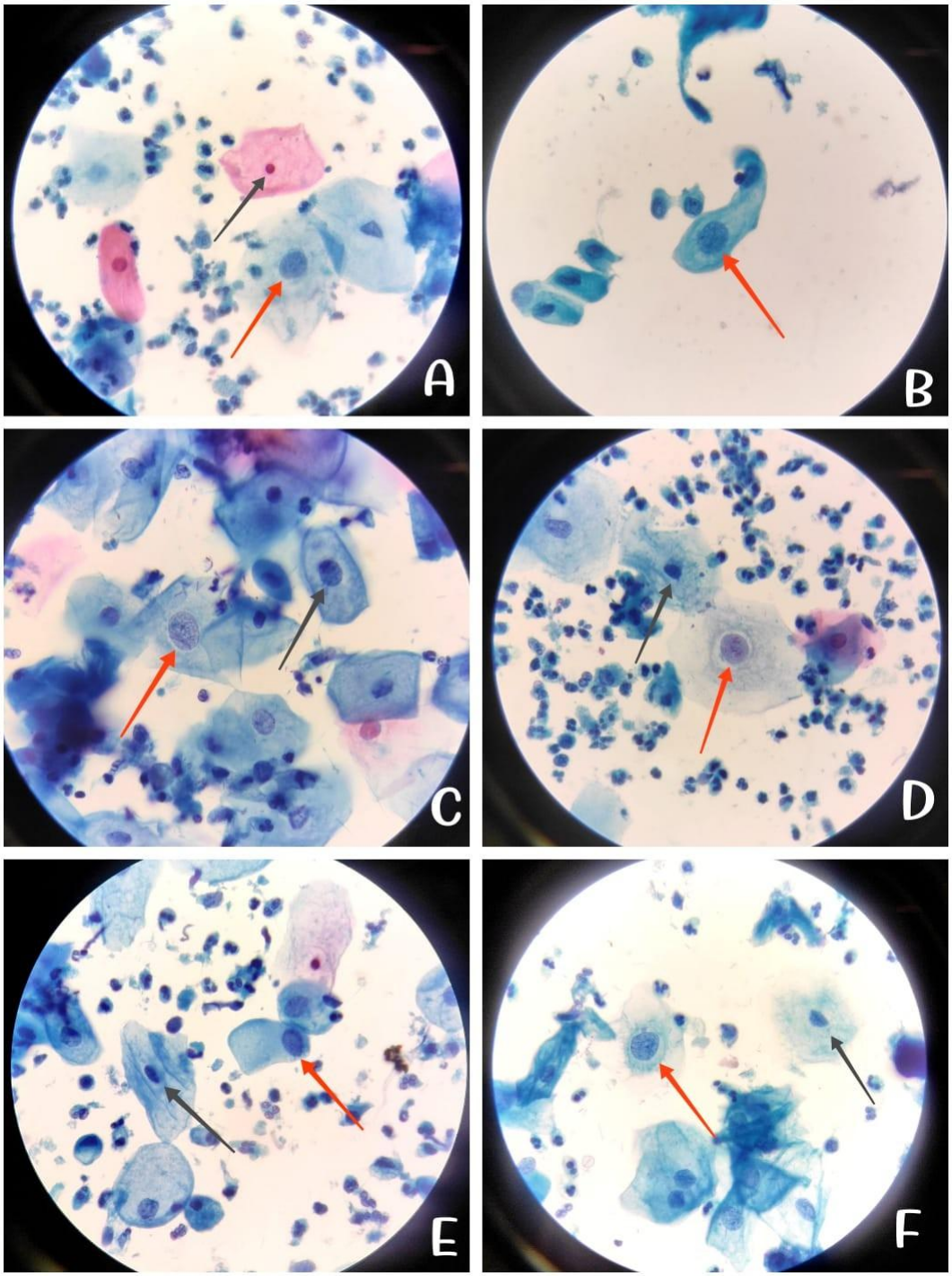

Figure 5.3: Representative micrographs of atypical cells of undetermined significance: (A to F) LBC at high magnification revealing a cell with an enlarged nucleus, 2-3 times (red arrow) compared to normal cells (black arrow).

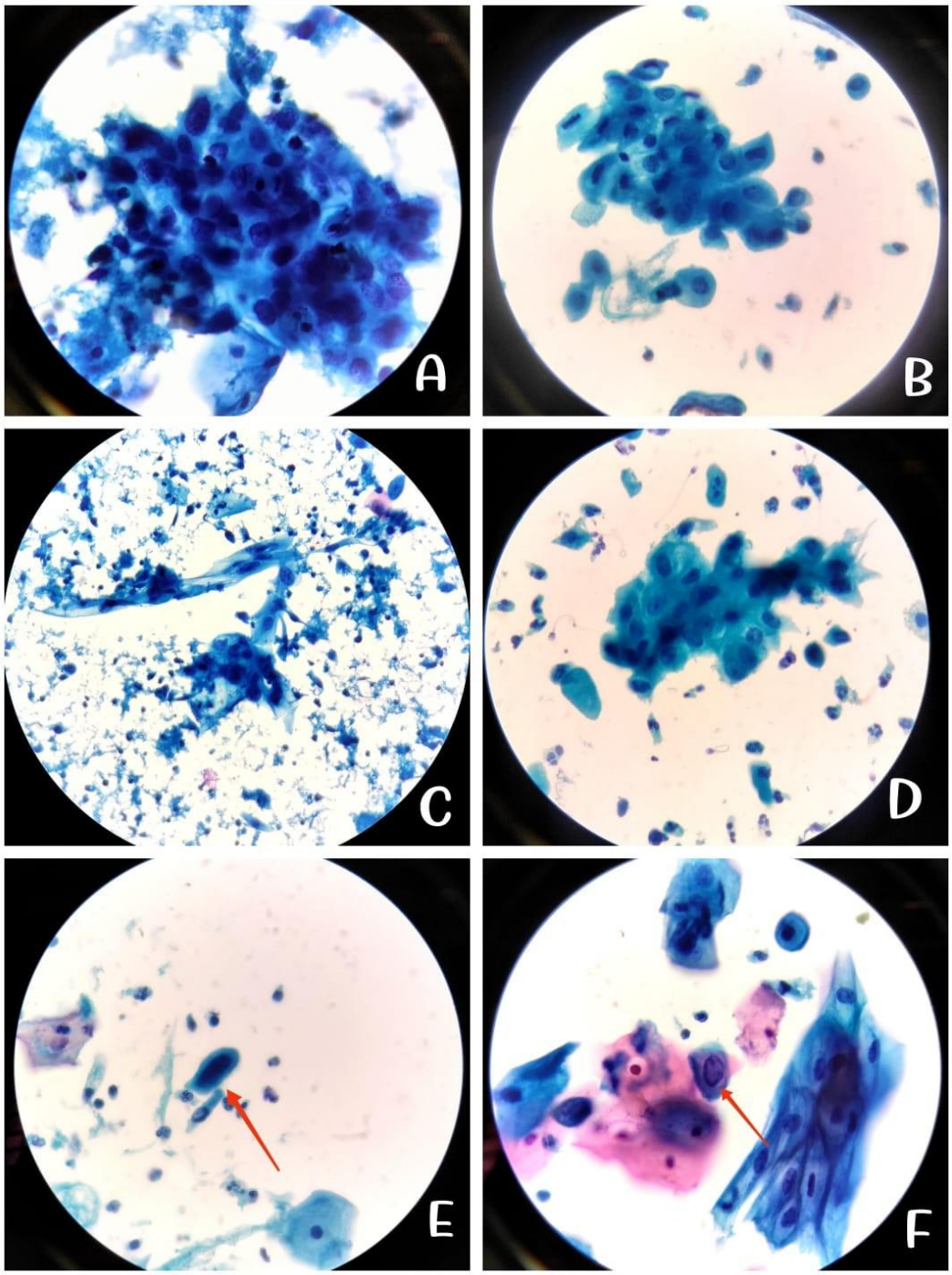

Figure 5.4: Representative micrographs of Low-grade squamous intraepithelial lesion: (A – F) (LBC at high magnification showing a cluster of intermediate cells having enlarged nuclei with irregular outlines (red arrow). The atypical cell has a nucleus greater than three times the size of an intermediate squamous cell.

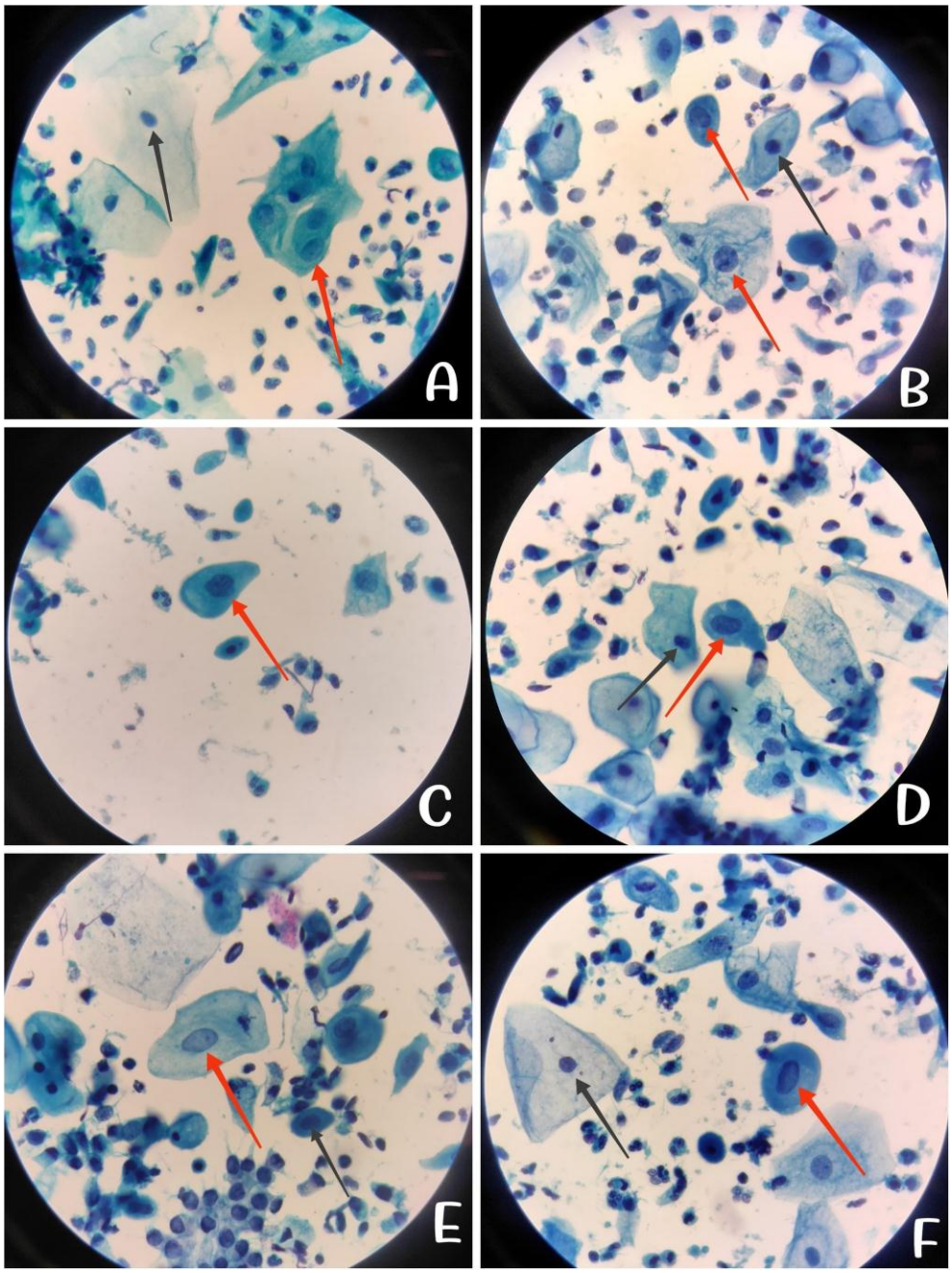

Figure 5.5: Representative micrographs of high-grade Squamous intraepithelial lesion: (A-F) LBC at high magnification showing Parabasal cells with enlarged hyperchromatic nuclei; Cell shows a high nuclear-to-cytoplasm ratio.

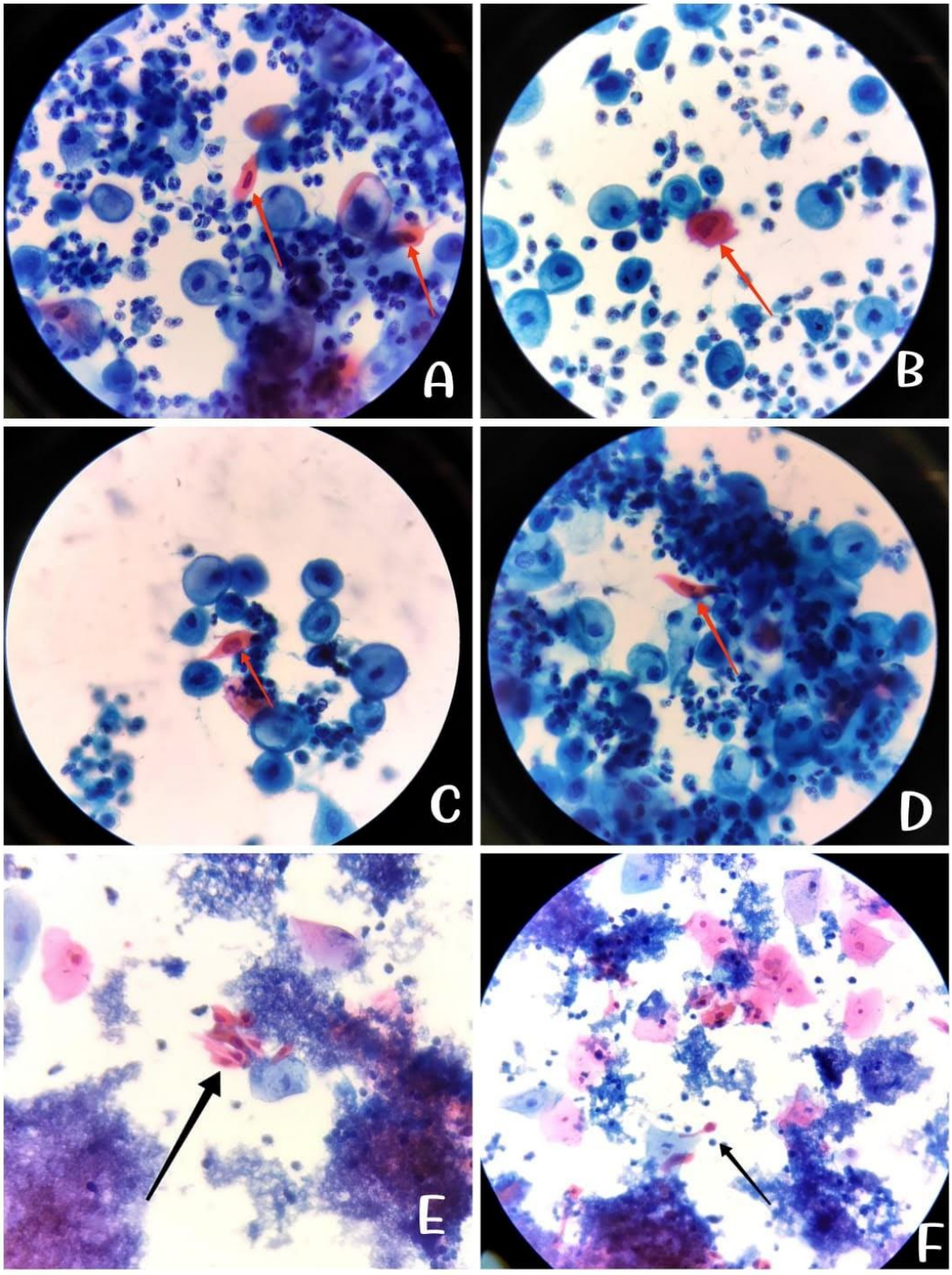

Figure 5.6: Representative micrographs of Squamous Cell Carcinoma: (A-F) LBC at high magnification showed a group of orangeophilic cells with irregular, hyperchromatic nuclei. (b) The arrow depicts a tadpole-like cell (indicative of SCC)

5.6 Analysis of Inflammatory Samples

The inflammatory samples in the primary samples were further subjected to secondary screening. Inflammatory samples in LBC were those in which white blood cells were present in the smear. Women with such samples were advised by the gynecologist to undergo screening again. The maximum number of such samples appeared negative, and some women did not appear for screening. As such inflammatory samples were ruled out, and samples with ASCUS, HSIL, LSIL, and SCC were carried forward for further analysis. A total of 224 abnormal samples out of 4000 were selected to study the symptoms.

5.7 Symptoms

Women with abnormal cytology reported experiencing certain symptoms. Pain in the lower abdomen was the most common complaint reported by 27.4% of women with abnormal cytology. The second most common complaint 25.5 % among women was vaginal discharge, followed by menstrual disorder reported in 22.6 %of women. 22.4% of women with abnormal cytology were experiencing itching and vaginal burning, while 2.1% of patients had other symptoms (Figure 5.7).

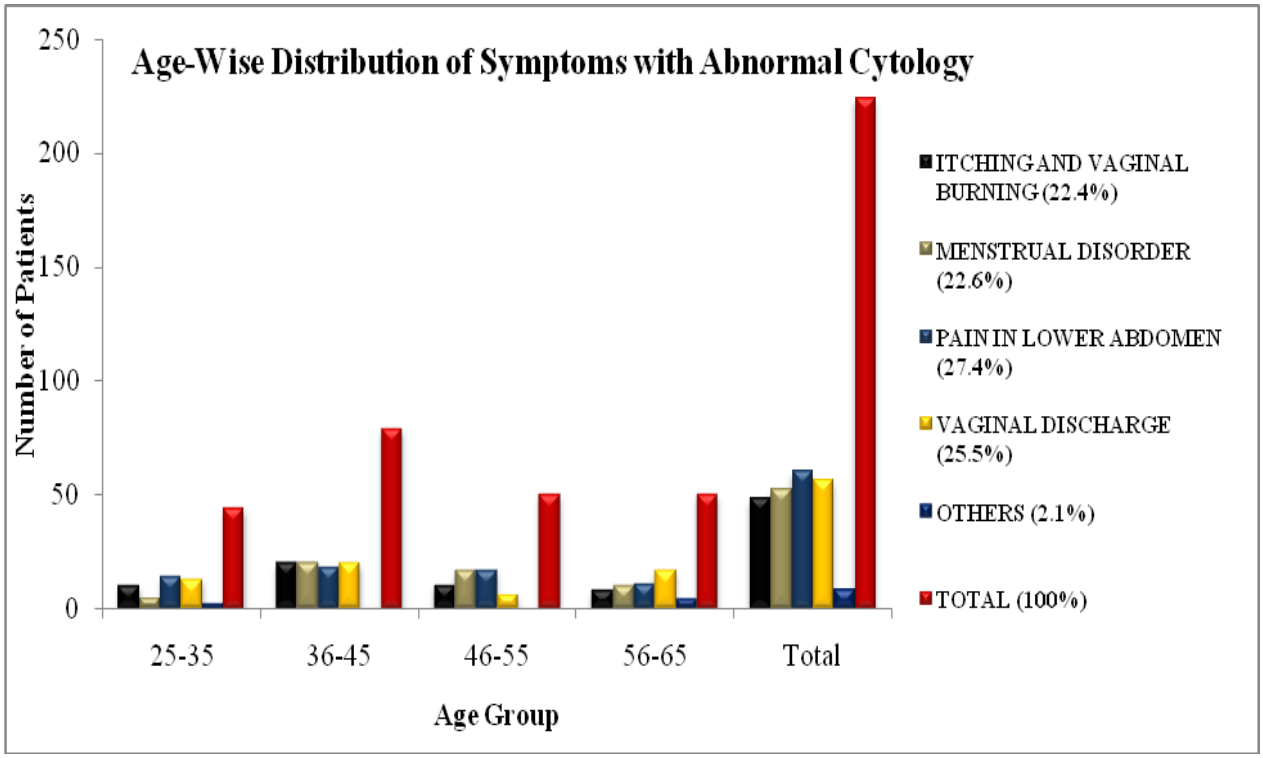

Figure 5.7: Bar graph of age-wise distribution of symptoms among abnormal patients.

Based on the findings of the study, indicated that there was variation in the reporting of symptoms among women across different age groups. The data suggested that the age group 36-45 years had the highest percentage of women reporting symptoms at 33.22%. Following closely behind, the age group 46-55 years had a slightly lower percentage of 23.77% of women reporting symptoms. Additionally, the age group 56-65 years also showed a substantial percentage, with 22.05% of women reporting symptoms. In contrast, the data indicates that there was a notably lower reporting of symptoms at 20.96%.

5.8 HPV DNA detection

4000 females enrolled in LBC were also subjected to HPV testing via Truenat HPV detection (MOLBIO KIT) for the HPV type present in these females. It was observed that 3.9% (155) of these females were HPV 16/31 type while 1.7% (66) females were HPV 18/45 positive. In 94.2% (3769) of the cases, no HPV strain was detected; in 0.3% (10) of the cases, HPV detection could not be done due to an inappropriate sample (Figure 5.8).

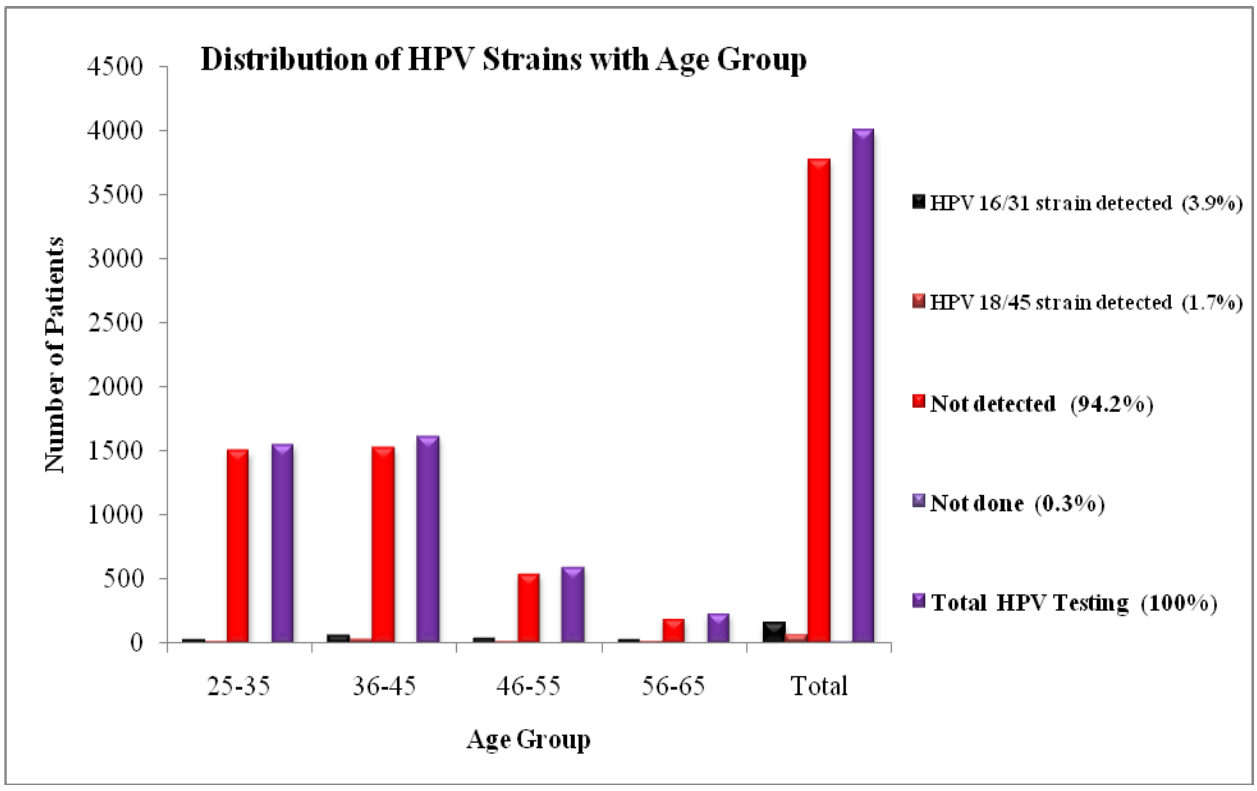

Figure 5.8: Bar graph of HPV DNA detection results in samples

5.8.1 Graph Illustration:

The Truelab Real-Time Quantitative Micro PCR Analyzer shows three amplification curves to check the test progress. These curves show the fluorescence signal produced when the target and internal positive control are amplified (IPC). When the fluorescence passes the threshold value in the case of positive samples, one or both of the target and the internal positive control (IPC) curves will follow a steep, exponential path. The quantity of viral genomes in the sample will determine the Ct. The target curve will stay horizontal for the whole test when a sample is negative and the IPC curve will follow an exponential route. If the IPC curve remains horizontal in a negative sample, the test is considered invalid. The graphs displayed three amplification curves, with the green line representing the control value, the blue line representing the 18/45 HPV strain, and the red lines representing the 16/31 HPV strain. The final result is displayed as "detected" or "not detected." Additionally, the viral load is categorized as "very low," "low, " "medium, " or "high." (Figure 5.9).

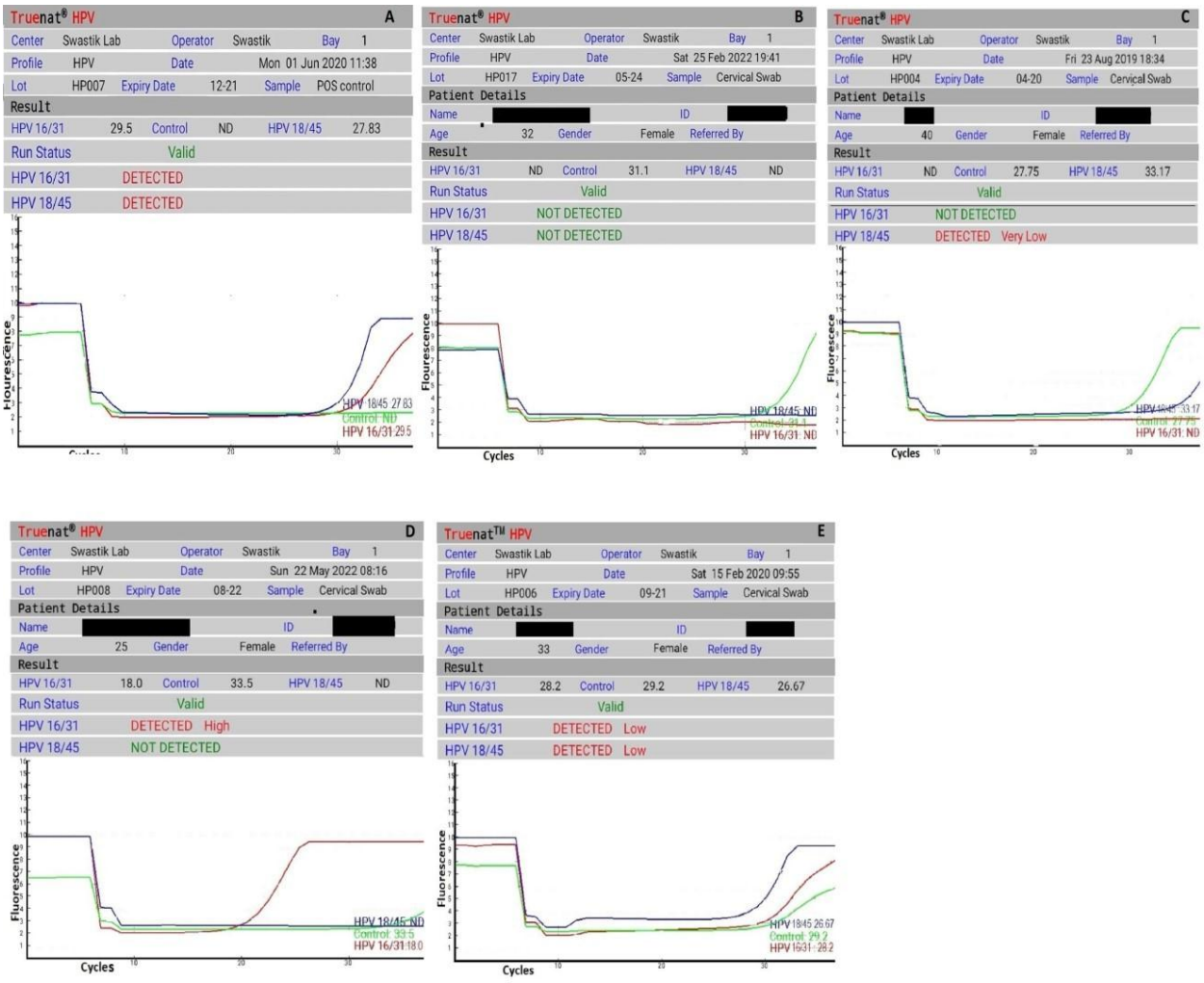

Figure 5.9: Representative panel of HPV genotype results by Truelab Uno Dx Real Time PCR Analyzer. (A) Graph illustrating positive control value where both the target (16/31) and (18/45) amplified and gave results. Control ensured the reliability of results. (B) Graph illustrating amplification only for internal control, the result displayed was not detected. In this graph both the targets did not amplify and remained horizontal throughout the test. (C) Graph depicting exponential curve, for the positive amplification of HPV 18/45 genotype in the sample with (26.67) Ct value (D) Graph illustrating positive amplification of 16/31 genotype. 18.0 was the calculated Ct value. (E) Graph illustrating positive amplification for both the 16/31 and 18/45 genotypes. 28.2 and 26.67 respectively were the Ct values for genotypes 16/31 and 18/45.

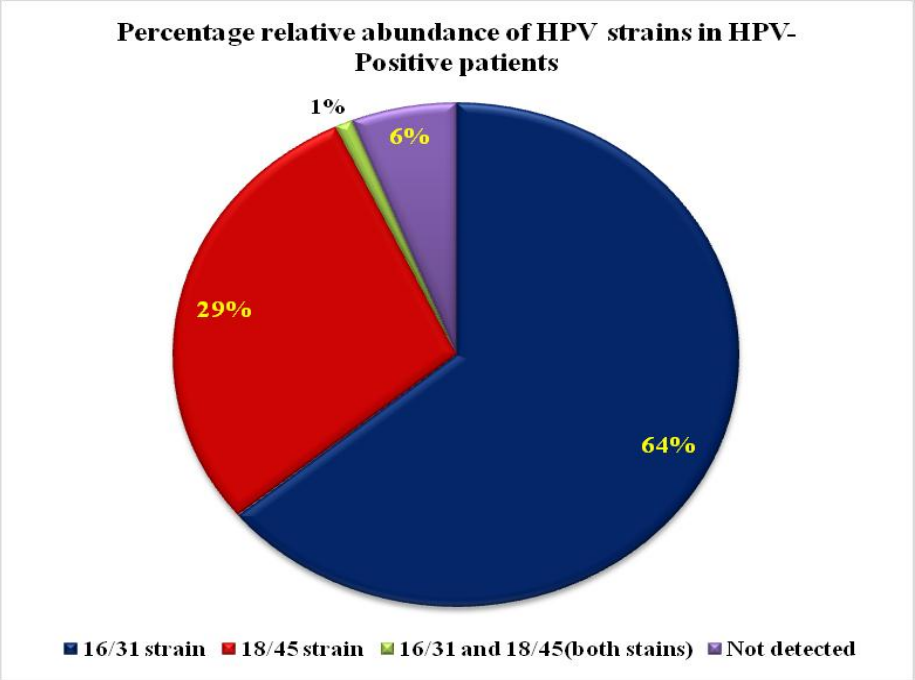

Figure 5.10: Pie chart of the percentage relative abundance of the strains detected in HPV-Positive patients.

In 224 abnormal cytology samples, it was found that 64% of women tested positive for the HPV 16/31 strain, indicating a significant presence of this particular strain within the sample population. Additionally, 29% of the women were found to be positive for the HPV 18/45 strain, highlighting another prevalent strain among the tested individuals. A notable observation was that both the HPV 16/31 and HPV 18/45 strains were detected in 16% of the women. Moreover, 6% of the samples did not show the presence of either the HPV 16/31 or HPV 18/45 strains, highlighting the diversity and complexity of HPV infections within the number of women included in the study (Figure 5.10).

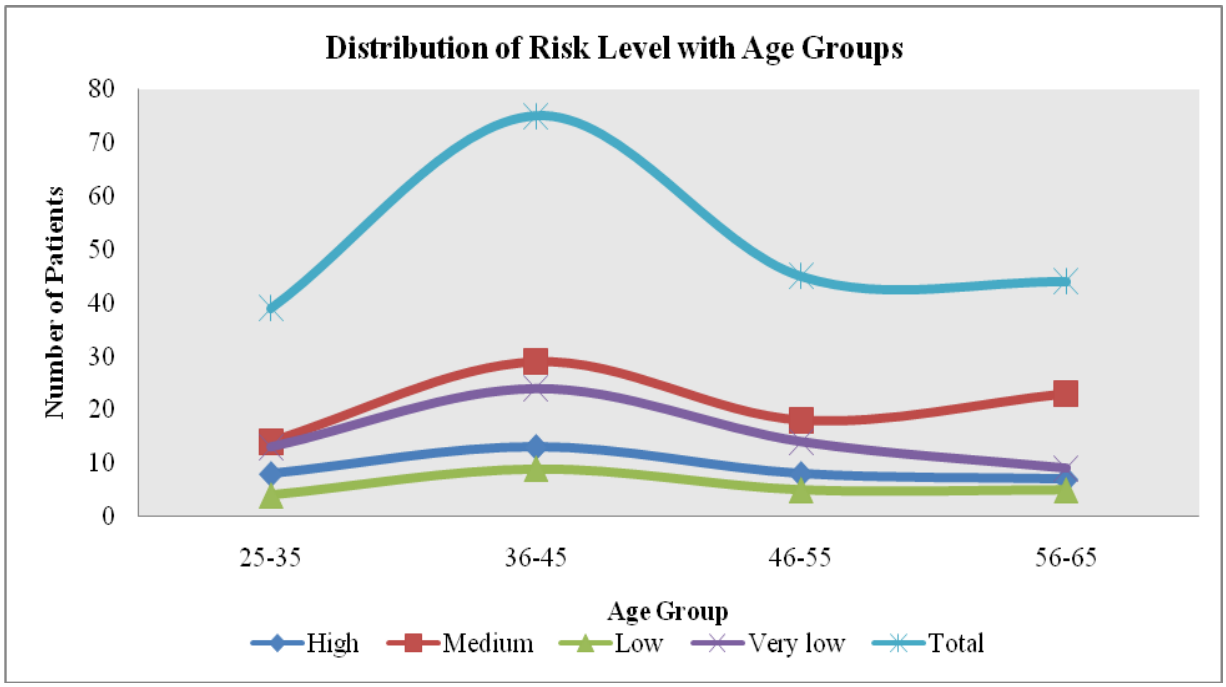

Figure 5.11: Scatter plot of age-wise distribution of the viral load in HPV-positive patients.

The number of women infected with HPV high, medium, low, and very low was mostly seen in the 36-45 age groups which is 36.95%. The HPV infection (19.21%) was found lowest in the age group of 25-35. In the age group of 46-55 and 56-65, HPV-infected women were 22.17% and 21.67% respectively (Figure 5.11).

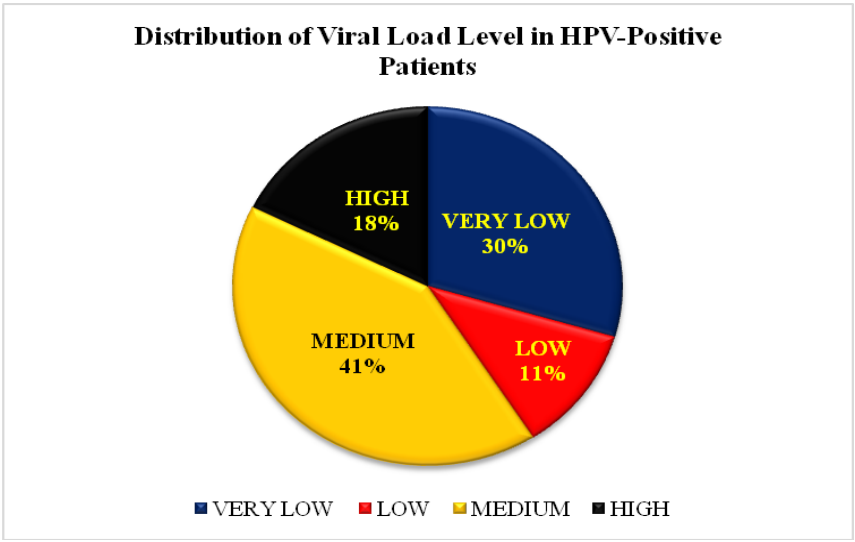

Figure 5.12: Pie chart of the percentage distribution of viral load in HPV-positive patients.

The distribution of viral loads and strain detection in 224 abnormal cytology patients with HPV diagnoses are shown in (Figure 5.12). Viral load levels had been identified by quantitative polymerase chain reaction (qPCR) analysis as high, low, medium, very low, or undetectable. Furthermore, genotyping assays were used to identify HPV strains. The group of patients under analysis in the study had a variable distribution of HPV (human papillomavirus) viral load levels. 18% of the population had a high viral load. On the other hand, 11% of patients had a low viral load, indicating a low relative concentration of HPV viruses. 41% percent and 30% percent respectively had medium and very low viral loads, suggesting that HPV infections with moderate to low viral presence are prevalent. In the study, high viral load was mainly present in patients who had been detected with HPV 16/31 strains.

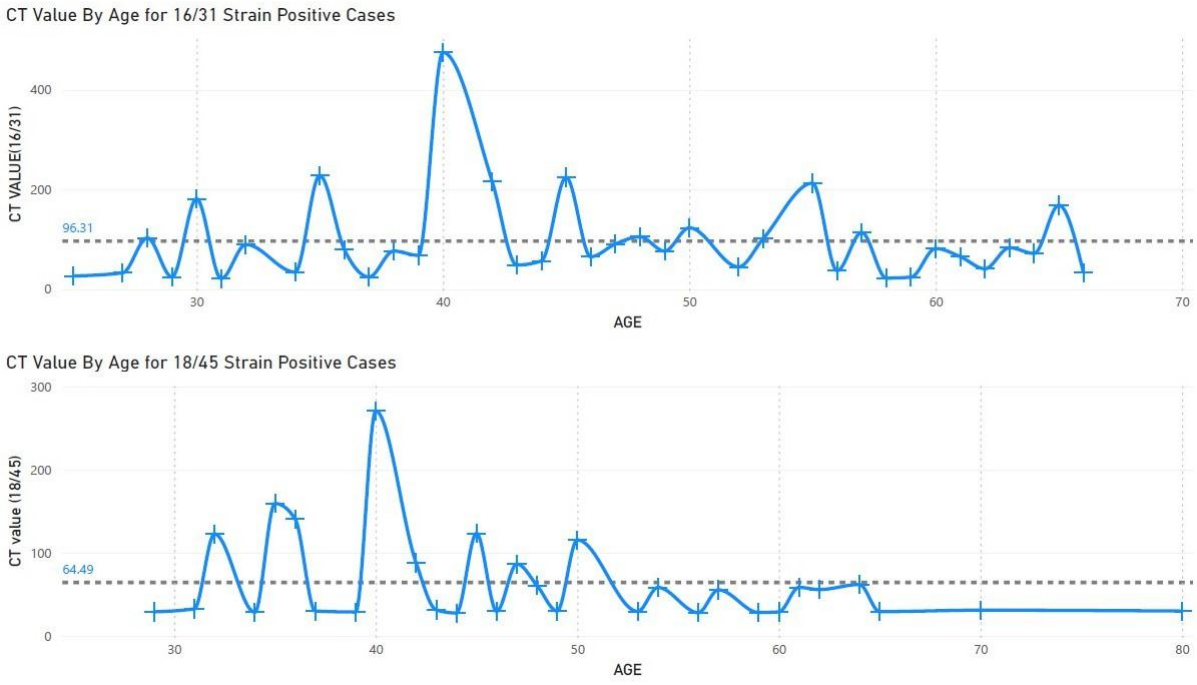

Figure 5.13: Scatter plot of the distribution of patients based on Ct values for genotypes 16/31 and 18/45 in HPV-positive cases across different age groups. Higher Ct values indicated lower viral load, with the highest value observed in women aged 40. Subsequently, the Ct values declined after age 40, suggesting a higher prevalence of HPV infection in individuals over 40 in the current study.

5.8.2 Qiagen HPV Testing

One of the significant findings of the study was that certain patients with abnormal cytology when tested for HPV16/31, 18/45 by TrueNat did not show the presence of any of these HPV strains. These samples were further processed in a Qiagen multistrain detector. In our study out of 12 patients having abnormal cytology when tested for other HPV strains on the Qiagen multistrain detector, only 4 samples were positive for others (strain numbers 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, 68) HPV strains. This is the first-ever report from the Jammu region where for the first time HPV strains other than HPV16/31, and 18/45 were reported. This finding can set a benchmark for further research analysis for the identification of more HPV strains in the population of the Jammu region. The Qiagen multistrain detector graph is explained below (Figure 5.14).

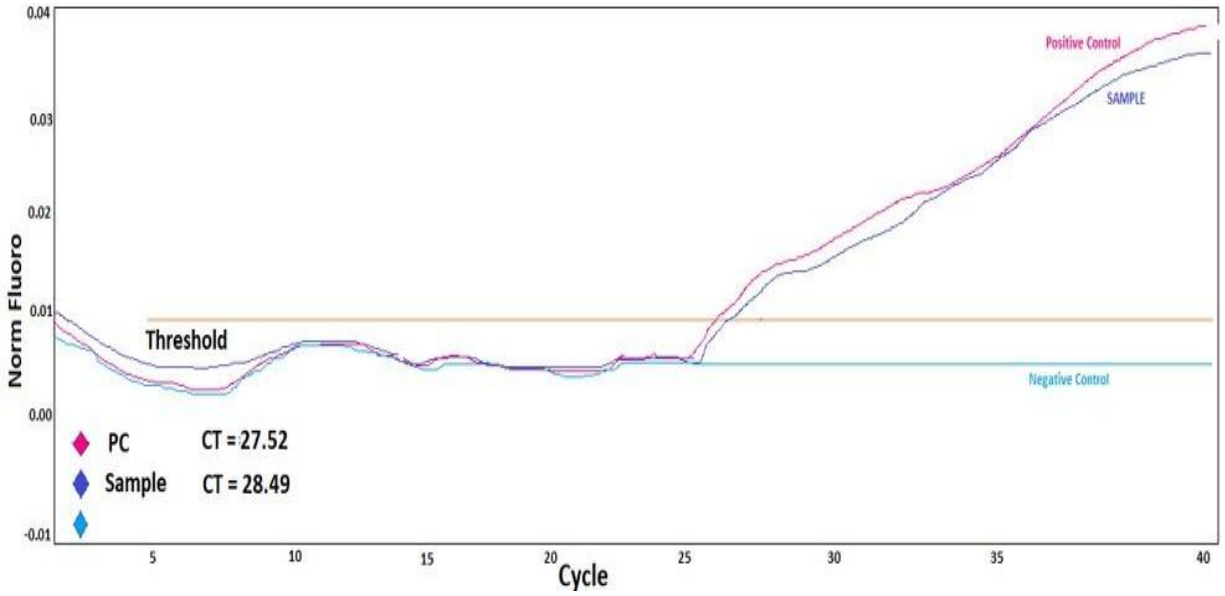

Figure 5.14: Amplification plot of the sample indicating the presence of HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67 and 68). The purple line indicates the sample has a Ct value of 28.49. The sky blue line indicated negative control and positive control was indicated by dark pink colour.

5.8.3 Distribution of HPV Strains among patients with normal cytology

In our study, twelve women were reported to have normal cytology but were positive for HPV strains. The discrepancy between cytology results (showing no abnormal cells) and HPV status (detecting the presence of the virus) can have far-reaching implications for patient care and management. Firstly, the detection of high-risk HPV strains in women with negative cytology results signified early identification of potential HPV infections, enabling timely monitoring and follow-up interventions to prevent the progression of cervical abnormalities or cancer (Figure 5.15).

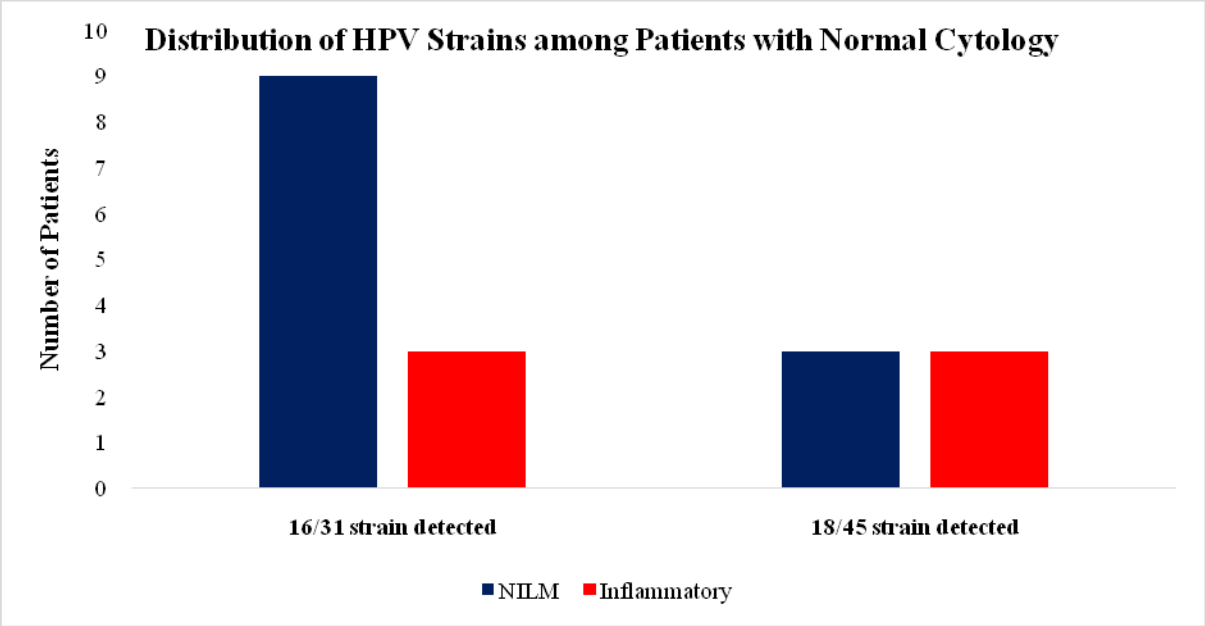

Figure 5.15: Bar graph of the distribution of patients with normal cytology results but positive HPV testing.

Table 5.2: Viral Load and HPV Positivity Among Cytology (LBC) Patients

Patient ID	LBC	AGE	Strain Detected	Viral Load	HPV16/31	HPV18/45	CTVALUE (16/31)	CTVALUE (18/45)	CONTROL VALUE
C1	ASCUS	61	16/31	MEDIUM	+	-	21.38	NA	30.22
C3	ASCUS	42	16/31	VERY LOW	+	-	32.11	NA	26.67
C4	ASCUS	47	18/45	MEDIUM	-	+	NA	23.67	31.4
C5	ASCUS	40	16/31	VERY LOW	+	-	32.5	NA	28.1
C6	ASCUS	30	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C7	ASCUS	36	18/45	MEDIUM	-	+	NA	21.69	30.29
C8	ASCUS	35	18/45	MEDIUM	-	+	NA	22.64	31.25
C9	ASCUS	40	18/45	MEDIUM	-	+	NA	22.67	31.3
C10	LSIL	46	16/31	HIGH	+	-	16.83	NA	32.75
C11	ASCUS	42	18/45	MEDIUM	-	+	NA	21.4	32.8
C12	ASCUS	50	16/31	VERY LOW	+	-	34.13	NA	26.14
C13	ASCUS	40	16/31	HIGH	+	-	18.5	NA	33.25
C15	ASCUS	36	18/45	MEDIUM	-	+	NA	21.7	30.17
C16	ASCUS	36	16/31	LOW	+	-	25.67	NA	29.7
C17	ASCUS	35	16/31	HIGH	+	-	16.33	NA	32.35
C18	ASCUS	36	18/45	MEDIUM	-	+	NA	24.25	31.15
C19	ASCUS	40	16/31	MEDIUM	+	-	24.5	NA	31.2
C20	ASCUS	44	18/45	VERY LOW	-	+	NA	33.17	27.75
C21	ASCUS	35	16/31	LOW	+	-	27.2	NA	29.5
C22	ASCUS	45	16/31	VERY LOW	+	-	34.67	NA	28.8
C23	ASCUS	40	18/45	VERY LOW	-	+	NA	34.64	27.57
C24	ASCUS	40	16/31	LOW	+	-	29.2	NA	28.46
C25	ASCUS	35	16/31	VERY LOW	+	-	30.94	NA	26.25
C26	ASCUS	36	16/31	MEDIUM	+	-	21.38	NA	30.22
C27	ASCUS	42	18/45	MEDIUM	-	+	NA	24	30.4
C28	ASCUS	60	16/31	MEDIUM	+	-	20.17	NA	31.4

C29	ASCUS	47	Both 16/31 and 18/45	LOW	+	+	28.2	26.67	29.2
C30	ASCUS	42	16/31	VERY LOW	+	-	36.5	NA	27.5
C32	LSIL	40	16/31	MEDIUM	+	-	24.3	NA	32.31
C33	ASCUS	35	18/45	MEDIUM	-	+	NA	22.6	30.4
C34	ASCUS	35	16/31	HIGH	+	-	19.5	NA	32.2
C35	SCC	64	16/31	HIGH	+	-	19.5	NA	33.2
C36	ASCUS	40	16/31	MEDIUM	+	-	23	NA	31.3
C37	HSIL	40	18/45	MEDIUM	-	+	NA	23	32
C38	HSIL	44	16/31	MEDIUM	+	-	24.25	NA	31.2
C39	LSIL	57	16/31	HIGH	+	-	14	NA	32.2
C40	ASCUS	40	18/45	VERY LOW	-	+	NA	36.5	26.5
C41	HSIL	56	16/31	MEDIUM	+	-	22.43	NA	30.25
C42	ASCUS	62	18/45	LOW	-	+	NA	26.69	29.2
C43	LSIL	59	16/31	MEDIUM	+	-	24	NA	32.33
C44	HSIL	47	16/31	MEDIUM	+	-	24.5	NA	30.5
C45	ASCUS	49	16/31	MEDIUM	+	-	23	NA	30.17
C46	HSIL	30	16/31	VERY LOW	+	-	35.13	NA	27.57
C47	ASCUS	64	16/31	VERY LOW	+	-	31.67	NA	27.6
C48	SCC	40	16/31	VERY LOW	+	-	36.5	NA	26.25
C49	LSIL	43	16/31	HIGH	+	-	14.23	NA	31.1
C50	ASCUS	49	16/31	HIGH	+	-	14.68	NA	31.5
C51	HSIL	47	16/31	HIGH	+	-	14.5	NA	33.8
C52	LSIL	64	18/45	VERY LOW	-	+	NA	32.82	27.42
C53	ASCUS	40	16/31	MEDIUM	+	-	23	NA	31.5
C54	LSIL	59	18/45	MEDIUM	-	+	NA	23.67	30.4
C55	ASCUS	42	16/31	MEDIUM	+	-	23	NA	32.2
C56	ASCUS	35	18/45	VERY LOW	-	+	NA	35.5	26.33
C57	SCC	45	16/31	HIGH	+	-	14.42	NA	32.25
C58	LSIL	62	16/31	HIGH	+	-	13.8	NA	33.32
C59	ASCUS	55	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C60	ASCUS	29	18/45	VERY LOW	-	+	NA	36.5	27.17

C61	ASCUS	38	16/31	VERY LOW	+	-	36.28	NA	26.17
C62	ASCUS	57	16/31	VERY LOW	+	-	34.51	NA	28
C63	HSIL	48	16/31	VERY LOW	+	-	35.79	NA	26.26
C64	ASCUS	53	16/31	MEDIUM	+	-	22.44	NA	30.12
C65	ASCUS	40	16/31	VERY LOW	+	-	34.67	NA	27.8
C66	ASCUS	61	18/45	MEDIUM	-	+	NA	23	30.43
C67	LSIL	35	18/45	MEDIUM	-	+	NA	22.34	31.41
C68	LSIL	50	16/31	HIGH	+	-	16.83	NA	31.75
C69	LSIL	35	Both 16/31 and 18/45	LOW	+	+	28.39	27.3	30.1
C70	ASCUS	40	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C71	ASCUS	60	16/31	LOW	+	-	29.75	NA	28.6
C72	LSIL	30	16/31	MEDIUM	+	-	24.25	NA	30.2
C73	HSIL	40	16/31	LOW	+	-	28.92	NA	27.2
C74	HSIL	56	18/45	MEDIUM	-	+	NA	21.4	31.17
C76	LSIL	54	18/45	VERY LOW	-	+	NA	33.17	27.75
C77	ASCUS	50	18/45	VERY LOW	-	+	NA	33.24	28.77
C79	ASCUS	45	16/31	LOW	+	-	29.75	NA	29
C80	ASCUS	45	16/31	HIGH	+	-	15.51	NA	33.75
C81	LSIL	50	18/45	MEDIUM	-	+	NA	20.5	30.67
C82	LSIL	50	18/45	MEDIUM	-	+	NA	23.57	31
C83	ASCUS	55	16/31	VERY LOW	+	-	32.5	NA	26.52
C84	LSIL	57	18/45	MEDIUM	-	+	NA	22.6	31
C85	HSIL	55	16/31	VERY LOW	+	-	34.13	NA	26.14
C86	HSIL	40	16/31	HIGH	+	-	16.83	NA	32.75
C87	ASCUS	36	18/45	MEDIUM	-	+	NA	22.4	31.6
C88	ASCUS	35	16/31	HIGH	+	-	18	NA	33.5
C89	LSIL	48	18/45	MEDIUM	-	+	NA	24.62	30.65
C90	ASCUS	40	18/45	VERY LOW	-	+	NA	32.11	26.67
C91	LSIL	37	18/45	MEDIUM	-	+	NA	22.7	30.17
C92	ASCUS	32	16/31	VERY LOW	+	-	33.13	NA	26.15
C93	ASCUS	42	16/31	LOW	+	-	26	NA	28.3

C94	ASCUS	32	Both 16/31 and 18/45	MEDIUM/VERY LOW	+	+	32	22.11	32.2
C95	ASCUS	32	16/31	MEDIUM	+	-	24.3	NA	30.5
C96	ASCUS	30	16/31	MEDIUM	+	-	24.15	NA	33.2
C97	ASCUS	36	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C98	HSIL	65	16/31	VERY LOW	+	-	34.11	NA	27.67
C99	ASCUS	63	16/31	LOW	+	-	29.63	NA	29.3
C10	LSIL	57	16/31	VERY LOW	+	-	32.13	NA	26.12
C101	LSIL	36	16/31	VERY LOW	+	-	32.4	NA	27.7
C102	HSIL	39	16/31	HIGH	+	-	16.81	NA	31.65
C103	LSIL	45	18/45	VERY LOW	-	+	NA	34.3	26.12
C104	ASCUS	34	16/31	LOW	+	-	29.1	NA	29.7
C105	ASCUS	40	16/31	HIGH	+	-	19.81	NA	32
C106	HSIL	50	16/31	HIGH	+	-	16.8	NA	34
C107	ASCUS	60	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C108	ASCUS	61	16/31	MEDIUM	+	-	21.31	NA	31.22
C109	ASCUS	56	16/31	HIGH	+	-	14.6	NA	32.1
C110	ASCUS	65	16/31	MEDIUM	+	-	23.25	NA	30.17
C111	ASCUS	40	16/31	VERY LOW	+	-	34.13	NA	27.42
C112	ASCUS	49	16/31	MEDIUM	+	-	21.24	NA	31.2
C113	ASCUS	57	16/31	LOW	+	-	27.8	NA	28.8
C114	LSIL	30	16/31	MEDIUM	+	-	24.35	NA	31.2
C115	ASCUS	62	16/31	MEDIUM	+	-	24.13	NA	30.8
C116	ASCUS	61	16/31	MEDIUM	+	-	23.33	NA	31.12
C117	ASCUS	49	18/45	MEDIUM	-	+	NA	21.75	30.17
C118	ASCUS	60	16/31	LOW	+	-	28.62	NA	29.4
C119	HSIL	40	16/31	MEDIUM	+	-	21.69	NA	30.29
C120	LSIL	42	18/45	LOW	-	+	NA	27.2	29.5
C121	ASCUS	65	16/31	HIGH	+	-	19.8	NA	34
C122	ASCUS	40	16/31	VERY LOW	+	-	35.23	NA	26.5
C123	HSIL	35	16/31	VERY LOW	+	-	33.62	NA	27.5
C124	LSIL	62	18/45	VERY LOW	-	+	NA	34.28	26.75

C125	ASCUS	58	16/31	MEDIUM	+	-	22.12	NA	31.35
C127	HSIL	57	18/45	MEDIUM	-	+	NA	20.8	30.57
C128	ASCUS	55	16/31	LOW	+	-	27.37	NA	29.7
C129	ASCUS	66	16/31	VERY LOW	+	-	33.24	NA	27.75
C130	ASCUS	80	18/45	VERY LOW	-	+	NA	35.9	26.12
C131	HSIL	61	18/45	MEDIUM	-	+	NA	21.5	30.15
C132	ASCUS	54	18/45	VERY LOW	-	+	NA	34	27.71
C133	ASCUS	65	16/31	MEDIUM	+	-	21	NA	30.7
C134	ASCUS	60	18/45	MEDIUM	-	+	NA	22.47	31.1
C135	ASCUS	50	18/45	MEDIUM	-	+	NA	21.4	30.29
C136	ASCUS	45	18/45	VERY LOW	-	+	NA	32.62	28
C138	ASCUS	46	16/31	MEDIUM	+	-	23.63	NA	30.5
C139	ASCUS	30	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C140	HSIL	56	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C141	ASCUS	40	18/45	VERY LOW	-	+	NA	34.9	27.2
C142	LSIL	64	18/45	MEDIUM	-	+	NA	21.9	31.62
C143	LSIL	55	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C144	ASCUS	48	16/31	VERY LOW	+	-	34.23	NA	26
C145	ASCUS	42	16/31	MEDIUM	+	-	21.73	NA	30.57
C146	HSIL	53	16/31	LOW	+	-	28.67	NA	29.6
C147	LSIL	48	18/45	VERY LOW	-	+	NA	34.2	27.12
C148	ASCUS	35	16/31	MEDIUM	+	-	22	NA	30.2
C149	ASCUS	28	16/31	HIGH	+	-	17.33	NA	32.5
C150	HSIL	38	16/31	MEDIUM	+	-	23.67	NA	31.9
C151	ASCUS	45	16/31	MEDIUM	+	-	22.78	NA	31.59
C152	LSIL	48	16/31	LOW	+	-	29.1	NA	29
C153	ASCUS	42	16/31	HIGH	+	-	16.28	NA	31.6
C154	ASCUS	42	16/31	MEDIUM	+	-	24.73	NA	30.26
C155	ASCUS	45	16/31	HIGH	+	-	15.17	NA	33.45
C156	ASCUS	30	16/31	MEDIUM	+	-	23.67	NA	27.1
C157	HSIL	45	16/31	MEDIUM	+	-	22.29	NA	31.5
C158	ASCUS	30	16/31	HIGH	+	-	17.33	NA	31.54

C159	LSIL	55	16/31	MEDIUM	+	-	23.61	NA	34.57
C160	ASCUS	63	16/31	HIGH	+	-	19.45	NA	31.32
C161	HSIL	32	18/45	MEDIUM	-	+	NA	22.12	31.3
C162	LSIL	61	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C163	ASCUS	55	16/31	VERY LOW	+	-	33.21	NA	27.57
C164	ASCUS	65	16/31	VERY LOW	+	-	33.87	NA	27
C165	ASCUS	64	16/31	MEDIUM	+	-	22.45	NA	30.1
C166	ASCUS	37	16/31	MEDIUM	+	-	24.38	NA	31.06
C167	HSIL	60	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C168	ASCUS	43	18/45	VERY LOW	-	+	NA	33.27	27.45
C169	ASCUS	47	16/31	MEDIUM	+	-	23.17	NA	32
C170	ASCUS	70	18/45	LOW	-	+	NA	25.57	31.2
C171	LSIL	44	16/31	VERY LOW	+	-	32.33	NA	27.17
C172	ASCUS	46	16/31	MEDIUM	+	-	24.5	NA	30.25
C173	ASCUS	30	16/31	HIGH	+	-	16.69	NA	33.2
C174	LSIL	27	16/31	VERY LOW	+	-	32.69	NA	26.75
C175	ASCUS	53	16/31	VERY LOW	+	-	31.32	NA	28.27
C176	LSIL	65	16/31	HIGH	+	-	14.8	NA	34.79
C177	ASCUS	52	16/31	MEDIUM	+	-	23.5	NA	31.63
C178	ASCUS	65	18/45	MEDIUM	-	+	NA	23.82	30.4
C179	ASCUS	53	18/45	VERY LOW	-	+	NA	34.62	27.7
C180	LSIL	49	16/31	HIGH	+	-	17.25	NA	31.2
C181	HSIL	31	16/31	MEDIUM	+	-	21.17	NA	31.67
C182	ASCUS	34	18/45	LOW	-	+	NA	27.17	28.65
C183	LSIL	43	16/31	VERY LOW	+	-	34.11	NA	28
C184	ASCUS	50	16/31	VERY LOW	+	-	33.42	NA	28.25
C185	LSIL	42	16/31	VERY LOW	+	-	36.67	NA	26.9
C186	HSIL	40	16/31	HIGH	+	-	19.13	NA	32
C187	LSIL	50	16/31	MEDIUM	+	-	21.67	NA	31.5
C188	ASCUS	31	18/45	VERY LOW	-	+	NA	33.37	27.82
C189	LSIL	45	16/31	MEDIUM	+	-	21.69	NA	30.22
C190	HSIL	38	16/31	HIGH	+	-	16.33	NA	34
C191	LSIL	39	16/31	MEDIUM	+	-	21.38	NA	31.2

C192	ASCUS	35	16/31	VERY LOW	+	-	33.69	NA	28.75
C194	HSIL	28	16/31	HIGH	+	-	16.28	NA	31.9
C195	LSIL	28	16/31	VERY LOW	+	-	34.67	NA	26.4
C196	SCC	40	18/45	VERY LOW	-	+	NA	36.2	27.75
C197	ASCUS	63	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C198	LSIL	65	16/31	MEDIUM	+	-	22.23	NA	33.65
C199	ASCUS	55	16/31	LOW	+	-	29	NA	29
C200	LSIL	40	18/45	MEDIUM	-	+	NA	23.79	31.43
C201	HSIL	47	18/45	VERY LOW	-	+	NA	35.65	29.14
C202	ASCUS	32	18/45	VERY LOW	-	+	NA	34.72	28.85
C203	LSIL	39	18/45	MEDIUM	-	+	NA	23	28.97
C204	ASCUS	39	16/31	LOW	+	-	29.5	NA	28.6
C205	LSIL	32	18/45	VERY LOW	-	+	NA	34.86	27.53
C206	HSIL	40	16/31	VERY LOW	+	-	35.83	NA	29.12
C207	ASCUS	52	16/31	MEDIUM	+	-	21.05	NA	35.8
C208	SCC	45	18/45	VERY LOW	-	+	NA	36.28	27.17
C209	LSIL	28	16/31	VERY LOW	+	-	34.28	NA	28
C210	ASCUS	53	16/31	HIGH	+	-	14.77	NA	33.13
C211	LSIL	46	18/45	MEDIUM	-	+	NA	24.12	30.8
C212	HSIL	45	16/31	MEDIUM	+	-	23.6	NA	31
C213	LSIL	31	None	NONE	NOT DETECTED	NOT DETECTED	NA	NA	NA
C214	ASCUS	40	16/31	HIGH	+	-	14.72	NA	33.28
C215	LSIL	30	16/31	HIGH	+	-	18.33	NA	32.2
C216	ASCUS	29	16/31	MEDIUM	+	-	24.5	NA	30.28
C217	SCC	36	18/45	VERY LOW	-	+	NA	33.7	27.75
C218	HSIL	40	18/45	LOW	-	+	NA	29.36	29.42
C219	ASCUS	63	16/31	MEDIUM	+	-	24.85	NA	31
C220	ASCUS	25	16/31	MEDIUM	+	-	24.12	NA	30.73
C221	ASCUS	55	16/31	HIGH	+	-	19.5	NA	33.29
C222	ASCUS	45	16/31	MEDIUM	+	-	24.24	NA	31
C223	SCC	45	18/45	HIGH	-	+	NA	16.7	33.53

5.9 Association of HPV with Epidemiological Risk Factors

5.9.1 Early Marriage:

Early marriage as a risk factor for HPV infection showed a considerable association with HPV positivity ($p < 0.05$) (Table 5.3). In cases of early marriage 47 HPV-positive women were seen in the age group 25-35 years. 82 positive women were seen in 36-45 years. 50 cases were found in the age group 46-55 years and 42 cases were seen in the age group 56-65 years. This shows maximum positivity of HPV was seen in the age group 36-45 years.

Table 5.3: Regression statistics for the comparison between the cases with early marriage and HPV-positive cases.

Regression Statistics	
Multiple R	0.027086414
R Square	0.000733674
Adjusted R Square	0.000483732
Standard Error	0.228440877
Observations	4000
P-value	0.0867357

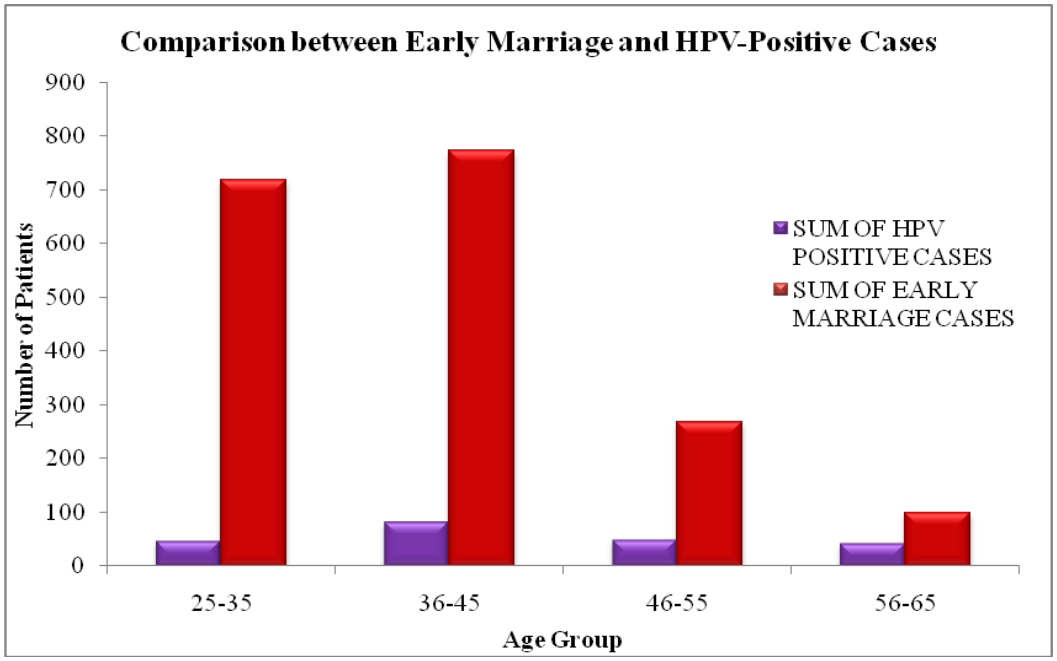

Figure 5.16: Bar graph of the comparison between the early marriage cases and HPV-positive cases.

5.9.2 History of STD

The correlation analysis between a history of sexually transmitted infections (STDs) and HPV-positive revealed a multiple R-value of 0.151 at a p-value of less than 0.05. 1233 women out of the 4000 that were studied had a history of STDs. The age range of 36 to 45 years had the highest HPV positivity among individuals with STDs, whereas the 55 to 65 years age group had the lowest HPV positivity, with 42 cases (Table 5.4).

Table 5.4: Regression statistics for the comparison between the cases with STD history and HPV-positive cases.

Regression Statistics	
Multiple R	0.151367313
R Square	0.022912063
Adjusted R Square	0.022667669
Standard Error	0.225891567
Observations	4000
P- value	0.000000000000000000000622
Observations	4000

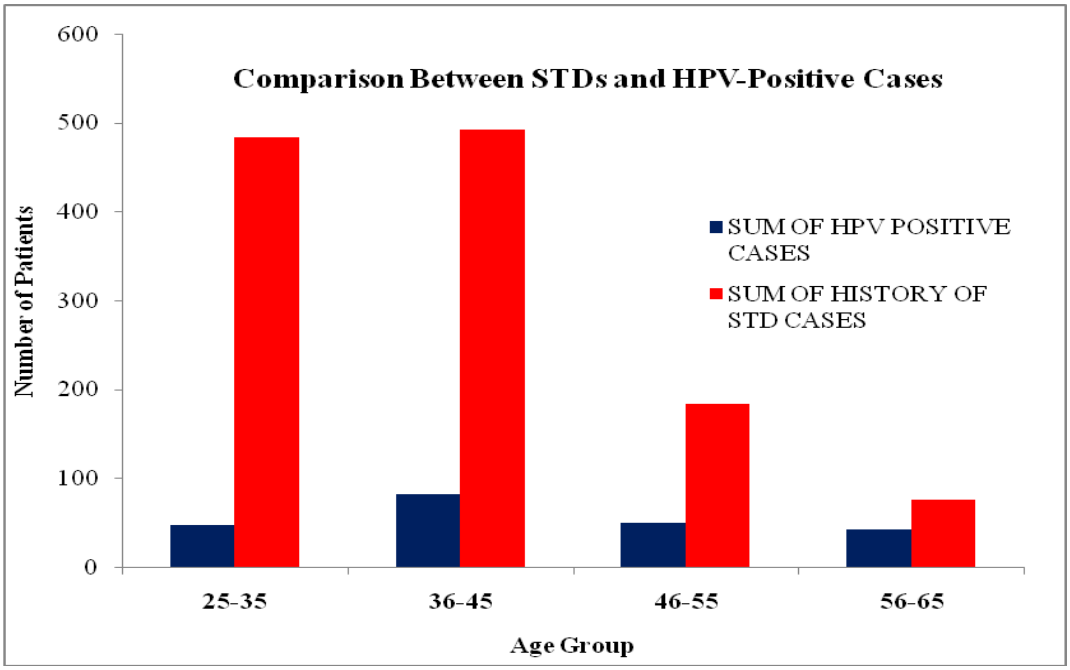

Figure 5.17: Bar graph of the comparison between the Sexually transmitted diseases cases and HPV-positive cases

5.9.3 Early pregnancy:

Early pregnancy was studied as an epidemiological factor for HPV infection and cervical cancer development showed a strong association with HPV positivity. Maximum patients (82) of HPV positivity were seen in the age group of 36-45 years in early pregnancy cases. Minimum cases of HPV positivity were seen age group of 55-65. A p-value <0.005 suggested an association of HPV positivity with early marriage (Table 5.5).

Table 5.5: Regression statistics for the comparison between the cases with early pregnancy and HPV-positive cases.

Regression Statistics	
Multiple R	0.046483166
R Square	0.002160685
Adjusted R Square	0.0019111
Standard Error	0.228277705
Observations	4000
P-value	0.003276574

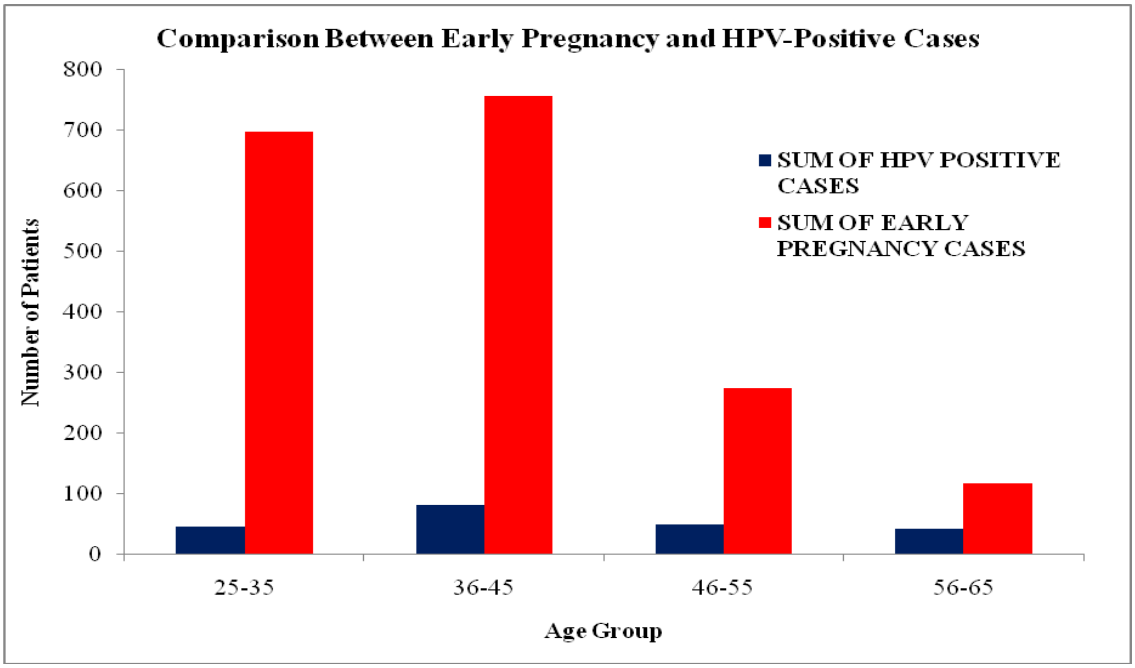

Figure 5.18: Bar graph of the comparison between the early pregnancy cases and HPV-positive Cases.

5.9.4 Vaginal bleeding

Vaginal bleeding as a risk factor showed a significant association with HPV DNA positivity and cervical cancer development. In our study, it was also observed that women experiencing vaginal bleeding had an enhanced chance of having HPV infection. Vaginal bleeding had a strong association with HPV positivity exhibiting a p-value <0.005 (Table 5.6). Among women with vaginal bleeding, the highest HPV positivity was observed in the 36-45 age groups, while the lowest cases of HPV positivity were found in the 56-65 age groups.

Table 5.6: Showing the regression statistics for the comparison between the cases with vaginal bleeding and HPV-positive cases.

Regression Statistics	
Multiple R	0.106947455
R Square	0.011437758
Adjusted R Square	0.011190494
Standard Error	0.22721406
Observations	4000
P-value	0.000000000011910

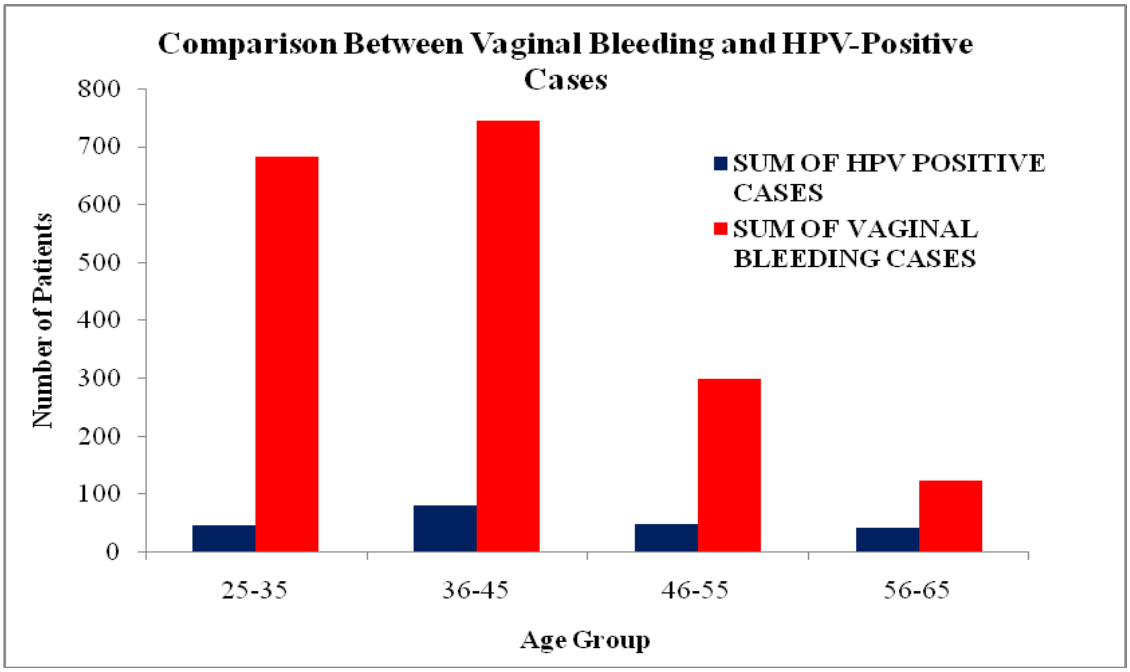

Figure 5.19: Bar graph of the comparison between the vaginal bleeding cases and HPV-positive cases

5.9.5 Smoking

Smoking was also a main risk factor for the enhancement of cervical cancer. Regression analysis between smoking and HPV positivity (Table 5.7) revealed a multiple R-value of 0.212 with a p-value<0.005, which implied that smoking is relatively more correlated with HPV positivity compared to a history of an STD. It was noted that women who smoked were more likely to become infected with HPV. The age group of 36–45 had the highest HPV positivity among the smoking patients, while the age group of 56–65 had the lowest HPV positivity.

Table 5.7: Regression Statistics for the comparison between cases with smoking and HPV-positive Cases

Regression Statistics	
Multiple R	0.212201709
R Square	0.045029565
Adjusted R Square	0.044790703
Standard Error	0.223320276
Observations	4000
P-value	0.00000059133

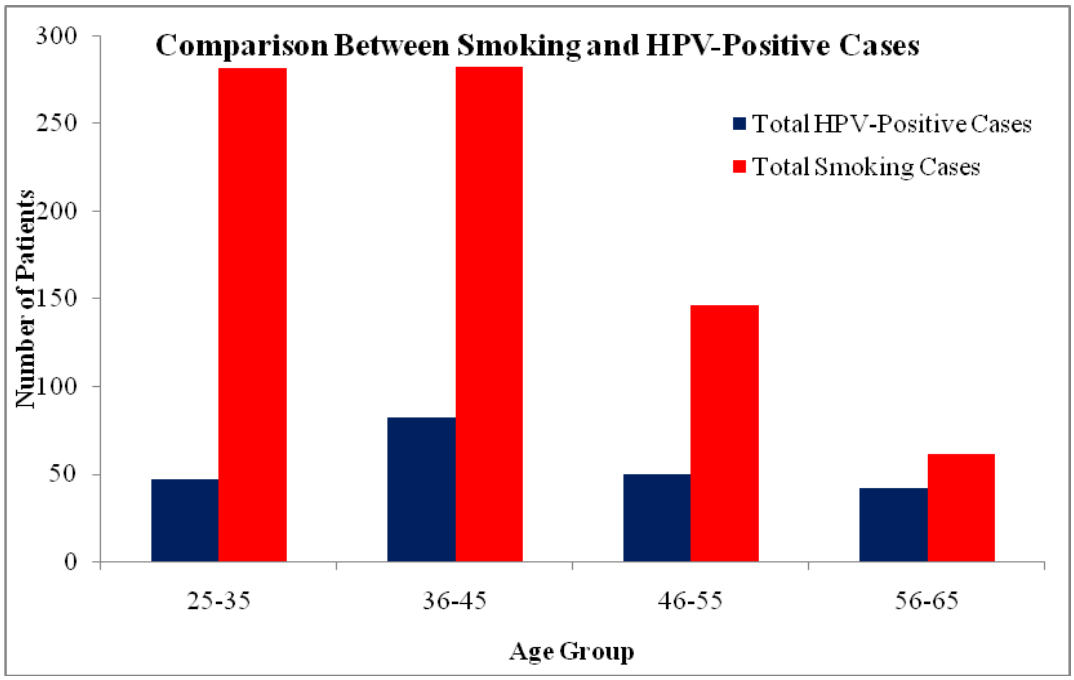

Figure 5.20: Bar graph of the comparison between the smoking cases and HPV-positive cases

5.9.6 Oral contraceptive

Regression analysis was employed to study the correlation between the usage of oral contraceptives and HPV-positive status. The results revealed a multiple R-value of 0.113 at a p-value <0.005. This implied that prolonged usage of oral contraceptives may be associated with some cases of HPV infection, which is known to be a risk factor for the development of cervical cancer (Table 5.8).

Table 5.8: Regression Statistics for the comparison between cases with Oral contraceptive pills and HPV-positive cases.

Regression Statistics	
Multiple R	0.11388991
R Square	0.012970912
AdjustedR Square	0.012724031
Standard Error	0.227037799
Observations	4000
P- value	0.00000000000050

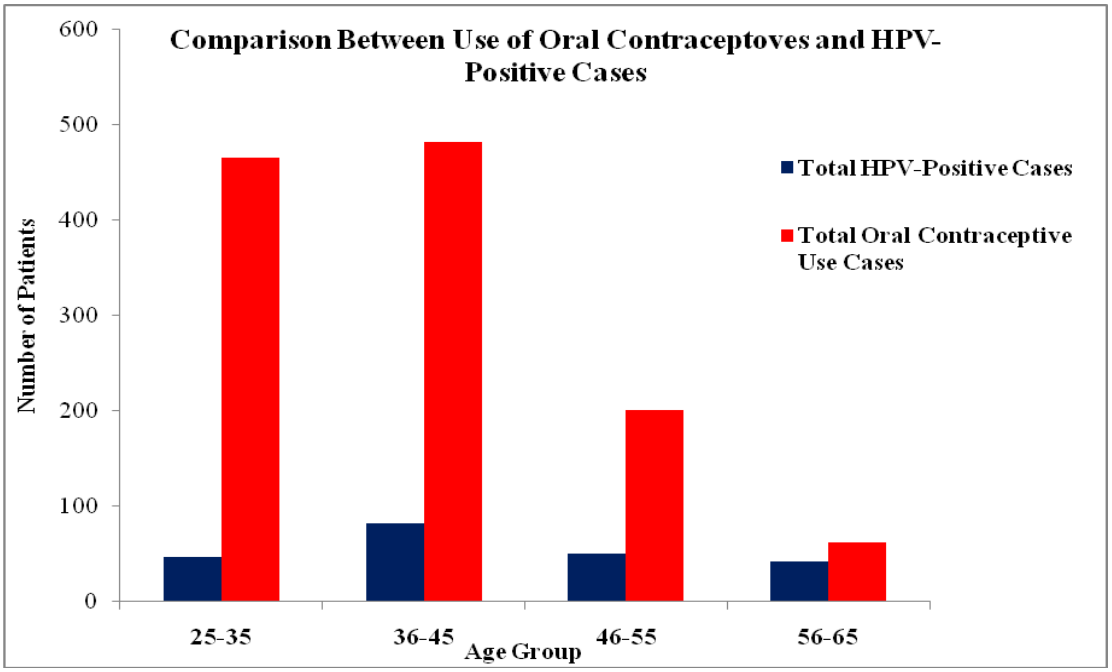

Figure 5.21: Bar graph of the comparison between the use of oral contraceptive cases and HPV-positive cases.

5.9.7 Low socioeconomic status

Low economic status was also one of the contributing factors in cervical cancer development. Low socio-economic status showed a positive association with HPV DNA positivity (p-value=<0.05) (Table 5.9). Among low socioeconomic status women maximum HPV positivity was seen in the age group of 36-45.

Table 5.9 Regression Statistics for the comparison between cases with Low socioeconomic cases and HPV-positive cases.

Regression Statistics	
Multiple R	0.11388991
R Square	0.012970912
AdjustedRSquare	0.012724031
Standard Error	0.227037799
Observations	4000
P- value	0.000000000321451

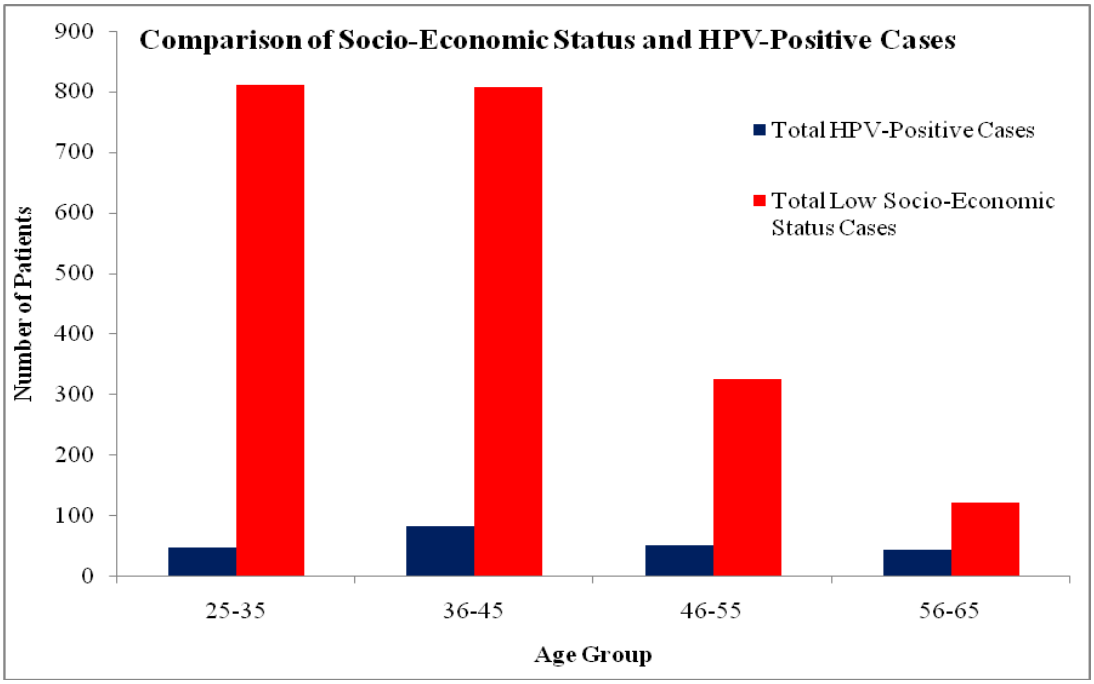

Figure 5.22: Bar graph of the comparison between the low socioeconomic status cases and HPV-positive cases.

5.9.8 **Multiparity**

A strong correlation was found in many research studies (Rani *et al*., 2016) between a woman's chance of getting HPV and developing cervical cancer and the number of children she gives birth to. However, in our study for the Jammu region, multiparity was not highly correlated with HPV positivity (p-value<0.005) highlighting a lower involvement of parity as a risk factor in the development of HPV-related diseases (Table 5.10).

Table 5.10: Regression Statistics for the comparison between cases with multiparity and HPV-positive Cases.

Regression Statistics	
Multiple R	0.073537909
R Square	0.005407824
Adjusted R Square	0.005159052
Standard Error	0.227905975
Observations	4000
P-value	0.00000322730

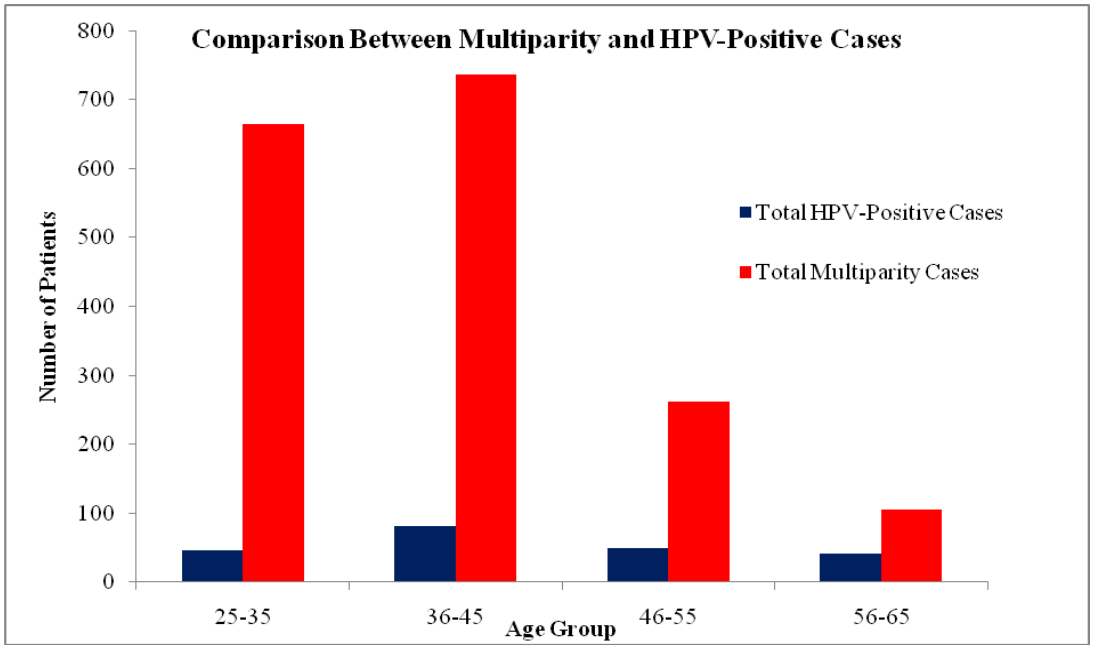

Figure 5.23: Bar graph of the comparison between the multiparity cases and HPV-positive cases.

5.10 Association of Cervical Cytology (LBC) and HPV

Results of cervical pap smear and HPV positivity were compared and the association between them was calculated by chi-square test. A significant association was found between abnormal pap smear and HPV positivity showing a p-value of (p= 0.00004, Table 5.11)

Table 5.11: Association of Abnormal Cervical Cytology and HPV

Chi-Square	NOT DETECTED	NOT DONE	16/31 STRAIN DETECTED	18/45 STRAIN DETECTED
Ascus	1.657	0.813	0	0.825
HSIL	1.614	1.076	0.857	0.216
LSIL	2.422	1.614	0.074	2.408
SCC	0.549	1.42	1.272	0.897

Test Values	
Chi-square	17.713
df (degrees of freedom)	9
p-value	0.04

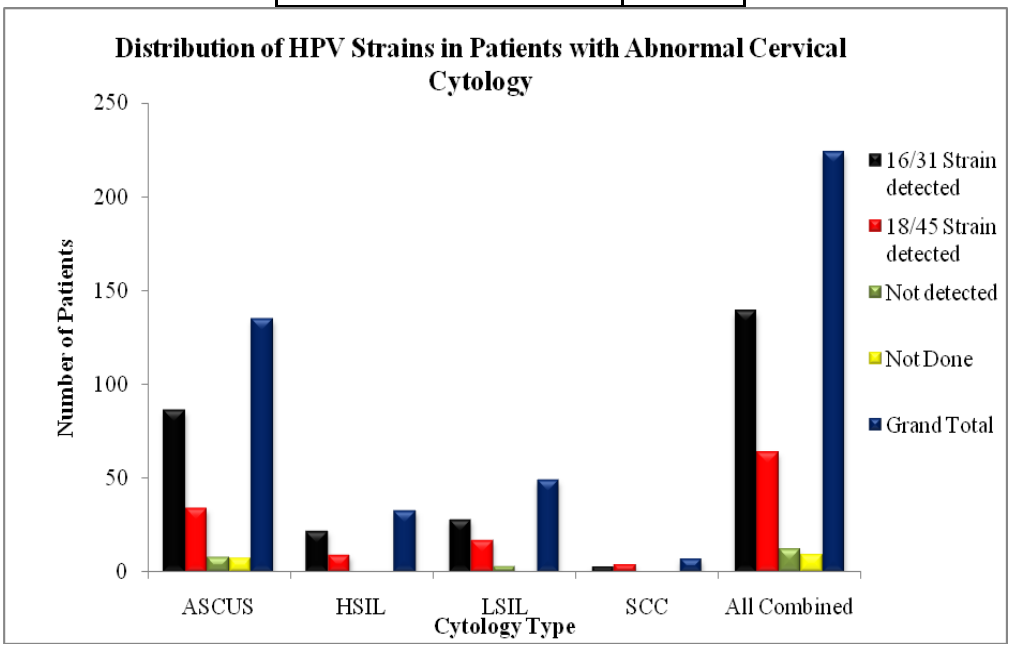

Figure 5.24: Bar graph of the number of HPV strains present in abnormal cases of cervical cytology.

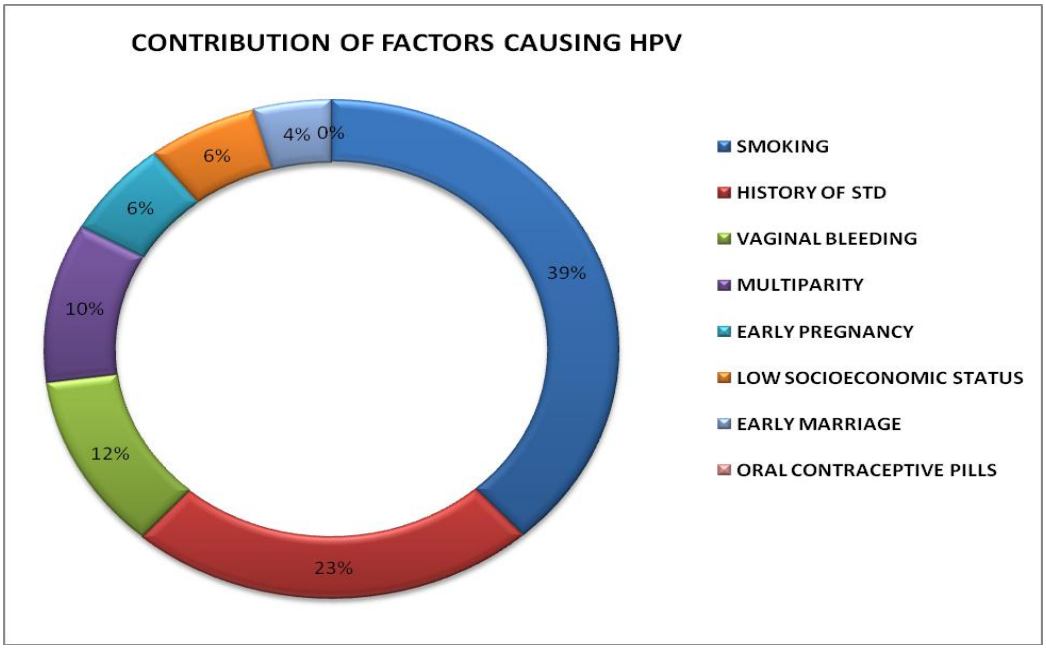

Figure 5.25: Donut plot of the impact of smoking on HPV incidence, with the highest contribution among all the factors studied. Following smoking, factors such as a history of STDs, vaginal bleeding, multiparity, early pregnancy, low socioeconomic status, early marriage, and the use of contraceptive pills were also identified as contributors to HPV.

5.11 Incidence of HPV strains

The incidence rates of the 16/31 and 18/45 strains were monitored over five years. There were 40 cases of the 16/31 strain and 15 cases of the 18/45 strain in 2018, indicating that the 16/31 strain was more common. Both variants had a rise the next year, with 20 cases of the 18/45 strain and 43 cases of the 16/31 strain. In 2020, there were 40 instances of the 16/31 strain, the same as in the previous year, and 16 cases of the 18/45 strain. This suggested that the incidence of the 18/45 strain was lower than it was the year before. Then, in 2021, the incidence of both strains decreased, with 29 instances of the 16/31 strain and 22 cases of the 18/45 strain reported.38 instances of the 16/31 strain and 14 cases of the 18/45 strain were reported in 2022. The data presented here demonstrated a trend of fluctuation in the incidence rates of both strains throughout the five years, indicating annual fluctuations in the prevalence of each strain (Table 5.12).

5.12 Incidence of Cervical Cancer

The detail provided in Table 5.12 represented the incidence rates of cervical cancer (SCC) per thousand individuals over five years. The incidence rate for 2018 was 3 cases per thousand individuals. The incidence rate fell to 1 case per thousand individuals in 2019, indicating a decline in the number of cases that were recorded. In 2020 also, only 1 case per thousand individuals was observed, which was the same incidence rate as in 2019. There were no SCC cases recorded in 2021, showing a sharp decline in the incidence rate to zero for that year. However, the incidence rate increased slightly to two cases per thousand persons in 2022 (Figure 5.26)

Table 5.12: Incidence of HPV Strain and cervical cancer in Jammu region

Year	Incidence Rate (16/31 strain) /per thousand people	Incidence Rate (18/45 strain) per thousand people	Incidence Rate of Cervical cancer (SCC) /per thousand people
2018	45	15	3
2019	32	10	1
2020	15	9	1
2021	33	18	0
2022	30	14	2

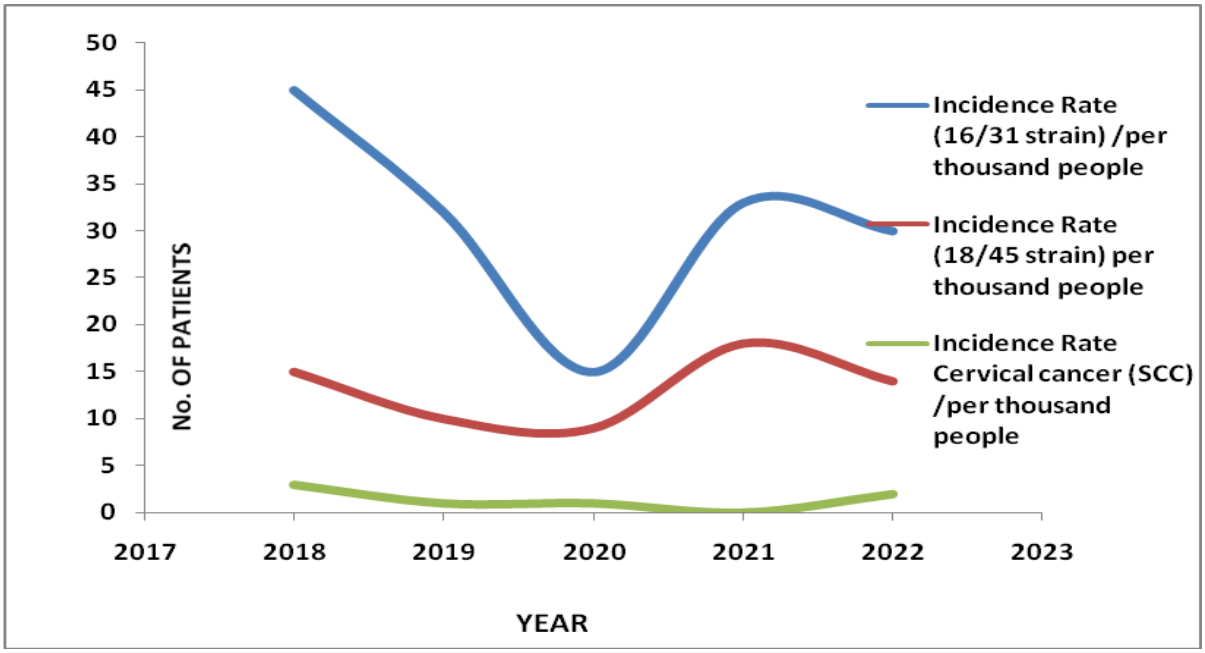

Figure 5.26: Trends in Incidence of HPV Strains 16/31 and 18/45, and Cervical Cancer Cases (2018-2022)

5.13 Prevalence of HPV strains and Cervical Cancer

The number of cases of a particular condition within a population at a given time is provided by the prevalence rates. The 16/31 strain's prevalence rate in this instance was 3.88%, meaning that roughly 3.88 out of every 100 people in the population carry this specific strain. Comparably, the 18/45 strain has a 1.65% prevalence rate, meaning that 1.65 out of every 100 people are impacted by this strain. Conversely, the prevalence rate of Squamous cell carcinoma (SCC) was 0.18%, which means that a smaller subset of the population—roughly 0.18 out of every 100 people—had received an SCC diagnosis (Table 5.13).

Table 5.13: Showing the prevalence rate of HPV strain and cervical cancer from the year 2018-2022.

Prevalence Rate (16/31 strain)	Prevalence Rate (18/45 strain)	Prevalence Rate of cervical cancer (SCC)
3.88%	**1.65%**	**0.18%**

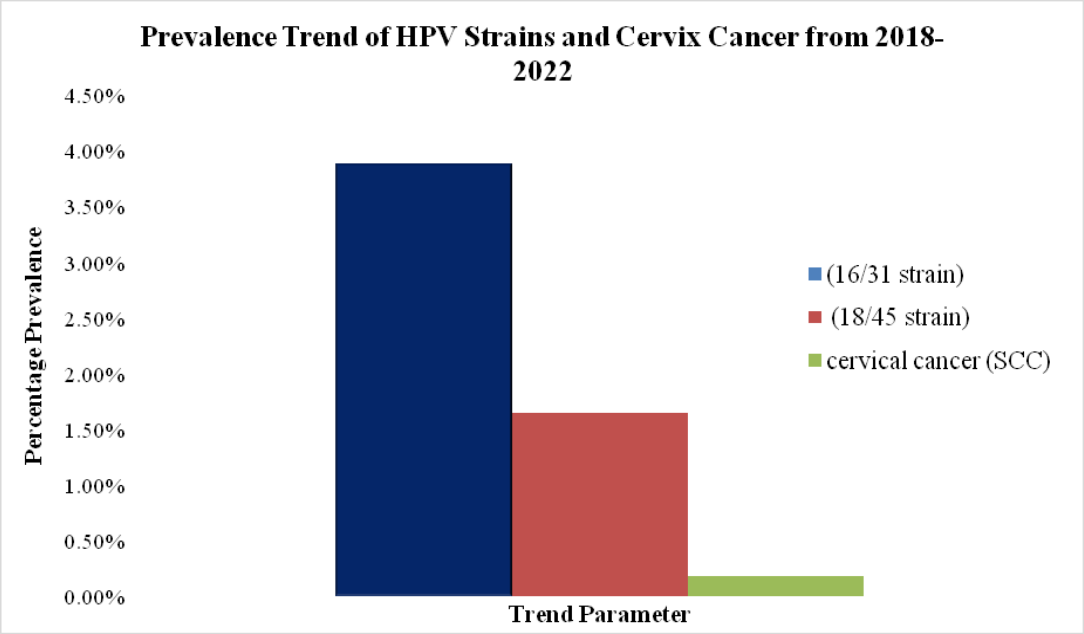

Figure 5.27: Prevalence Trends of HPV Strains 16/31, 18/45, and Cervical Cancer Cases (2018-2022).

CHAPTER 6

DISCUSSION

Early detection is pivotal in cancer therapeutics as it improves prognosis and overall survival. Cervical cancer is highly preventable, yet in 2020, 6, 04, 127 fresh cervical cancer cases were registered; and 3, 41, 831 females died. The worldwide incidence of cervical cancer is highly skewed towards low and low/middle-income countries. Asian sub-continent alone books more than 58% of cervical cancer incidences. Very few studies have been conducted regarding cervical cancer in northern India, particularly in the Jammu region.

6.1 LBC and HPV

During the early onset of sexual activity women often get infected with HPV. However, most of the time this infection is transient and clears up in due course of time. Screening with cytology is a simple and preferred method in cervical cancer diagnosis. Yet, screening with cytology alone during the initial stages of HPV infection is amenable to false positive results and thus warrants unnecessary radio-chemo treatments and unjustified colposcopies. Thus, screening alone with cytology is not enough to predict or design the most appropriate course of action that would benefit the patient. Several studies have compared liquid-based cytology and HPV testing as screening methods for cervical cancer. In a study by Feldstein and his coworkers in 2023 compared two screening methods viz. LBC and HPV testing among 42, 244 women enrolled for the study. 20, 997 women were screened with LBC while 21, 247 women were screened with HPV. It was observed that HPV screening resulted in higher positive screening results (18%) as compared with LBC where positive results i.e. abnormal cytology were found in 9.4% of women. Non-16/18 (other hrHPV) was the most prevalent HPV type detected in 85% of cases, HPV 16 was detected in 23% of women while HPV 18 in 5% of women. Further, it was observed that CIN3+ detection was higher in women screened with hrHPV (1.93) as compared to women screened with LBC (1.35). It was concluded that hrHPV detected 43% more CIN3+ as compared to LBC screening. A similar study by Rebolj and his team in 2019 also established the importance of hrHPV detection as a preferred method of screening compared to LBC in the age group 25-30. It was shown that hrHPV screening enhanced the chances of CIN3+ detection by 40% and cervical cancer by 30% as compared to LBC (Rebolj *et al*., 2019)

In a similar study, Schiffman and his coworkers in 2018 evaluated the significance of HPV testing and cervical cytology in the diagnosis of cervical cancer. It was observed that HPV testing yielded more positive results (83.8%) as compared to cytology (61.9%) while co-testing HPV and/or cytology yielded even higher positive results (87.3%). Further, HPV testing was more efficient in detecting CIN3+ (83.9%) as compared to cytology (62.8%), and adenocarcinoma *in situ* (82.2%) by HPV testing, 53.2% by cytology. The study concluded that HPV testing was more efficient than cytology in diagnosing pre-cancer as well as cancer at any time duration before diagnosis. Further, HPV testing was only marginally more efficient than co-testing i.e. HPV and/or cytology in diagnosis pre-cancer and cancers at any time interval before diagnosis. Similar results have been corroborated in a study by Poljak and his team in 2020 showed that HPV-assisted cervical cancer screening had higher sensitivity in diagnosing CIN2+, CIN3+, and cervical cancer as compared to cervical cytology which is less accurate and subjective, requires highly trained professionals, low reproducibility, and yields high false positives. In a recent study, Abbas and his coworkers 2022 analyzed the role of conventional cervical cytology versus HPV-genotyping as a screening method in diagnosing dysplastic cervical epithelial changes. It was concluded that both morphological screening via cytological examination along with immunocytochemistry and HPV-Genotyping should be employed as a preferred screening method in detecting dysplastic cervical epithelial changes.

The findings of the above Western studies were also corroborated in the Indian setup which envisaged an immediate paradigm shift from cervical cytology to HPV-based screening in cervical cancer diagnosis. In a Uttrakhand-based study, the correlation between cervical cytology, HPV-based testing, and colposcopy in cervical cancer screening was analyzed. A total of 324 patients were included in the study among which 263 underwent colposcopy, 214 had pap smear test and 100 patients were screened with HPV DNA testing. It was observed that HPV-based diagnosis was highly sensitive (90%) as compared to colposcopy (87%) and cytology (66. 6%) but specificity was marginally lower (84. 6%) as compared to cytology (93. 5%) and colposcopy (86. 32%). It also suffered from fewer false positives as compared to other diagnostic procedures. It was concluded that HPV DNA testing is a more efficient method of cervical cancer screening as compared to cytology and should replace cytology as the preferred primary screening method. However, it can be complemented with other diagnostic methods such as colposcopy to enhance the diagnostic efficacy. The results of our study show a significant association between abnormal pap smear and

HPV positivity with a p-value of (p-value=0. 00004). We suggest that cervical cytology alone is not efficient in the detection of cervical cancer as it suffers from a high degree of false positives and false negatives. It must be complemented with HPV testing to improve the efficacy of diagnosing cervical lesions. In a low-to-middle-income country like India, it is not plausible to immediately shift from cytology-based screening to HPV-based screening but gradually India should adopt HPV bases screening as the primary screening method for cervical cancer. It may be further complemented with immunohistochemistry (p16) based markers or colposcopy for meticulous detection of cervical cancer.

6.2 Viral load distribution and cervical lesions

There is a lot of ambiguity regarding the correlation between HPV viral load and cervical cancer and the significance of viral load distribution in the diagnosis and prognosis of cervical cancer (Vinodhini *et al*., 2012; Long *et al*., 2018) . The majority of the studies have established that HPV viral load is indeed related to the severity of cervical lesions but this is not a generalization. Viral load of only HPV 16 can predict CIN2+ lesions and this correlation has not been found in HPV 18 or other HPV genotypes. However contrary to this, a Canadian study showed that in addition to HPV 16, HPV18 and HPV31 viral load were good predictors of high-grade cervical lesions. A Colombian cohort study by Del Rio-Ospina exhibited more conflicting results showing an inverse relation between HPV16 viral load and level of cervical lesions. It was documented that high-grade cervical lesions in women corresponded to a lower HPV 16 viral load. Therefore, our study aimed to analyze the significance of high-risk HPV viral load distribution in cervical cancer diagnosis. It was observed that the highest viral load of HPV cases (6. 40%) was found predominantly in the age group of 36-45. Medium viral load instances (14. 29%) were also recorded in the same age group while a low viral load (3. 94%) was more prevalent in the 46-65 age group. 17% of the population had a high viral load, while 11% of patients had a low viral load, indicating a low relative concentration of HPV viruses. 39% of patients were detected with a medium viral load while 28 percent patients were detected with very low viral loads, suggesting that HPV infections with moderate to low viral presence are prevalent and subdue gradually in due course of time. In our study, it was found that high viral load was mainly present in patients who have been detected with high-risk HPV 16/31 and HPV18/45 strains. It was concluded that the risk of high-grade cervical lesions increased with an increase in high-risk HPV viral load. The results of our study were in

alignment with earlier studies signifying the importance of viral load distribution in predicting cervical cancer outcome.

A study by Liu and his coworkers on the significance of high-risk HPV viral load in diagnosing and predicting cervical lesions. It was observed that out of the 265 patients enrolled in the study, 139 (52. 5%) had a low viral load, 90 (34 %) exhibited moderate viral load and 36 (13. 4%) exhibited high viral load. A high viral load of high-risk HPV was an independent factor for CIN 1, CIN 2, and CIN 3 lesions and cervical cancer. Similarly, the study also evaluated the diagnostic value of high-risk HPV viral load in cervical lesion assessment. Spearman rank correlation analysis depicted a positive correlation between high-risk HPV16/18 viral loads with the grade of the cervical lesion. Further, it was observed that HPV16/18 viral load was positively correlated with cervical lesion grades in HPV16/18 positive cases, irrespective of the other 12 high-risk HPV types. A similar study showed the association between HPV16 and 18 viral loads and abnormal cytology in Saudi Arabian women. It was observed that a high viral load distribution of HPV16 and 18 were predominantly found in samples detected with HSIL or that had cervical cancer. Viral load of both HPV types varied with the severity of cytology grade and abnormality i. e. patients with high grade of cytology generally had higher viral loads of high-risk HPV types. Patients with cervical cancer had the highest viral load of HPV 16 and 18 types in comparison to other cytology grades. A Chinese retrospective study carried out by Tao and his co-workers in 2020 compared the viral load of eight high-risk HPV genotypes (HPV16/18/31/33/45/52/58/82) in ≤ CIN1 and CIN2 + patients. It was observed that a statistical significance value was detected only in CIN2+ patients having high viral loads of the HPV16 genotype while other genotypes did not exhibit any significant association between viral loads and cytology grades. A 15-year prospective cohort study carried out in China also showed that the difference in HPV viral load over time was significantly correlated with higher cervical-grade lesions (CIN2+/CIN3+). Women with elevated levels of viral load (15. 4%) exhibited a 38-fold enhanced risk of being CIN2+ in comparison to HPV-negative women (0. 4%).

6.3 Early marriage

Age at marriage has a significant impact on the development of cervical cancer. Various studies have established a positive correlation between marriage age and cervical cancer. Cervical cancer is a disease of married as it is seldom seen in unmarried women and almost absent among those performing celibacy. Various studies have shown that early age at marriage corresponds to early

and prolonged sexual activity which augments, the risk of getting HPV infection and sexually transmitted diseases. Young age at first mating is linked with risky (sexual behavior, high behaviors of sexual partners, early pregnancy, and less consistent condom use, which augments the risk of acquiring sexually transmitted infections. Sexual activity at a young age makes them susceptible to the onset of cytopathological changes in the cervix and develop other pre-cancerous lesions which may ultimately progress to cancer (Monica & Mishra, 2020) . Another study showed that the onset of cervical dysplasia was asymmetrically skewed in young women (Carson & DeMay, 1993) . A study on the association between early marriage and the outcome of the cervical pap smear results found that early marriage is a potent gradient responsible for the occurrence of abnormal Pap results (Khalaf *et al*., 2015) . A study by Fotra and his coworkers in 2014 showed that age at marriage (age at first intercourse) resulted in a skewed distribution of HPV across various age groups. HPV distribution was higher in the young age group as compared to older age groups. A substantial association (p-value= 0. 0081) was found between HPV infection and age at marriage of women. Younger women (<18 years) represented 2. 5-fold higher chances of getting HPV infection as compared to other age groups (Fotra *et al.*, 2014) .

A study on various risk factors associated with cervical cancer showed that about 86. 5% of women marrying before 17 years of age represented a maximum risk of cervical cancer development (Mayavati & Jawadekar, 2011) . The epidemiological risk factors related to cervical cancer found that early age of marriage and age at first intercourse ≤20 years were as positively associated with cancer of the cervix (Kashyap *et al*., 2019a) . Various other studies also depicted considerable association among women initiating their sexual life at a younger age. Early marriage results in longer sexual life in women and more pregnancies, thus augmenting the chances of developing cervical cancer in these females owing to immature cervix and more wear and tear owing to prolonged sexual life and higher pregnancies (Baudu *et al*., 2014) . The results of our study, are in correspondence to earlier studies showing a significant association between women who married at an early age and HPV infection. The majority of the HPV strains were detected in females who married before 22 years of age while females who married after 22 years of age exhibited a lower risk of HPV positivity. HPV16/31 was the predominant strain detected in females in contrast to HPV18/45.

6.4 History of STD

History of sexually transmitted diseases in females also has a great impact on the initiation and progression of cervical cancer. In our study, it was found that females with a prior incidence of STD showed an increased chance of getting HPV and cervical cancer. HPV infection when studied about females having an early exposure to sexually transmitted disease showed that 33. 56% of females who had a history of STD were HPV-positive. HPV infection was dependent on STD history and a significant association was found between STD history in females and HPV infection (p-value <0. 05). Cunha and his team workers in 2020 reported that in females with a prior incidence of STD, HPV infection was more prevalent and linked with reduced ability to clear HPV infection. Concurrent infections with gonorrhea, chlamydia, and herpes have been linked with HPV persistence and a higher chance of progressing into cervical neoplasia. Gonorrhoea was the most common infection followed by syphilis, herpes, and HIV in HPV-positive women. Further, HIV infection predisposes women to acquire HPV and stimulates the growth of cervical neoplasia by up-regulating HPV oncogene expression (Cunha *et al*., 2020).

In a study, it was found that the overall prevalence of STI was 26. 78 % and the mostprevalentpathogenswere*Ureplasmaparvum*/*Ureplasmaurealyticum and Mycoplasmahominis* It was observed that infections transferred via the sexual route were considerably related to abnormal cervical cytology grades, HPV persistence, and neoplasia progression. Sexually transmitted infections probably facilitate the entry and persistence of HPV by reducing host cell-mediated immunity and by chronic inflammation and ulceration of the cervical epithelium (Alotaibi *et al*., 2020) . Abebe and his coworkers 2021 also reported a higher prevalence of STDs among cancer-suspected patients. HIV, HBV, HCV, and syphilis were the most common STDs found in the patients. The higher burden of sexually transmitted diseases in cancer-suspected patients was because both HPV and STDs share a common route of infection and augment cancer development and progression. However the role of STDs as an autonomous risk factor for cervical cancer is still debatable (Abebe *et al.*, 2021) . A study by Mariam and his coworkers in 2019 also reported that the history of any sexually transmitted disease, sex partners throughout life, and oral contraceptive use were significant elements of HPV acquisition and augmented the development of cervical cancer (Mariam *et al.*, 2019). A similar study also described sexually transferred infections among females to be an important determinant responsible for the formation of pre-cancerous cervical lesions. Females with a past of sexually transmitted diseases exhibited a 2. 6-fold increased

probability of evolving pre-cancerous lesions in the cervix in contrast to women without a sexually transmitted disease history (Teklehaimanot *et al.*, 2022). In our study, we also found that women who were suffering from sexually transmitted diseases were more susceptible to HPV infections.

6.5 Early pregnancy

Early pregnancy is also one of the potent contributors to the progress of cervical cancer. One of the possible explanations for this could be the trauma to the immature region of the cervix's transformation zone, which promotes viral access to mitotically active epithelial cells. Infection of the mitotically active epithelial cell allows the virus to cause latent infection and form dysplastic lesions of the cervix which may persist and progress to carcinoma in certain cases. Fotra*et al.,* in a study, depicted that the women who had delivered their first child before 18 years of age showed the maximum percentage of HPV infection which decreased with an increase in age. About 46. 4% of females were married at the age of 21-25 and 54. 3% of females had their first child in the age group 18-23. It was further observed that women who had their first childbirth between 25-31 years had a 5-fold lesser chance of getting HPV infection than women who became mothers <18 years of age (Fotra *et al.*, 2014) .

A similar study identified that first pregnancy at a young age (>20 years) is a significant risk factor in cervical cancer development. It was seen that also in women aged above 25 years, age at child delivery was related to cervical cancer progression. Thus, it was concluded that it is not the delivery age but rather the breaks between deliveries that may be requisite for the initiation of cervical cancer (Bezabih *et al.,* 2015) . This was proved by studies showing that women who married late but delivered more children had cervical cancer. Hence, it is not the number of parity that augments the cervical cancer risk; rather it is the quickness of multiple pregnancies that substantially augments cervical cancer risk. Females with initial pregnancy between 15-19 years carried a double higher risk of cervical cancer development compared to those with first pregnancy at ≥ 25 years (Green *et al.*, 2003).

The study depicted that age at first mating, marriage at early age, and young age at first pregnancy were highly correlated and exhibited similar invasive cervical cancer threat in developing countries. Females reporting age at first mating and age at first pregnancy at ≤ 16 years exhibited a 2. 4 fold higher cervical cancer risk as compared to those with age at first mating and first pregnancy age ≥21 years (Louie *et al.*, 2009) . The impact of pregnancy on the development of cervical cancer is

highly debated with some reports predicting that levels of hormones like progesterone, estrogen, and human chorionic gonadotropin during pregnancy have a positive association with HPV 16 and18 infection and thus promote cervical cancer progression. In some studies, it has been found that an increase in the lymphatic circulation and blood flow to the reproductive organs of pregnant women, cervical dilation, and a decrease in the body immunity during the initial stage of pregnancy may accelerate tumor metastasis and cervical cancer development (Beharee *et al.*, 2019) . In our research also a positive connection between HPV infection and early pregnancy was found. This might be due to the damage to the transformation zone of the cervix which becomes increasingly susceptible to viral infections which might progress to cervical lesions and ultimately develop into cervical cancer (Bezabih *et al.*, 2015). In our research also majority of HPV strains about 6. 65% were found in females who showed their first pregnancy before 22 years of age while only about 4. 14% of HPV strains were found in females who got pregnant after the age of 22. A p-value of 0. 003276574 suggested a very strong association of early pregnancy with HPV positivity.

6.6 Vaginal discharge

Most of the patients in our study reported more than one complaint. The most common complaints in our study were pain in the lower abdomen (PLA), followed by menstrual disorder, vaginal bleeding and itching, and vaginal burning. Vaginal bleeding when studied as a risk factor showed considerable association with cervical cancer development as the p-value was significant (p-value=0. 001). A similar study showed that vaginal discharge was the chief complaint among 36. 96% of the women, followed by pain in the abdomen occurring in 25. 63% of women and irregular menses among 12. 78% of women (Sachan *et al.* , 2018). In a similar study, Fotra *and* his coworkers in 2014 presented that women having genital warts had a 100% chance of getting HPV infection, and women with inter-menstrual bleeding had 87. 5%, women with post-menopausal bleeding having 57. 89%, women with vaginal discharge with 56. 25 % and women with pain in the lower abdomen having 31. 25% chance of getting HPV infection. All the complaints were having significant association with HPV infection (p-value<0. 0001) (Fotra *et al.*, 2014). A similar study also established abnormal vaginal discharge as one of the primary symptoms among women visiting for cervical cancer screening. It was found that abdominal vaginal discharge characterized by red-colored discharges and consistency and smells was strongly associated with abnormal cytopathological findings (Salih *et al.*, 2017).

Mwaka and his coworkers in 2016 also emphasized the impact of vaginal bleeding as a vital risk factor in the initiation of cervical cancer among women in northern Uganda. It was found that inter-menstrual bleeding was the most frequently reported symptom in these women followed by post-menopausal bleeding, vaginal discharge, and post-coital bleeding (Mwaka *et al.*, 2016). A study while working on cervical cancer screening in the Alappuzha region of Kerela showed that pain in the lower abdomen (43. 98%) and vaginal discharges were the primary complaints of women visiting screening programs. It was further observed that the vaginal discharge was strongly related to atypical pap smear with a statistically significant p-value<0. 01. It is speculated that the excessive vaginal discharge could be from the fragile blood vessels as a result of lower genital tract infections causing inflammation of colpos or due to neo angiogenesis seen in malignancy (Mohan *et al.*, 2022). Our findings are consistent with previous research showing a greater chance of HPV infection in women exhibiting symptoms like vaginal bleeding. A p-value of 0. 000000000011910 was found by statistical analysis; this value is less than 0. 005. Based on the study population, it appears that there is a correlation between HPV infection and vaginal bleeding.

6.7 Smoking

In the present study, HPV infection when studied in relation to smoking exhibited a positive association with a p-value of 0. 016. It was observed that non-smokers had the least risk of infection while frequent smokers exhibited maximum risk of HPV infection. Similar results were found while studying the relationship between smoking and the risk of cervical cancer development. It was seen that the number of years of smoking was strongly linked with the risk of evolving CIS, CIN3, and ICC as compared to the number of cigarettes smoked. Smoking status, intensity, and duration displayed a two times higher CIS, CIN3, and invasive cervical cancer risk. However, ever since last use was related to two times decreased risk while the rising number of cigarettes smoked daily and smoking years had a positive association with cervical cancer risk (Roura *et al.*, 2014) . Fang and his coworkers showed that current smoking and those who have ever smoked had an enhanced risk for CIN3+ compared to non-smokers. Further, in women having high-risk HPV the possibility of cervical cancer progression was strongly associated with extensive smoking duration but in the case of infection with persistent HPV, no such trend was seen. Those females infected with high-risk HPV and who began smoking between 16 to 19 years of age exhibited a significantly enhanced risk for CIN3 compared to those who never smoked, while those

women having persistent HPV infection and began smoking below 15 years carried the highest risk of CIN3 development (Fang *et al.*, 2018) .

A study by Alam and his team in 2008 found that benzo[*a*]pyrene (BaP), a carcinogen found in cigarette smoke undergoes molecular interaction with HPV synthesis, and results in increased virion production and enhances viral persistence necessary for progression. High concentrations of BaP increased HPV31 viral titers by almost 10 fold while low concentrations caused higher HPV genome copies including high templates which produced E6 and E7 oncogene transcripts (Alam *et al.*, 2008) . The study presented that current tobacco smoking was related to an enhanced risk of persistent HPV infection while the growing number of cigarettes smoked daily enhanced HPV-positivity risk. In contrast to never smokers, females who smoked 15 or more cigarettes daily carried a two-fold risk of HPV positivity (Cohen *et al.*, 2019) . The study stated that high viral loads of high-risk HPV 16/18 were linked with current smokers and not with former smokers. However, the intensity and period of smoking in current smokers did not result in any variation in the viral load (Xi *et al.*, 2009) . The study reported that irrespective of the status of HPV infection in women, smoking independently regulated cervical cancer progression. The mechanism behind such a phenomenon is that smoking induces epigenetic changes in the tumor suppressor genes. The substances found in cigarettes, like nicotine, cotinine, and BaP were also identified in the mucus of the cervical area of female smokers and as such predicted to cause DNA damage in squamous epithelial cells (Collins *et al.*, 2010) . Another possible mechanism of smoking-induced cancer is the initiation of a resident immune-suppressing effect produced by tobacco metabolites. These metabolites reduce the host's ability to induce a potent immune response against viral infections thereby enhancing the susceptibility of the cervical region to persistent HPV infections (Aguayo *et al.*, 2020) .

The results of our study are in line with earlier studies, showing that women who smoke have a greater prevalence of HPV infection than non-smokers. A p-value of 0. 00000059133 was found by statistical analysis, which is significantly less than the accepted significance level of 0. 005. This supports the findings from previous research and indicates an association between smoking and higher HPV-positive among study participants.

6.8 Oral contraception

Prolonged use of oral contraceptives has long been debated to have a role in cervical cancer development. Females using oral contraceptives for a quite long time had an enhanced threat of HPV infection and cervical cancer. Various studies have described oral contraceptive use as an independent predictor of HPV infection (Ley *et al.,* 1991). A study exhibited that extended oral contraceptive use results in a decrease in cervical HPV acquisition and a regular use of intrauterine devices as a contraceptive method imparted a protective effect against HPV infection (M. T. Goodman *et al*., 2009). Fotra and his coworkers obtained statistically significant results showing that barrier methods of contraception provided the highest degree of protection against HPV infection, followed by women who use oral contraceptives and least in women with no use of contraception (Fotra *et al.,* 2014) . A multicentric case study revealed that every use of oral contraceptives did not result in an appreciable growth in invasive cervical cancer or cervical cancer *in situ*. However, the extent of oral contraceptive use was considerably related to increased cancer incidence. HPV-positive women using oral contraceptives for a period of 5 to 9 years and ≥10 years highly augmented cervical cancer risk as compared with never users. Women using oral contraceptives for a period of fewer than 5 years did not affect cervical cancer risk and time since first or last use also had negligible impact (Kamani *et al.*, 2022) . A similar study analyzed the association between oral contraceptive use and oral cancer risk among HPV-positive U. S women. It was described that a rise in the duration of use of oral contraceptives resulted in a reduced threat of cervical cancer and such a tendency was substantial only in women who used contraceptive pills for fewer than 5 years as compared with never users (Shields *et al.*, 2004). Various studies have registered a 1. 5-to-3-fold higher cervical cancer threat in females who used oral contraception for a duration exceeding five years. The relative cervical cancer risk reduced as time since last use increased and beyond 10 years did not vary significantly from that of never users. The mechanism behind such a trend can be attributed to the fact that steroid contraceptives may promote HPV - DNA ligation with the host genome and binding at specific HPV-DNA sequences inside transcriptional regulatory regions thereby modulating the cell cycle (Gierisch *et al.,* 2013) . There also have been some reports showing no considerable relation between oral contraceptives and cervical cancer risk. A study on North Indian women did not find any considerable association between the type of contraceptive used and HPV infection risk (Garg *et al.,* 2016) .

The results of our study, are in line with that of (Gierisch *et al.*, 2013) showing a considerable association between oral contraception use and cervical cancer risk (p-value= 0. 00000000000050). A plausible explanation behind this could be the longer duration of use of oral contraceptives resulting in DNA damage and inhibition of apoptosis.

6.9 Socioeconomic status of symptomatic women

Low economic status is also a contributing element in cervical cancer progression. The impact of socioeconomic status on HPV infection and cervical cancer development is highly debatable with various studies reporting contradicting results. In our study, it was found that females with low socioeconomic status had a greater chance of getting HPV infection as compared to high socioeconomic background. Low socioeconomic status showed a positive association with HPV positivity (p-value <0. 05). The study showed that the low socioeconomic status of women was directly associated with the incidence and prevalence of HPV infection. It was seen that the prevalence of HPV infection surged with a decrease in socioeconomic status. Most of the HPV-positive women had low socioeconomic status and lacked knowledge about HPV infection and vaccination and thus carried the highest risk of cervical cancer (Fotra *et al.*, 2014) . A similar study by Vinoda and his coworkers in 2015 showed a high prevalence of cervical cancer in rural areas and low socio-economic groups. This was primarily due to poor genital hygiene, poor nutritional status, poor health, poverty, low education level, dearth of knowledge and awareness among people, absence of screening programs, and social stigma which unable affected individuals from seeking medical care. The study exhibited that education, residing area, use of old cloth, sanitary napkins, personal hygiene, and health services availability have a significant relation with cervical cancer (p-value < 0. 05). Daily bathing and bathing during menses were found to be protective for cervical cancer. Females having low socioeconomic status and poverty carry the maximum probability of HPV infection as such women do not opt for cervical cancer screening and are unable to exploit health services and vaccinations available for preventing cervical cancer (Vinoda *et al.*, 2015) . Kops and his coworkers in 2021 while studying the impact of socioeconomic status on HPV infection described a similar HPV prevalence among different social classes in the Brazilian population. The overall HPV prevalence among the lowest class was slightly greater than the highest, but this increase was non-significant (p=0. 48). High-risk HPV strain distribution among various classes was also similar (Kops *et al.*, 2021) . The study indicated that the resident country and socioeconomic elements like little education, and low family income, were strongly related to

an elevated threat of developing cervical cancer (Broberg *et al.*, 2018) . Low socioeconomic status women in our sample had significant HPV-positive rates, which is consistent with previous research.

6. 10 Multiparty

Parity is one of the pivotal factors in cervical cancer development. Studies have established that the number of children delivered is directly correlated with an increased risk of developing cervical cancer. In our study also, it was observed that parity (number of births given) when studied in relation to HPV infection showed a positive association (p-value= 0. 0005). Further HPV infection in women increased as the number of children increased, with nulliparity (no children) having the lowest risk of infection. It was concluded that multiparty carried the highest risk of cervical cancer development while nulliparity had the least risk or no risk of getting cervical cancer. Our study, results corroborate with the results of Fotra and his coworkers exhibited that women with 4 or more children had double the risk of getting HPV infection in contrast to the females with fewer children (Fotra *et al.*, 2014) . High parity in Indian women is a result of a high portion of the rural population in whom it is customary to marry girls at a younger age which leads to more children. The study found that high parity in HPV-infected females considerably increased the possibility of squamous cell cervical carcinoma (Sarma *et al.*, 2015) .

A similar study reported a 3-5 times higher risk associated with women with higher parity as compared to nulliparous women (Raj & Srivastava, 2022) . In situ carcinoma and invasive cervical cancer data from case-control studies of four continents showed that women with three or four complete pregnancies had 2. 6 fold cervical cancer risk whereas females with seven or greater pregnancies had 4 times the cervical cancer risk as compared with women who had never given birth (Bosch *et al.*, 2002) . It was found that the number of HPV cases and squamous intraepithelial lesion rate increased with increasing parity in adult females however no statistically significant relation was found between differences in SIL rate in different parity groups (Mishra *et al.*, 2020). The study showed multiparity as a risk factor for cervix cancer among female patients where the average parity was 4. 8. In Muslim cervical cancer patients, the average parity was higher than in Hindu patients and thus exhibited an early initiation and augmented risk of getting cervical cancer in contrast to Hindu patients (Rani *et al.*, 2016) .

A study depicted that multiparous females with more than 4 childbirths and first pregnancy before 25 years were recognized as major risk factors in the progression of cervical cancer (Bezabih *et al.*, 2015) . The mechanism behind the role of multiparity in accelerating cervical cancer is due to a surge in the hormonal concentration of progesterone and estrogen which reach the highest level during the final gestational weeks. Such hormonal fluctuations alter the transformation zone throughout pregnancy and increase the transformation zone's squamous metaplasia (Tekalegn *et al.*, 2019) . Some studies have speculated trauma to the cervix uteri at the time of vaginal deliveries results in various cervical abnormalities, increases susceptibility to viral infections, and thus augments cervical cancer development and progression. The idea was strengthened by the fact that cesarean delivery did not augment cervical cancer risk as vaginal delivery does (Muñoz *et al.*, 2006) . Our study findings are in line with those of Srivastava *et al*., 2022 indicating that multiparity is associated with HPV infection.

6. 11 Incidence and prevalence

In our study, 5-year incidence of both HPV16 and HPV 18 and cervical cancer (SCC) was monitored. The incidence of both HPV strains showed a decrease from 2019 to 2021 and again increased in 2022. A similar trend was observed in the case of cervical cancer. The annual incidence in 2022 of HPV16 was 38 cases per 1000 females while the incidence of HPV18 was 14 cases per 1000 females. The incidence rate of SCC in the year 2022 was 2 cases per 1000 females which is in accordance with contemporary studies carried out in the North Indian region. Various earlier population-based cancer registries from areas like Chennai, Bengaluru, Bhopal, Delhi, and Barshi Rural reported an incidence rate in the range of 13 to 16/100, 000 (3-yr PBCR). A detailed study on the incidence of cervical cancer among different regions of India was carried out in 2016. The highest average age-adjusted incidence rate is 23. 07/100000 was recorded in Mizoram state and the lowest 4. 91/100000 in the Dibrugarh district. Various northern regions of India like Delhi recorded an incidence rate of 15. 53/100000 and Patiala recorded an incidence rate of 11. 46/100000 (Bobdey *et al*., 2016) . Another study carried out in 2016 to assess the cervical cancer burden throughout India showed that cervical cancer was the second most common cancer in India after breast cancer with 77000 incident cases in India. Sikkim recorded the lowest cervical cancer incidence among all the states followed by J&K (India State-Level Disease Burden Initiative Cancer Collaborators 2018). A detailed study showed that Jammu and Kashmir have recorded a declining trend of cervical cancer incidence in recent decades with 7. 24 in 1990, 6.31 in 2000,

6. 21 in 2010 and 6.13 in 2019). J&K recorded the lowest cervical cancer incidence of 6. 13 in 2019 throughout India and was less than half of the Indian average of 13. 1. Similarly, mortality of J&K also showed a declining trend (4.59 per 100, 000 women in 1990, 3. 93 in 2000, 3. 57 in 2010, and 3. 38 in 2019) and the lowest among all Indian states. The mortality rate recorded in 2019 3. 39, too less than half of the Indian average 7. 38 (Singh *et al*., 2022) . Studies reported similar trends regarding the frequency and incidence of cervical cancer in the Jammu region (Fotra *et al.*, 2014) , (Harper & Demars, 2017; Yaqoob & Kaul, 2018) . Most of the females were in the age group of 25-35 which varies as per the study sample.

The number of cases of a particular condition within a population at a given time is provided by the prevalence rates. The prevalence of the 16/31 strain in our study was found to be 3. 88%, meaning that roughly 3. 88 out of every 100 people in the population carry this specific strain. Comparably, the 18/45 strain exhibited a lower prevalence of 1. 65% as compared to HPV 16. The prevalence rate of Squamous cell carcinoma (SCC) was found to be 0. 18%, which means that a smaller subset of the population—roughly 0. 18 out of every 100 people were diagnosed with SCC (Table 5. 13). A study by Bhardwaj showed the high prevalence of HPV in the Jammu region. A prevalence of 40. 2 % was recorded in this region. In addition to this it was observed that high-risk HPV types 16/18/31/33 were more prevalent (11%) as compared to other HPV types (Bhardwaj *et al*., 2015) In a similar study Kaur and her coworkers 2014 assessed the prevalence of various HPV types in Amritsar region of Punjab. It was observed that HPV 18 (6%) was more prevalent in this region as compared to HPV 16 type (2%). Other HPV types recorded were HPV 52 and HPV 39 (Kaur *et al*., 2014) .

6. 12 Limitation of the Study

One of the major limitations of the study was that the most vulnerable section of the society viz. sexual workers both married and unmarried could not be included in the study. As a small city, there is no registered area where such kind of activities is permissible as in metro cities like Delhi where such activities are regulated and data can be easily accessed. Further, women hesitated to disclose such details to us even for the research work.

- 4000 females were subjected to primary screening by LBC testing. Out of the total females screened by liquid-based cytology maximum of 3094 (77. 4%) were normal, and 682 (17. 0%) females had inflammatory cytology. Rest 224 (5. 6%) females were LBC positive i. e. . cervicitis or another benign lesion.
- The highest number of patients was 40. 4% (1615) were seen in the age group 36-45, followed by 38. 6% (1553) in the age group 25-35, 14. 9% (597) in the age group 46-55 and 5. 9% (235) in the age group 56-65 respectively. Out of 4000 women, 46% were early married, 31% had a history of STD, 46% of women had an early pregnancy, 46% of women had a problem with vaginal bleeding, 52% of women had low socioeconomic factors, 30% of women were using an oral contraceptive at an early stage, 39% of women had more than 2 children and smoking been reported in 19% of women.
- Among these LBC-positive female samples, 135 (3. 4%) female samples were atypical squamous cells of undetermined significance type (ASCUS), 49 (1. 2%) female samples were LSIL type, 33 (0. 8%) female samples were HSIL type and 7 (0. 2%) female samples were SCC type.
- All the samples were subjected to HPV testing for the HPV type present in these females. It was observed that 62. 33% (139) of the females with abnormal cytology were HPV 16/31 positive while 28. 6% (64) of females with abnormal cytology were HPV 18/45 positive. A significant association (p-value= 0. 005) was observed between the results of HPV-positive and abnormal cytology women. HPV strains were detected in 91% of the abnormal cytology cases. In 5. In 38% (12) of the cases of abnormal cytology, no HPV strain was detected and in 3. 58% (8) of the cases of abnormal cytology, no HPV test could not be done due to an inappropriate sample.
- Early marriage exhibited a significant association with HPV positivity ($p<0.05$).
- Early pregnancy showed a significant association with HPV positivity showing a p-value of 0. 003276574.
- Use of oral contraceptives also exhibited significant association with HPV positivity (p-value= 0. 00000000000050).

- History of STD had a significant association with HPV positivity (p-value= 0. 000000000000000000000622)
- Vaginal bleeding had a strong association with HPV positivity exhibiting a p-value of 0. 000000000011910.
- Smoking had a considerable relation with HPV positivity (p-value= 0. 0000000000000000059133).
- Low socio-economic status showed a positive relation with HPV positivity (p-value= 0. 000321451).
- Multiparity showed a positive association with HPV positivity exhibiting a p-value of 0. 00000322730.
- A significant association was detected between cervical cancer and HPV detection exhibiting a p-value of 0. 04.
- PAP/LBC and HPV testing combined had a greater efficiency than individual LBC or HPV DNA testing. It is very essential for efficient screening of cervical cancer patients and can detect false negatives that often appear in LBC testing. It also eliminated false positives that sometimes appeared in HPV testing. Co-testing has also been recommended by WHO in screening cervical cancer.
- A strong correlation was observed between LBC and HPV testing highlighting the importance of both these tests in cervical cancer screening programmes. Both these tests complemented each other's attributes and were pivotal in the screening of cervical cancer patients.
- Thus, our study corroborates studies of the West signifying the importance of combination testing in cervical screening programs, and proposes to adopt it as a regimen in cervical cancer detection.
- The incidence rates of the 16/31 and 18/45 strains were monitored over five years. There were 155 cases of the 16/31 strain and 66 cases of the 18/45 strain in 2018 -2024 indicating that the 16/31 strain was a more common strain found.
- The prevalence rate of Squamous cell carcinoma (SCC) was found to be 0. 18%, which means that a smaller subset of the population, roughly 18 out of every 10,000 people was diagnosed with SCC.

REFERENCES

1. Abbas, M. , de Jonge, J. , & Bettendorf, O. (2022). HPV-genotyping versus conventional cervical cytology as a screening method to detect dysplastic cervical epithelial changes. *Scientific Reports, 12*(1), 1–7. https://doi. org/10. 1038/s41598-022-22438-z
2. Abbas, M. , Erduran, I. , de Jonge, J. , & Bettendorf, O. (2022). Evaluation of P16/Ki67 (CINtecPlus) and L1-capsid compared with HPV-genotyping in cervical cytology in women ≥35 years old focusing on patients with atypical squamous cells of undetermined significance. *Oncology Letters, 24*(1), 1–6. https://doi. org/10. 3892/ol. 2022. 13362
3. Abebe, M. , Eshetie, S. , & Tessema, B. (2021). Prevalence of sexually transmitted infections among cervical cancer suspected women at University of Gondar Comprehensive Specialized Hospital, North-west Ethiopia. *BMC Infectious Diseases, 21*(1), 1–7. https://doi. org/10. 1186/s12879-021-06074-y
4. Abreu, A. L. P. , Souza, R. P. , Gimenes, F. , & Consolaro, M. E. L. (2012). A review of methods for detecting human papillomavirus infection. *Virology Journal, 9*(1), 262. https://doi. org/10. 1186/1743-422X-9-262
5. Aguayo, F. , Muñoz, J. P. , Perez-Dominguez, F. , Carrillo-Beltrán, D. , Oliva, C. , Calaf, G. M. , Blanco, R. , & Nuñez-Acurio, D. (2020). High-risk human papillomavirus and tobacco smoke interactions in epithelial carcinogenesis. *Cancers, 12*(8), 1–18. https://doi. org/10. 3390/cancers12082201
6. Akram Husain, R. S. , Rajakeerthana, R. , Sreevalsan, A. , Prema Jayaprasad, P. , Ahmed, S. S. S. J. , & Ramakrishnan, V. (2018). Prevalence of human papilloma virus with risk of cervical cancer among south Indian women: A genotypic study with meta-analysis and molecular dynamics of HPV E6 oncoprotein. *Infection, Genetics and Evolution, 62*, 130–140. https://doi. org/10. 1016/J. MEEGID. 2018. 04. 029
7. Alam, S. , Conway, M. J. , Chen, H. -S. , & Meyers, C. (2008). The cigarette smoke carcinogen benzo[a]pyrene enhances human papillomavirus synthesis. *Journal of Virology, 82*(2), 1053–1058. https://doi. org/10. 1128/jvi. 01813-07
8. Al-Daraji, W. I., & Smith, J. H. F. (2009). Infection and cervical neoplasia: Facts and fiction. *International Journal of Clinical and Experimental Pathology, 2*(1), 48–64.

9. Ali, M. A. M. , Bedair, R. N. , & Abd El Atti, R. M. (2019). Cervical high-risk human papillomavirus infection among women residing in the Gulf Cooperation Council countries: Prevalence, type-specific distribution, and correlation with cervical cytology. *Cancer Cytopathology*, *127*(9), 567–577. https://doi. org/10. 1002/cncy. 22165
10. Alizon, S. , Murall, C. L. , & Bravo, I. G. (2017). Why human papillomavirus acute infections matter. *Viruses*, *9*(10), Article 293. https://doi. org/10. 3390/v9100293
11. Alotaibi, H. J. , Almajhdi, F. N. , Alsaleh, A. N. , Obeid, D. A. , Khayat, H. H. , Al-Muammer, T. A. , Tulbah, A. M. , Alfageeh, M. B. , Al-Ahdal, M. N. , & Alhamlan, F. S. (2020). Association of sexually transmitted infections and human papillomavirus co-infection with abnormal cervical cytology among women in Saudi Arabia. *Saudi Journal of Biological Sciences*, *27*(6), 1587–1595. https://doi. org/10. 1016/j. sjbs. 2020. 03. 021
12. Alrajjal, A. , Pansare, V. , Choudhury, M. S. R. , Khan, M. Y. A. , & Shidham, V. B. (2021). Squamous intraepithelial lesions (SIL: LSIL, HSIL, ASCUS, ASC-H, LSIL-H) of Uterine Cervix and Bethesda System. *CytoJournal*, *18*,16 https://doi. org/10. 25259/Cytojournal_24_2021
13. Arbyn, M. , & Xu, L. (2018). Efficacy and safety of prophylactic HPV vaccines: A Cochrane review of randomized trials. *Expert Review of Vaccines*, *17*(12), 1085–1091. https://doi. org/10. 1080/14760584. 2018. 1548282
14. Artika, I. M. , Dewi, Y. P. , Nainggolan, I. M. , Siregar, J. E. , & Antonjaya, U. (2022). Real-time polymerase chain reaction: Current techniques, applications, and role in COVID-19 diagnosis. *Genes*, *13*(12). https://doi. org/10. 3390/genes13122387
15. Asiaf, A. , Ahmad, S. T. , Mohammad, S. O. , & Zargar, M. A. (2014). Review of the current knowledge on the epidemiology, pathogenesis, and prevention of human papillomavirus infection. *European Journal of Cancer Prevention*, *23*(3), 206–224. https://doi. org/10. 1097/CEJ. 0b013e328364f273
16. Athanassiadou, P. , Kavantzas, N. , Gonidi, M. , Athanassiades, P. , Liossi, A. , Nakopoulou, L. , Petrakakou, E. , & Davaris, P. (2001). Correlations between nuclear/cytoplasmic area ratio and classification of cervical smears. *Journal of Experimental & Clinical Cancer Research, 20*(4), 481–486.

17. Ault, K. A. (2006). Epidemiology and natural history of human papillomavirus infections in the female genital tract. *Infectious Diseases in Obstetrics and Gynecology, 2006*, 1-5. https://doi. org/10. 1155/IDOG/2006/40470
18. Bajaj, P. S. , Baviskar, P. , Rajyaguru, D. M. , & Chaudhari, R. A. (2022). Application of Bethesda system for cervical cytology at a tertiary care centre. *MVP Journal of Medical Sciences, 8*(2), 171–178. https://doi. org/10. 18311/mvpjms/2021/v8i2/320
19. Bamanikar, S. , Baravkar, D. , Chandanwale, S. , Dharwadkar, A. , & Paranjape, S. (2016). Study of cervical cytology and its correlation with clinical and histopathological findings. *Clinical Cancer Investigation Journal, 5*(5), 403. https://doi. org/10. 4103/2278-0513. 197869
20. Baudu, A. , Prétet, J. L. , Riethmuller, D. , Chotard, M. , Mougin, C. , & Mercier, M. (2014). Prevalence and risk factors of human papillomavirus infection types 16/18/45 in a cohort of French females aged 15-23 years. *Journal of Epidemiology and Global Health, 4*(1), 35–43. https://doi. org/10. 1016/j. jegh. 2013. 11. 003
21. Baumann, A. , Henriques, J. , Selmani, Z. , Meurisse, A. , Lepiller, Q. , Vernerey, D. , Valmary-Degano, S. , Paget-Bailly, S. , Riethmuller, D. , Ramanah, R. , Mougin, C. , & Prétet, J. -L. (2021). HPV16 load is a potential biomarker to predict risk of high-grade cervical lesions in high-risk HPV-infected women: A large longitudinal French hospital-based cohort study. *Cancers, 13*(16),. https://doi. org/10. 3390/cancers13164149
22. Beharee, N. , Shi, Z. , Wu, D. , & Wang, J. (2019). Diagnosis and treatment of cervical cancer in pregnant women. *Cancer Medicine, 8*(12), 5425–5430. https://doi. org/10. 1002/cam4. 2435
23. Bezabih, M. , Tessema, F. , Sengi, H. , & Deribew, A. (2015). Risk factors associated with invasive cervical carcinoma among women attending Jimma University Specialized Hospital, Southwest Ethiopia: A case control study. *Ethiopian Journal of Health Sciences, 25*(4), 345–352. https://doi. org/10. 4314/ejhs. v25i4. 8
24. Bhardwaj, R. , Bhagat, M. , Kumar, R. , Kuldeep, P. , Sharma, K. , & Kumar, P. (2015). Human papillomavirus detection in the women of Jammu region of Jammu and Kashmir: A hospital-based study. *Indian Journal of Applied Research, 57*(7), 2249–2555. http://www. internationalijar. com/indian-journal-of-applied-research-(IJAR)/file. php?val=July_2015_1437828971_15. pdf

25. Bhattacharya, A. , Sen, S. , Mandal, P. , Sharma Saha, S. , Sarkar, S. , Pathak, O. P. , Biswas, L. , Roy, J. , Banerjee, R. , Roy Chowdhury, R. , Pal, M. , Mukherjee, A. , & Sengupta, S. (2018). Prevalence and age-wise distribution of human papillomavirus type 16/18 infections among hospital screened women of a peri-urban area in West Bengal: Impact of socio-demographic factors. *Cancer Epidemiology, 54*, 31–37. https://doi. org/10. 1016/J. CANEP. 2018. 03. 005
26. Bobdey, S. , Sathwara, J. , Jain, A. , & Balasubramaniam, G. (2016). Burden of cervical cancer and role of screening in India. *Indian Journal of Medical and Paediatric Oncology, 37*(4), 278–285. https://doi. org/10. 4103/0971-5851. 195751
27. Bontkes, H. J. , de Gruijl, T. D. , van den Muysenberg, A. J. C. , Verheijen, R. H. M. , Stukart, M. J. , Meijer, C. J. L. M. , Scheper, R. J. , Stacey, S. N. , Duggan-Keen, M. F. , Stern, P. L. , Man, S. , Borysiewicz, L. K. , & Walboomers, J. M. M. (2000). Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes in women with cervical neoplasia. *International Journal of Cancer, 88*(1), 92–98. https://doi. org/10. 1002/1097-0215(20001001)88:1
28. Borruto, F. , & De Ridder, M. (2011). HPV and cervical cancer: Achievements in prevention and future prospects. *Springer*. https://doi. org/10. 1007/978-1-4614-1988-4
29. Bosch, F. X. , Lorincz, A. , Muñoz, N. , Meijer, C. J. L. M. , & Shah, K. V. (2002). The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology*, *55*(4), 244–265. https://doi. org/10. 1136/jcp. 55. 4. 244
30. Braaten, K. P. , & Laufer, M. R. (2008). Human papillomavirus (HPV), HPV-related disease, and the HPV vaccine. *Reviews in Obstetrics & Gynecology, 1*(1), 2–10.
31. Bray, F. , Ferlay, J. , Soerjomataram, I. , Siegel, R. L. , Torre, L. A. , & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394–424. https://doi. org/10. 3322/caac. 21492
32. Brisson, M. , Kim, J. J. , Canfell, K. , Drolet, M. , Gingras, G. , Burger, E. A. , Martin, D. , Simms, K. T. , Bénard, É. , Boily, M. C. , Sy, S. , Regan, C. , Keane, A. , Caruana, M. , Nguyen, D. T. N. , Smith, M. A. , Laprise, J. F. , Jit, M. , Alary, M. , … Hutubessy, R. (2020). Impact of HPV vaccination and cervical screening on cervical cancer elimination:

A comparative modelling analysis in 78 low-income and lower-middle-income countries. *The Lancet, 395*(10224), 575–590. https://doi. org/10. 1016/S0140-6736(20)30068-4

33. Broberg, G. , Wang, J. , Östberg, A. L. , Adolfsson, A. , Nemes, S. , Sparén, P. , & Strander, B. (2018). Socio-economic and demographic determinants affecting participation in the Swedish cervical screening program: A population-based case-control study. *PLoS ONE, 13*(1), 1-14. https://doi. org/10. 1371/journal. pone. 0190171
34. Burd, E. (2003). Human papillomavirus and cervical cancer. *Clinical Microbiology Reviews, 16*(1), 1–17. https://doi. org/10. 1128/CMR. 16. 1. 1
35. Cameron, R. L. , Kavanagh, K. , Watt, D. C. , Robertson, C. , Cuschieri, K. , Ahmed, S. , & Pollock, K. G. (2017). The impact of bivalent HPV vaccine on cervical intraepithelial neoplasia by deprivation in Scotland: Reducing the gap. *Journal of Epidemiology and Community Health, 71*(10), 954–960. https://doi. org/10. 1136/jech-2017-209113
36. Capalash, N. , & Sobti, R. C. (1999). Epidemiology of cervical cancer: A case-control study on north Indian population. *Indian Journal of Cancer, 36*(2–4), 179–185. http://europepmc. org/abstract/MED/10921222
37. Carson, H. J. , & DeMay, R. M. (1993). The mode ages of women with cervical dysplasia. *Obstetrics and Gynecology, 82*(3), 430–434.
38. Castle, P. E. , Pierz, A. J. , Adcock, R. , Aslam, S. , Basu, P. S. , Belinson, J. L. , Cuzick, J. , El-Zein, M. , Ferreccio, C. , Firnhaber, C. , Franco, E. L. , Gravitt, P. E. , Isidean, S. D. , Lin, J. , Mahmud, S. M. , Monsonego, J. , Muwonge, R. , Ratnam, S. , Safaeian, M. , … Xi, L. F. (2020). A pooled analysis to compare the clinical characteristics of human papillomavirus–positive and -negative cervical precancers. *Cancer Prevention Research, 13*(10), 829–840. https://doi. org/10. 1158/1940-6207. CAPR-20-0182
39. Chakravarthy, A. , Reddin, I. , Henderson, S. , Dong, C. , Kirkwood, N. , Jeyakumar, M. , Rodriguez, D. R. , Martinez, N. G. , McDermott, J. , Su, X. , Egawa, N. , Fjeldbo, C. S. , Skingen, V. E. , Lyng, H. , Halle, M. K. , Krakstad, C. , Soleiman, A. , Sprung, S. , Lechner, M. , … Fenton, T. R. (2022). Integrated analysis of cervical squamous cell carcinoma cohorts from three continents reveals conserved subtypes of prognostic significance. *Nature Communications, 13*(1), 1–17. https://doi. org/10. 1038/s41467-022-33544-x

40. Chan, C. K. , Aimagambetova, G. , Ukybassova, T. , Kongrtay, K. , & Azizan, A. (2019). Human papillomavirus infection and cervical cancer: Epidemiology, screening, and vaccination - Review of current perspectives. *Journal of Oncology, 2019*,. https://doi. org/10. 1155/2019/3257939
41. Chatzistamatiou, K. (2013). Smoking and genital human papilloma virus infection in women attending cervical cancer screening in Greece. *World Journal of Obstetrics and Gynecology*, *2*(3), 53. https://doi. org/10. 5317/wjog. v2. i3. 53
42. Choi, Y. J. , Hur, S. Y. , Kim, T. J. , Hong, S. R. , Lee, J. K. , Cho, C. H. , Park, K. S. , Woo, J. W. , Sung, Y. C. , Suh, Y. S. , & Park, J. S. (2020). A phase II, prospective, randomized, multicenter, open-label study of GX-188E, an HPV DNA vaccine, in patients with cervical intraepithelial neoplasia 3. *Clinical Cancer Research*, *26*(7), 1616–1623. https://doi. org/10. 1158/1078-0432. CCR-19-1513
43. Clifford, G. M. , Smith, J. S. , Plummer, M. , Muñoz, N. , & Franceschi, S. (2003). Human papillomavirus types in invasive cervical cancer worldwide: A meta-analysis. *British Journal of Cancer*, *88*(1), 63–69. https://doi. org/10. 1038/sj. bjc. 6600688
44. Cohen, P. A. , Jhingran, A. , Oaknin, A. , & Denny, L. (2019). Cervical cancer. *The Lancet*, *393*(10167), 169–182. https://doi. org/10. 1016/S0140-6736(18)32470-X
45. Collins, S. , Rollason, T. P. , Young, L. S. , & Woodman, C. B. J. (2010). Cigarette smoking is an independent risk factor for cervical intraepithelial neoplasia in young women: A longitudinal study. *European Journal of Cancer*, *46*(2), 405–411. https://doi. org/10. 1016/j. ejca. 2009. 09. 015
46. Coomarasamy, A. , Williams, H. , Truchanowicz, E. , Seed, P. T. , Small, R. , Quenby, S. , Gupta, P. , Dawood, F. , Koot, Y. E. M. , Bender Atik, R. , Bloemenkamp, K. W. M. , Brady, R. , Briley, A. L. , Cavallaro, R. , Cheong, Y. C. , Chu, J. J. , Eapen, A. , Ewies, A. , Hoek, A. , … Rai, R. (2015). A Randomized Trial of Progesterone in Women with Recurrent Miscarriages. *New England Journal of Medicine*, *373*(22), 2141–2148. https://doi. org/10. 1056/nejmoa1504927
47. Cunha, A. P. A. , Belfort, I. K. P. , Mendes, F. P. B. , dos Santos, G. R. B. , de Lima Costa, L. H. , de Matos Monteiro, P. , Gaspar, R. L. , Ferreira, M. B. , de Sá Ferreira, A. , Monteiro, S. C. M. , & Vidal, F. C. B. (2020). Human papillomavirus and Its Association with Other Sexually Transmitted Coinfection among Sexually Active Women from the

Northeast of Brazil. *Interdisciplinary Perspectives on Infectious Diseases*, *2020*. https://doi. org/10. 1155/2020/8838317

48. Cutts, F. T. , Franceschi, S. , Goldie, S. J. , Castellsagué, X. , De Sanjosé, S. , Garnett, G. P. , Edmunds, W. J. , Claeys, P. , Goldenthal, K. L. , Harper, D. M. , & Markowitz, L. E. (2007). Human papillomavirus and HPV vaccines: A review. *Bulletin of the World Health Organization*, *85*(9), 719–726. https://doi. org/10. 2471/BLT. 06. 038414
49. Cuzick, J. , Sasieni, P. , Davies, P. , Adams, J. , Normand, C. , Frater, A. , van Ballegooijen, M. , & van den Akker-van Marle, E. (2000). A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening programme: summary and conclusions. *British journal of cancer*, *83*(5), 561–565. https://doi. org/10. 1054/bjoc. 2000. 1375
50. Das, B. C. , Gopalkrishna, V. , Das, D. K. , Sharma, J. K. , Singh, V. , & Luthra, U. K. (1993). Human papillomavirus DNA sequences in adenocarcinoma of the uterine cervix in Indian women. *Cancer*, *72*(1), 147–153. https://doi. org/10. 1002/1097-0142(19930701)72:1<147::aid-cncr2820720128>3. 0. co;2-7
51. Das, B. C. , Gopalkrishna, V. , Hedau, S. , & Katiyar, S. (2000). Cancer of the uterine cervix and human papillomavirus infection. *Journal of Biosciences*, *25*(1), 1–10.
52. Datta, P. , Bhatla, N. , Pandey, R. M. , Dar, L. , Patro, A. R. , Vasisht, S. , Kriplani, A. , & Singh, N. (2012). Type-specific incidence and persistence of HPV infection among young women: A prospective study in North India. *Asian Pacific Journal of Cancer Prevention*, *13*, 1019–1024.
53. De Villiers, E. M. (1989). Heterogeneity of the human papillomavirus group. *Journal of Virology*, *63*(11), 4898–4903. https://doi. org/10. 1128/jvi. 63. 11. 4898-4903. 1989
54. Del Río-Ospina, L. , Soto-De León, S. C. , Camargo, M. , Moreno-Pérez, D. A. , Sánchez, R. , Pérez-Prados, A. , Patarroyo, M. E. , & Patarroyo, M. A. (2015). The DNA load of six high-risk human papillomavirus types and its association with cervical lesions. *BMC Cancer*, *15*(1), 1–11. https://doi. org/10. 1186/s12885-015-1126-z
55. Djurisic, S. , Jakobsen, J. C. , Petersen, S. B. , Kenfelt, M. , & Gluud, C. (2017). Aluminium adjuvants used in vaccines versus placebo or no intervention. *Cochrane Database of Systematic Reviews*, *2017*(9). https://doi. org/10. 1002/14651858. CD012805

56. Doorbar, J. (2013). The E4 protein; structure, function and patterns of expression. *Virology*, *445*(1–2), 80–98. https://doi. org/10. 1016/j. virol. 2013. 07. 008

57. Ehehalt, D. , Lener, B. , Pircher, H. , Dreier, K. , Pfister, H. , Kaufmann, A. M. , Frangini, S. , Ressler, S. , Müller-Holzner, E. , Schmitt, M. , Höfler, D. , Rostek, U. , Kaiser, A. , Widschwendter, A. , Zwerschke, W. , & Jansen-Dürra, P. (2012). Detection of human papillomavirus type 18 E7 oncoprotein in cervical smears: A feasibility study. *Journal of Clinical Microbiology*, *50*(2), 246–257. https://doi. org/10. 1128/JCM. 01108-11

58. Elsheikh, T. M. , Kirkpatrick, J. L. , & Wu, H. H. (2006). The significance of "low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion" as a distinct squamous abnormality category in Papanicolaou tests. *Cancer*, *108*(5), 277–281. https://doi. org/10. 1002/cncr. 22169

59. Fang, J. H. , Yu, X. M. , Zhang, S. H. , & Yang, Y. (2018). Effect of smoking on high-grade cervical cancer in women on the basis of human papillomavirus infection studies. *Journal of Cancer Research and Therapeutics, 14*(8), S184–S189. https://doi. org/10. 4103/0973-1482. 179190

60. Feldstein, O. , Gali-Zamir, H. , Schejter, E. , Feinberg, T. , Yehuda-Shnaidman, E. , Bornstein, J. , & Levy, T. (2023). High-risk HPV testing vs liquid-based cytology for cervical cancer screening among 25- to 30-year-old women: A historical cohort study. *Acta Obstetricia et Gynecologica Scandinavica, 102*(2), 226–233. https://doi. org/10. 1111/aogs. 14482

61. Felix, J. C. , Lacey, M. J. , Miller, J. D. , Lenhart, G. M. , Spitzer, M. , & Kulkarni, R. (2016). The clinical and economic benefits of co-testing versus primary HPV testing for cervical cancer screening: A modeling analysis. *Journal of Women's Health, 25*(6), 606–616. https://doi. org/10. 1089/jwh. 2015. 5708

62. Fotra, R. , Gupta, S. , & Gupta, S. (2014). Sociodemographic risk factors for cervical cancer in Jammu region of Jand K state of India: First ever report from Jammu. *Indian Journal of Scientific Research, 9*(1), 105–110. https://doi. org/10. 5958/2250-0138. 2014. 00018. 2

63. Franceschi, S. , Rajkumar, T. , Vaccarella, S. , Gajalakshmi, V. , Sharmila, A. , Snuders, P. J. F. , Muñoz, N. , Meijer, C. J. L. M. , & Herrero, R. (2003). Human papillomavirus

and risk factors for cervical cancer in Chennai, India: A case-control study. *International Journal of Cancer, 107*(1), 127–133. https://doi. org/10. 1002/ijc. 11350

64. Franco, E. L. (2003). Primary screening of cervical cancer with human papillomavirus tests. *Journal of the National Cancer Institute Monographs, 6*(31), 89–96. https://doi. org/10. 1093/oxfordjournals. jncimonographs. a003488
65. Garbuglia, A. R. , Lapa, D. , Sias, C. , Capobianchi, M. R. , & Del Porto, P. (2020). The use of both therapeutic and prophylactic vaccines in the therapy of papillomavirus disease. *Frontiers in Immunology, 11*, 1–14. https://doi. org/10. 3389/fimmu. 2020. 00188
66. Garg, A. , Suri, V. , Nijhawan, R. , Aggarwal, N. , Aggarwal, R. , Guleria, C. , & Thakur, M. (2016). Prevalence of human papillomavirus infection in young primiparous women during postpartum period: Study from a tertiary care center in northern India. *Journal of Clinical and Diagnostic Research, 10*(10), QC06–QC09. https://doi. org/10. 7860/JCDR/2016/20235. 8607
67. Garnock-Jones, K. P. , & Giuliano, A. R. (2011). Quadrivalent human papillomavirus (HPV) types 6, 11, 16, 18 vaccine. *Drugs, 71*(5), 591–602. https://doi. org/10. 2165/11205980-000000000-00000
68. Garon, N. , Morel, S. , Didierlaurent, A. , Descamps, D. , Wettendorff, M. , & Van Mechelen, M. (2011). Development of an AS04-adjuvanted HPV vaccine with the adjuvant system approach. *BioDrugs, 25*(4), 217–226. https://doi. org/10. 2165/11591760-000000000-00000
69. Geetha, B. , & Santhy, K. S. (2013). Sexual risk factors for cervical carcinogenesis: A case-control study. *International Journal of Current Microbiology and Applied Sciences, 2*(8), 394–399.
70. Gheit, T. , Vaccarella, S. , Schmitt, M. , Pawlita, M. , Franceschi, S. , Sankaranarayanan, R. , Sylla, B. S. , Tommasino, M. , & Gangane, N. (2009). Prevalence of human papillomavirus types in cervical and oral cancers in central India. *Vaccine, 27*(5), 636–639. https://doi. org/10. 1016/j. vaccine. 2008. 11. 041
71. Ghittoni, R. , Accardi, R. , Hasan, U. , Gheit, T. , Sylla, B. , & Tommasino, M. (2010). The biological properties of E6 and E7 oncoproteins from human papillomaviruses. *Virus Genes, 40*(1), 1–13. https://doi. org/10. 1007/s11262-009-0412-8

72. Gierisch, J. M. , Coeytaux, R. R. , Urrutia, R. P. , Havrilesky, L. J. , Moorman, P. G. , Lowery, W. J. , Dinan, M. , McBroom, A. J. , Hasselblad, V. , Sanders, G. D. , & Myers, E. R. (2013). Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: A systematic review. *Cancer Epidemiology, Biomarkers & Prevention, 22*(11), 1931–1943. https://doi. org/10. 1158/1055-9965. EPI-13-0298

73. Ginsburg, O. , Bray, F. , Coleman, M. P. , Vanderpuye, V. , Eniu, A. , Kotha, S. R. , Sarker, M. , Huong, T. T. , Allemani, C. , Dvaladze, A. , Gralow, J. , Yeates, K. , Taylor, C. , Oomman, N. , Krishnan, S. , Sullivan, R. , Kombe, D. , Blas, M. M. , Parham, G. , … Conteh, L. (2017). The global burden of women's cancers: A grand challenge in global health. *The Lancet, 389*(10071), 847–860. https://doi. org/10. 1016/S0140-6736(16)31392-7

74. Gnanamony, M. , & Peedicayil, A. (2007). An overview of human papillomaviruses and current vaccine strategies. *Indian Journal of Medical Microbiology, 25*(1). http://www. ijmm. org

75. Goodman, A. (2015). HPV testing as a screen for cervical cancer. *BMJ, 350*, h2372. https://doi. org/10. 1136/bmj. h2372

76. Goodman, M. T. , Shvetsov, Y. B. , McDuffie, K. , Wilkens, L. R. , Zhu, X. , Thompson, P. J. , Ning, L. , Killeen, J. , Kamemoto, L. , & Hernandez, B. Y. (2009). Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii human papillomavirus cohort study. *Cancer Research, 69*(1), 381. https://doi. org/10. 1158/0008-5472. CAN-69-1-COR1

77. Griffiths M. (1991). 'Nuns, virgins, and spinsters'. Rigoni-Stern and cervical cancer revisited. *British journal of obstetrics and gynaecology*, *98*(8), 797–802. https://doi. org/10. 1111/j. 1471-0528. 1991. tb13485. x

78. Gupta, A. , Bhatt, M. L. B. , & Misra, M. K. (2009). Lipid peroxidation and antioxidant status in head and neck squamous cell carcinoma patients. *Oxidative Medicine and Cellular Longevity, 2*(2), 68–72. https://doi. org/10. 4161/oxim. 2. 2. 8160

79. Gutiérrez-Xicoténcatl, L. , Plett-Torres, T. , Madrid-González, C. L. , & Madrid-Marina, V. (2009). Molecular diagnosis of human papillomavirus in the development of cervical cancer. *Salud Pública de México, 51*(SUPPL. 3), 479–488. https://doi. org/10. 1590/S0036-36342009000900014

80. Hariprasad, R. , Tulsyan, S. , Babu, R. , Dhanasekaran, K. , Thakur, N. , Hussain, S. , Tripathi, R. , Sreenivas, V. , Sharma, S. , Sriram, L. , Singh, S. , & Mehrotra, R. (2020). Evaluation of a chip-based, point-of-care, portable, real-time micro PCR analyzer for the detection of high-risk human papillomavirus in uterine cervix in India. *JCO Global Oncology, 6*, 1147–1154. https://doi. org/10. 1200/go. 20. 00024
81. Harper, D. M. , & Demars, L. R. (2017). HPV vaccines: A review of the first decade. *Gynecologic Oncology, 146*(1), 196-204. https://doi. org/10. 1016/j. ygyno. 2017. 04. 004
82. Heideman, D. A. , Hesselink, A. T. , van Kemenade, F. J. , Iftner, T. , Berkhof, J. , Topal, F. , Agard, D. , Meijer, C. J. , & Snijders, P. J. (2013). The Aptima HPV assay fulfills the cross-sectional clinical and reproducibility criteria of international guidelines for human papillomavirus test requirements for cervical screening. *Journal of Clinical Microbiology, 51*(11), 3653–3657. https://doi. org/10. 1128/JCM. 01517-13
83. Herrero, R. , González, P. , Markowitz, L. E. , & Rica, C. (2014). Present status of human papillomavirus vaccine development and implementation. *The Lancet Oncology, 15*(3), 206–216. https://doi. org/10. 1016/S1470-2045(14)70481-4
84. Hn, H. K. , & Tanya, S. (2014). A study on knowledge and screening for cervical cancer among women in Mangalore city. *Annals of Medical and Health Sciences Research, 4*(5), 751–756. https://doi. org/10. 4103/2141-9248. 141547
85. Hoffman, M. S. , Cardosi, R. J. , Roberts, W. S. , Fiorica, J. V. , Grendys, E. C. , Griffin, D. , & Underwood, P. (2004). Accuracy of pelvic examination in the assessment of patients with operable cervical cancer. *American Journal of Obstetrics and Gynecology, 190*(4), 986–992. https://doi. org/10. 1016/j. ajog. 2004. 01. 019
86. Hull, R. , Mbele, M. , Makhafola, T. , Hicks, C. , Wang, S. M. , Reis, R. M. , Mehrotra, R. , Mkhize-Kwitshana, Z. , Kibiki, G. , Bates, D. O. , & Dlamini, Z. (2020). Cervical cancer in low and middle-income countries: A review. *Oncology Letters, 20*(3), 2058–2074. https://doi. org/10. 3892/ol. 2020. 11754
87. International Collaboration of Epidemiological Studies of Cervical Cancer (2006). Cervical carcinoma and reproductive factors: collaborative reanalysis of individual data on 16,563 women with cervical carcinoma and 33,542 women without cervical carcinoma from 25 epidemiological studies. *International journal of cancer, 119*(5), 1108–1124. https://doi. org/10. 1002/ijc. 21953

88. Jantunen, I. T. , Flander, M. K. , Heikkinen, M. I. , Kuoppala, T. A. , Teerenhovi, L. , & Kataja, V. V. (1993). Comparison of ondansetron with customary treatment in the prophylaxis of nausea and emesis induced by non-cisplatin containing chemotherapy. *Acta Oncologica*, *32*(4), 413–415. https://doi. org/10. 3109/02841869309093619
89. Jefferies, S. , & Foulkes, W. D. (2001). Genetic mechanisms in squamous cell carcinoma of the head and neck. *Oral Oncology*, *37*(2), 115–126. https://doi. org/10. 1016/s1368-8375(00)00065-8
90. Jensen, E. (2014). Technical review: In situ hybridization. *The Anatomical Record*, *297*(8), 1349–1353. https://doi. org/10. 1002/ar. 22944
91. Kaarthigeyan, K. (2012). Cervical cancer in India and HPV vaccination. *Indian Journal of Medical and Paediatric Oncology*, *33*(1), 7–12. https://doi. org/10. 4103/0971-5851. 96961
92. Kahn, J. A. (2007). High-risk human papillomavirus infection. *Pediatrics*, *110*(1), 87–95. https://doi. org/10. 1542/peds. 110. 1. 87
93. Kaku, M. , Mathew, A. , & Rajan, B. (2008). Prevalence of high-risk HPV among women with cervical cancer in South India. *Asian Pacific Journal of Cancer Prevention*, *9*(4), 589–594.
94. Kamani, M. , Akgor, U. , & Gültekin, M. (2022). Review of the literature on combined oral contraceptives and cancer. *Ecancermedicalscience*, *16*, 1416. https://doi. org/10. 3332/ecancer. 2022. 1416
95. Kantathavorn, N. , Mahidol, C. , Sritana, N. , Sricharunrat, T. , Phoolcharoen, N. , Auewarakul, C. , Teerayathanakul, N. , Taepisitpong, C. , Saeloo, S. , Sornsamdang, G. , Udomchaiprasertkul, W. , & Krongthong, W. (2015). Genotypic distribution of human papillomavirus (HPV) and cervical cytology findings in 5906 Thai women undergoing cervical cancer screening programs. *BMC Cancer*, *15*, 1–9. https://doi. org/10. 1186/s12885-015-1505-2
96. Kapoor, C. S. , & Sharma, M. (2020). Prevalence of HPV infection in reproductive aged females in Delhi NCR region. *Clinical Epidemiology and Global Health*, *8*(2), 612–615. https://doi. org/10. 1016/j. cegh. 2019. 12. 012

97. Kashyap, N. , Krishnan, N. , Kaur, S. , & Ghai, S. (2019). Risk factors of cervical cancer: A case-control study. *Asia-Pacific Journal of Oncology Nursing, 6*(3), 308–314. https://doi. org/10. 4103/apjon. apjon
98. Kaur, P. , Aggarwal, A. , Nagpal, M. , Oberoi, L. , & Sharma, S. (2014). Prevalence and clinical utility of human papillomavirus genotyping in patients with cervical lesions. *Journal of Obstetrics and Gynecology of India, 64*(4), 279–283. https://doi. org/10. 1007/s13224-014-0508-5
99. Kawana, K. , Adachi, K. , Kojima, S. , Kozuma, S. , & Fujii, T. (2012). Therapeutic human papillomavirus (HPV) vaccines: A novel approach. *The Open Virology Journal, 6*, 264–269. https://doi. org/10. 2174/1874357901206010264
100. Khajuria, A. , Dogra, S. , & Mahajan, B. (2021). Knowledge and awareness of cervical cancer risk factors among educated youth: A cross-sectional, e-questionnaire based survey among the college students of Jammu City. *Cross Current International Journal of Medical and Biosciences, 3*(3), 36–41. https://doi. org/10. 36344/ccijmb. 2021. v03i03. 003
101. Khalaf, M. K. , Rasheed, F. A. , & Hussain, S. A. (2015). Association between early marriage and other socio-medical characteristics with the cervical pap smear results in Iraqi women. *Advances in Sexual Medicine, 5*(4), 73–82. https://doi. org/10. 4236/asm. 2015. 54009
102. Khan, M. J. , & Smith-McCune, K. K. (2014). Treatment of cervical precancers: Back to basics. *Obstetrics and Gynecology, 123*(6), 1339–1343. https://doi. org/10. 1097/AOG. 0000000000000287
103. Kops, N. L. , Caierão, J. , Bessel, M. , Horvath, J. D. C. , Domingues, C. M. , Benzaken, A. S. , Villa, L. L. , de Souza, F. M. A. , Pereira, G. F. M. , & Wendland, E. M. (2021). Behavioral factors associated with multiple-type HPV genital infections: Data from a cross-sectional study in young women in Brazil. *Reproductive Health, 18*(1), 1–9. https://doi. org/10. 1186/s12978-021-01244-2
104. Kour, P. , Lal, M. , Panjaliya, R. , Dogra, V. , & Gupta, S. (2010). Study of the risk factors associated with cervical cancer. *Biomedical and Pharmacology Journal, 3*(1), 179–182.

105. Krawczyk, E. , Suprynowicz, F. A. , Liu, X. , Dai, Y. , Hartmann, D. P. , Hanover, J. , & Schlegel, R. (2008). A cooperative interaction between the human papillomavirus E5 and E6 oncoproteins. *American Journal of Pathology, 173*(3), 682–688. https://doi. org/10. 2353/ajpath. 2008. 080280
106. Krings, A. , Dückelmann, A. M. , Moser, L. , Gollrad, J. , Wiegerinck, M. , Schweizer, J. , & Kaufmann, A. M. (2018). Performance of OncoE6 cervical test with collection methods enabling self-sampling. *BMC Women's Health, 18*(1), 1–7. https://doi. org/10. 1186/s12905-018-0559-3
107. Kroger, A. T. , & Orenstein, W. A. (2015). Capsid protein L1 immunization papillomaviruses. *Clinical Infectious Diseases, 61*(3), 350–355.
108. Latsuzbaia, A. , Arbyn, M. , Tapp, J. , Fischer, M. , Weyers, S. , Pesch, P. , & Mossong, J. (2019). Effectiveness of bivalent and quadrivalent human papillomavirus vaccination in Luxembourg. *Cancer Epidemiology, 63,* 101593. https://doi. org/10. 1016/j. canep. 2019. 101593
109. Laudadio, J. (2013). Human papillomavirus detection: Testing methodologies and their clinical utility in cervical cancer screening. *Advances in Anatomic Pathology*, *20*(3), 155–164. https://doi. org/10. 1097/PAP. 0b013e31828c
110. LaVigne, A. W. , Triedman, S. A. , Randall, T. C. , Trimble, E. L. , & Viswanathan, A. N. (2017). Cervical cancer in low- and middle-income countries: Addressing barriers to radiotherapy delivery. *Gynecologic Oncology Reports, 22,* 16–20. https://doi. org/10. 1016/j. gore. 2017. 08. 004
111. Lee, S. , Yang, A. , Wu, T. , Hung, C. , & Yang, A. (2016). Immunotherapy for human papillomavirus-associated disease and cervical cancer: Review of clinical and translational research. *Journal of Cancer*, *27*(5), 1–17.
112. Lewis, J. S. , Beadle, B. , Bishop, J. A. , Chernock, R. D. , Colasacco, C. , Lacchetti, C. , Moncur, J. T. , Rocco, J. W. , Schwartz, M. R. , Seethala, R. R. , Thomas, N. E. , Westra, W. H. , & Faquin, W. C. (2018). Human papillomavirus testing in head and neck carcinomas: Guideline from the College of American Pathologists. *Archives of Pathology and Laboratory Medicine, 142*(5), 559–597. https://doi. org/10. 5858/arpa. 2017-0286-CP
113. Ley, C. , Bauer, H. M. , Reingold, A. , Schiffman, M. H. , Chambers, J. C. , Tashiro, C. J. , & Manos, M. M. (1991). Determinants of genital human papillomavirus infection in

young women. *JNCI: Journal of the National Cancer Institute, 83*(14), 997–1003. https://doi. org/10. 1093/jnci/83. 14. 997

114. Lim, A. W. W. , Landy, R. , Castanon, A. , Hollingworth, A. , Hamilton, W. , Dudding, N. , & Sasieni, P. (2016). Cytology in the diagnosis of cervical cancer in symptomatic young women: A retrospective review. *British Journal of General Practice, 66*(653), e871–e879. https://doi. org/10. 3399/bjgp16X687937
115. Lim, T. Y. K. , Lin, K. K. M. , Wong, W. L. , Aggarwal, I. M. , & Yam, P. K. L. (2019). Surgical and oncological outcome of total laparoscopic radical hysterectomy versus radical abdominal hysterectomy in early cervical cancer in Singapore. *Gynecology and Minimally Invasive Therapy, 8*(2), 53–58. https://doi. org/10. 4103/GMIT. GMIT_43_18
116. Liu, Y. , Xu, C. , Pan, J. , Sun, C. , Zhou, H. , & Meng, Y. (2021). Significance of the viral load of high-risk HPV in the diagnosis and prediction of cervical lesions: A retrospective study. *BMC Women's Health, 21*(1), 4–9. https://doi. org/10. 1186/s12905-021-01493-0
117. Long, W. , Yang, Z. , Li, X. , Chen, M. , Liu, J. , Zhang, Y. , & Sun, X. (2018). HPV-16, HPV-58, and HPV-33 are the most carcinogenic HPV genotypes in Southwestern China and their viral loads are associated with severity of premalignant lesions in the cervix. *Virology Journal, 15*(1), 1–8. https://doi. org/10. 1186/s12985-018-1003-x
118. Lopes, A. , Vandermeulen, G. , & Préat, V. (2019). Cancer DNA vaccines: Current preclinical and clinical developments and future perspectives. *Journal of Experimental and Clinical Cancer Research, 38*(1), 1–24. https://doi. org/10. 1186/s13046-019-1154-7
119. Louie, K. S. , De Sanjose, S. , Diaz, M. , Castellsagué, X. , Herrero, R. , Meijer, C. J. , Shah, K. , Franceschi, S. , Mũoz, N. , & Bosch, F. X. (2009). Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries. *British Journal of Cancer, 100*(7), 1191–1197. https://doi. org/10. 1038/sj. bjc. 6604974
120. Ma, G. X. , Fang, C. Y. , Feng, Z. , Tan, Y. , Gao, W. , Ge, S. , & Nguyen, C. (2012). Correlates of cervical cancer screening among Vietnamese American women. *Infectious Diseases in Obstetrics and Gynecology, 2012*. https://doi. org/10. 1155/2012/617234
121. Mackay, I. M. , Arden, K. E. , & Nitsche, A. (2002). Real-time PCR in virology. *Nucleic Acids Research, 30*(6), 1292–1305. https://doi. org/10. 1093/nar/30. 6. 1292

122. Malagón, T. , Louvanto, K. , Ramanakumar, A. V. , Koushik, A. , Coutlée, F. , & Franco, E. L. (2019). Viral load of human papillomavirus types 16/18/31/33/45 as a predictor of cervical intraepithelial neoplasia and cancer by age. *Gynecologic Oncology, 155*(2), 245–253. https://doi. org/10. 1016/j. ygyno. 2019. 09. 010
123. Markowitz, L. E. , Drolet, M. , Perez, N. , Jit, M. , & Brisson, M. (2018). Human papillomavirus vaccine effectiveness by number of doses: Systematic review of data from national immunization programs. *Vaccine*. https://doi. org/10. 1016/j. vaccine. 2018. 01. 057
124. Martín-Vallejo, J. , Laforga, J. B. , Molina-Bellido, P. , & Clemente-Pérez, P. A. (2022). Superficial spreading cervical squamous cell carcinoma in situ involving the endometrium: A case report and review of the literature. *Journal of Medical Case Reports, 16*(1), 1–7. https://doi. org/10. 1186/s13256-022-03433-4
125. Massarelli, E. , William, W. , Johnson, F. , Kies, M. , Ferrarotto, R. , Guo, M. , Feng, L. , Lee, J. J. , Tran, H. , Kim, Y. U. , Haymaker, C. , Bernatchez, C. , Curran, M. , Zecchini Barrese, T. , Rodriguez Canales, J. , Wistuba, I. , Li, L. , Wang, J. , Van Der Burg, S. H. , & Glisson, B. (2019). Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16-related cancer: A phase 2 clinical trial. *JAMA Oncology, 5*(1), 67–73. https://doi. org/10. 1001/jamaoncol. 2018. 4051
126. Mathur, P. , Sathishkumar, K. , Chaturvedi, M. , Das, P. , Sudarshan, K. L. , Santhappan, S. , Nallasamy, V. , John, A. , Narasimhan, S. , & Roselind, F. S. (2020). Cancer statistics, 2020: Report from the National Cancer Registry Programme, India. *JCO Global Oncology, 6*, 1063–1075. https://doi. org/10. 1200/go. 20. 00122
127. Maver, P. J. , & Poljak, M. (2020). Primary HPV-based cervical cancer screening in Europe: Implementation status, challenges, and future plans. *Clinical Microbiology and Infection, 26*(5), 579–583. https://doi. org/10. 1016/j. cmi. 2019. 09. 006
128. McLaughlin, C. (2008). Emotional well-being and its relationship to schools and classrooms: A critical reflection. *British Journal of Guidance & Counselling, 36*(4), 353–366. https://doi. org/10. 1080/03069880802364486
129. Mhaske, M. , & Jawadekar, S. J. (2011). Study of association of some risk factors & cervical dysplasia/cancer among rural women. *National Journal of Community Medicine,*

2(2), 209–212. http://eds. b. ebscohost. com. ezproxy. lib. usf. edu/eds/pdfviewer/pdfviewer?vid=15&sid=aadcfa9d-7aeb-4547-9b5e-79812332c280@sessionmgr198&hid=112

130. Mo, Y. , Ma, J. , Zhang, H. , Shen, J. , Chen, J. , Hong, J. , Xu, Y. , Qian, C. , Smalley Rumfield, C. , Pellom, S. T. , Morillon, Y. M. , Schlom, J. , Jochems, C. , Strauss, J. , Gatti-Mays, M. E. , Cho, B. C. , Hill, A. , Salas, S. , McClay, E. , & Jochems, C. (2020). Prophylactic and therapeutic HPV vaccines: Current scenario and perspectives. *Journal for ImmunoTherapy of Cancer, 8*(2), 1–19. https://doi. org/10. 3389/fcimb. 2022. 909223
131. Monica, & Mishra, R. (2020). An epidemiological study of cervical and breast screening in India: District-level analysis. *BMC Women's Health, 20*(1), 1–15. https://doi. org/10. 1186/s12905-020-01083-6
132. Monie, A. , Hung, C. F. , Roden, R. , & Wu, T. C. (2008). Cervarix™: A vaccine for the prevention of HPV 16, 18-associated cervical cancer. *Biologics: Targets and Therapy, 2*(1), 107–113. https://doi. org/10. 2147/BTT. S1877
133. Morshed, K. , Polz-Gruszka, D. , Szymañski, M. , & Polz-Dacewicz, M. (2014). Human papillomavirus (HPV) - Structure, epidemiology and pathogenesis. *Otolaryngologia Polska, 68*(5), 213–219. https://doi. org/10. 1016/j. otpol. 2014. 06. 001
134. Muñoz, N. , Castellsagué, X. , de González, A. B. , & Gissmann, L. (2006). Chapter 1: HPV in the etiology of human cancer. *Vaccine, 24*(3), S1–S10. https://doi. org/10. 1016/J. VACCINE. 2006. 05. 115
135. Muwonge, R. , Basu, P. , Gheit, T. , Anantharaman, D. , Verma, Y. , Bhatla, N. , Joshi, S. , Esmy, P. O. , Poli, U. R. R. , Shah, A. , Zomawia, E. , Shastri, S. S. , Pimple, S. , Prabhu, P. R. , Hingmire, S. , Chiwate, A. , Sauvaget, C. , Lucas, E. , Malvi, S. G. , & Sankaranarayanan, R. (2020). Acquisition, prevalence and clearance of type-specific human papillomavirus infections in young sexually active Indian women: A community-based multicentric cohort study. *PLoS ONE, 15*(12), 1–18. https://doi. org/10. 1371/journal. pone. 0244242
136. Mwaka, A. D. , Orach, C. G. , Were, E. M. , Lyratzopoulos, G. , Wabinga, H. , & Roland, M. (2016). Awareness of cervical cancer risk factors and symptoms: Cross-sectional community survey in post-conflict northern Uganda. *Health Expectations, 19*(4), 854–867. https://doi. org/10. 1111/hex. 12382

137. Nagarsheth, N. B. , Norberg, S. M. , Sinkoe, A. L. , Adhikary, S. , Meyer, T. J. , Lack, J. B. , Warner, A. C. , Schweitzer, C. , Doran, S. L. , Korrapati, S. , Stevanović, S. , Trimble, C. L. , Kanakry, J. A. , Bagheri, M. H. , Ferraro, E. , Astrow, S. H. , Bot, A. , Faquin, W. C. , Stroncek, D. , & Hinrichs, C. S. (2021). TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. *Nature Medicine, 27*(3), 419–425. https://doi. org/10. 1038/s41591-020-01225-1

138. Nanda, A. , Bansal, N. , Gupta, V. , Sharma, A. , Tandon, A. , & Singhal, M. (2014). Correlation between cytology, HPV-DNA test, and colposcopy in evaluation of cervical intraepithelial lesions. *Journal of SAFOMS, 2*(2), 71–74. https://doi. org/10. 5005/jp-journals-10032-1045

139. Natphopsuk, S. , Settheetham-Ishida, W. , Sinawat, S. , Pientong, C. , Yuenyao, P. , & Ishida, T. (2012). Risk factors for cervical cancer in Northeastern Thailand: Detailed analyses of sexual and smoking behavior. *Asian Pacific Journal of Cancer Prevention, 13*(11), 5489–5495. https://doi. org/10. 7314/APJCP. 2012. 13. 11. 5489

140. Nayar, R. , & Wilbur, D. C. (2017). The Bethesda system for reporting cervical cytology: A historical perspective. *Acta Cytologica, 61*(4–5), 359–372. https://doi. org/10. 1159/000477556

141. Nene, B. , Jayant, K. , Arrossi, S. , Shastri, S. , Budukh, A. , Hingmire, S. , Muwonge, R. , Malvi, S. , Dinshaw, K. , & Sankaranarayanan, R. (2007). Determinants of women's participation in cervical cancer screening trial, Maharashtra, India. *Bulletin of the World Health Organization, 85*(4), 264–272. https://doi. org/10. 2471/BLT. 06. 031195

142. Ngamkham, J. , Boonmark, K. , & Phansri, T. (2016). Detection and type-distribution of human papillomavirus in vulvar and vaginal abnormal cytology lesions and cancer tissues from Thai women. *Asian Pacific Journal of Cancer Prevention, 17*(3), 1129–1134. https://doi. org/10. 7314/APJCP. 2016. 17. 3. 1129

143. Nielson, C. M. , Flores, R. , Harris, R. B. , Abrahamsen, M. , Papenfuss, M. R. , Dunne, E. F. , Markowitz, L. E. , & Giuliano, A. R. (2007). Human papillomavirus prevalence and type distribution in male anogenital sites and semen. *Cancer Epidemiology Biomarkers and Prevention, 16*(6), 1107–1114. https://doi. org/10. 1158/1055-9965. EPI-06-0997

144. Obeid, D. A. , Almatrrouk, S. A. , Khayat, H. H. , Al-Muammer, T. A. , Tulbah, A. M. , Albadawi, I. A. , Al-Ahdal, M. N. , & Alhamlan, F. S. (2020). Human papillomavirus type 16 and 18 viral loads as predictors associated with abnormal cervical cytology among women in Saudi Arabia. *Heliyon*, *6*(2), e03473. https://doi. org/10. 1016/j. heliyon. 2020. e03473

145. Ogilvie, G. S. , Van Niekerk, D. , Krajden, M. , Smith, L. W. , Cook, D. , Gondara, L. , Ceballos, K. , Quinlan, D. , Lee, M. , Martin, R. E. , Gentile, L. , Peacock, S. , Stuart, G. C. E. , Franco, E. L. , & Coldman, A. J. (2018). Effect of screening with primary cervical HPV testing vs cytology testing on high-grade cervical intraepithelial neoplasia at 48 months: The HPV FOCAL randomized clinical trial. *JAMA - Journal of the American Medical Association*, *320*(1), 43–52. https://doi. org/10. 1001/jama. 2018. 7464

146. Origoni, M. , Cristoforoni, P. , Costa, S. , Mariani, L. , Scirpa, P. , Lorincz, A. , & Sideri, M. (2012). HPV-DNA testing for cervical cancer precursors: From evidence to clinical practice. *Ecancermedicalscience*, *6*(1), 1–15. https://doi. org/10. 3332/ecancer. 2012. 258

147. Ortiz, A. P. , Romaguera, J. , Pérez, C. M. , González, D. , Muñoz, C. , González, L. , Marrero, E. , Tortolero-Luna, G. , Suárez, E. , & Palefsky, J. (2016). Prevalence, genotyping, and correlates of anogenital HPV infection in a population-based sample of women in Puerto Rico. *Papillomavirus Research*, *2*, 89–96. https://doi. org/10. 1016/j. pvr. 2016. 04. 002

148. Overbergh, L. , Giulietti, A. P. , Valckx, D. , & Mathieu, C. (2009). Real-time polymerase chain reaction. In *Molecular Diagnostics: Second Edition* (87–105). https://doi. org/10. 1016/B978-0-12-374537-8. 00007-9

149. Paengchit, K. , Kietpeerakool, C. , & Lalitwongsa, S. (2014). Prevalence and genotype distribution of HPV among women attending a cervical cancer screening mobile unit in Lampang, Thailand. *Asian Pacific Journal of Cancer Prevention*, *15*(15), 6151–6154. https://doi. org/10. 7314/APJCP. 2014. 15. 15. 6151

150. Palmer, M. , Katanoda, K. , Saito, E. , Acuti Martellucci, C. , Tanaka, S. , Ikeda, S. , Sakamoto, H. , Machelek, D. , ML Brotherton, J. , & Hocking, J. S. (2022). Genotype prevalence and age distribution of human papillomavirus from infection to cervical cancer in Japanese women: A systematic review and meta-analysis. *Vaccine*, *40*(41), 5971–5996. https://doi. org/10. 1016/j. vaccine. 2022. 07. 052

151. Petca, A. , Borislavschi, A. , Zvanca, M. E. , Petca, R. -C. , Sandru, F. , & Dumitrascu, M. C. (2020). Non-sexual HPV transmission and role of vaccination for a better future (Review). *Experimental and Therapeutic Medicine*, *20*(6), 186. https://doi. org/10. 3892/etm. 2020. 9316
152. Pitts, M. , & Clarke, T. (2002). Human papillomavirus infections and risks of cervical cancer: What do women know? *Health Education Research*, *17*(6), 706–714. https://doi. org/10. 1093/her/17. 6. 706
153. Pizzichini, M. M. M. , Patino, C. M. , & Ferreira, J. C. (2020). Measures of frequency: Calculating prevalence and incidence in the era of COVID-19. *Jornal Brasileiro de Pneumologia*, *46*(3), 1. https://doi. org/10. 36416/1806-3756/e20200243
154. Prakash, P. , Patne, S. C. U. , Singh, A. K. , Kumar, M. , Mishra, M. N. , & Gulati, A. K. (2016). PCR and genotyping for HPV in cervical cancer patients. *Journal of Global Infectious Diseases*, *8*(3), 100–107. https://doi. org/10. 4103/0974-777X. 188591
155. Prigge, E. -S. , Arbyn, M. , von Knebel Doeberitz, M. , & Reuschenbach, M. (2017). Diagnostic accuracy of p16INK4a immunohistochemistry in oropharyngeal squamous cell carcinomas: A systematic review and meta-analysis. *International Journal of Cancer*, *140*(5), 1186–1198. https://doi. org/10. 1002/ijc. 30516
156. Qurieshi, M. A. , Khan, S. M. S. , Masoodi, M. A. , Qurieshi, U. , Ain, Q. , Jan, Y. , Haq, I. , & Ahmad, S. Z. (2016). Epidemiology of Cancers in Kashmir, India: An Analysis of Hospital Data. *Advances in Preventive Medicine*, *2016*, 1–6. https://doi. org/10. 1155/2016/1896761
157. Raj, S. , & Srivastava, M. (2022). Comparative analysis of HPV DNA and LBC for screening of cervical cancer in women with unhealthy cervix. *The New Indian Journal of OBGYN*, *8*(2), 166–173. https://doi. org/10. 21276/obgyn. 2022. 8. 2. 4
158. Rani, R. , Kumar, R. , Trivedi, V. , Singh, U. , Chauhan, R. , Ali, M. , & Kumar, A. (2016). Age, parity and stages of cervix cancer: A hospital-based study. *British Journal of Medical and Health Research*, *3*(4), 73–82.
159. Rebolj, M. , Rimmer, J. , Denton, K. , Tidy, J. , Mathews, C. , Ellis, K. , Smith, J. , Evans, C. , Giles, T. , Frew, V. , Tyler, X. , Sargent, A. , Parker, J. , Holbrook, M. , Hunt, K. , Tidbury, P. , Levine, T. , Smith, D. , Patnick, J. , & Kitchener, H. (2019). Primary cervical

screening with high-risk human papillomavirus testing: Observational study. *BMJ*, *364*, 1–21. https://doi. org/10. 1136/bmj. l240

160. Reguero-Cadilla, L. , Dominicci-Maura, A. , Godoy-Vitorino, F. , & Romaguera, J. (2022). Effect of menopause on genital human papillomavirus infection and the microbiota in a Hispanic cohort [A320]. *Obstetrics & Gynecology, 139*(1, Suppl.), 92S. https://doi. org/10. 1097/01. AOG. 0000825600. 95480. 64
161. Reid, R. , & Scalzi, P. (1985). Genital warts and cervical cancer. *American Journal of Obstetrics and Gynecology, 153*(6), 611–618. https://doi. org/10. 1016/s0002-9378(85)80244-1
162. Rohan, T. , Mann, V. , McLaughlin, J. , Harnish, D. G. , Yu, H. , Smith, D. , Davis, R. , Shier, R. M. , & Rawls, W. (1991). PCR- detected genital papillomavirus infection: Prevalence and association with risk factors for cervical cancer. *International Journal of Cancer, 49*(6), 856–860. https://doi. org/10. 1002/ijc. 2910490609
163. Rosolowsky, E. W. , Pineda, J. E. , Kauffmann, J. , & Goodman, A. A. (2008). Structural analysis of molecular clouds: Dendrograms. *The Astrophysical Journal, 679*(2), 1338–1351. https://doi. org/10. 1086/587685
164. Roura, E. , Castellsagué, X. , Pawlita, M. , Waterboer, T. , Uria Margall, N. , Bosch, F. X. , De Sanjos, S. , Travis, R. C. , Rinaldi, S. , Tommasino, M. , Franceschi, S. , & Riboli, E. (2014). HPV and cervical cancer screening: The importance of both HPV testing and cytology. *International Journal of Cancer, 134*(2), 453–466. https://doi. org/10. 1002/ijc. 28666
165. Sachan, P. , Singh, M. , Patel, M. , & Sachan, R. (2018). A study on cervical cancer screening using Pap smear test and clinical correlation. *Asia-Pacific Journal of Oncology Nursing*, *5*(3), 337–341. https://doi. org/10. 4103/APJON. APJON_15_18
166. Sanjose, S. De, Brotons, M. , Lamontagne, D. S. , & Bruni, L. (2019). Human papillomavirus vaccine: Disease impact beyond expectations. *Current Opinion in Virology, 39*, 16–22. https://doi. org/10. 1016/j. coviro. 2019. 06. 006
167. Sarma, U. , Mahanta, J. , Borkakoty, B. , & Sarmah, B. (2015). Distribution of human papilloma virus infections of uterine cervix among women of reproductive age: A cross-sectional hospital-based study from northeast India. *Asian Pacific Journal of Cancer Prevention, 16*(4), 1519–1523. https://doi. org/10. 7314/APJCP. 2015. 16. 4. 1519

168. Schiffman, M. , Castle, P. E. , Jeronimo, J. , Rodriguez, A. C. , & Wacholder, S. (2007). Human papillomavirus and cervical cancer. *The Lancet, 370*(9590), 890–907. https://doi. org/10. 1016/S0140-6736(07)61416-0

169. Schiffman, M. , Kinney, W. K. , Cheung, L. C. , Gage, J. C. , Fetterman, B. , Poitras, N. E. , Lorey, T. S. , Wentzensen, N. , Befano, B. , Schussler, J. , Katki, H. A. , & Castle, P. E. (2018). Relative performance of HPV and cytology components of cotesting in cervical screening. *Journal of the National Cancer Institute, 110*(5), 501–508. https://doi. org/10. 1093/jnci/djx225

170. Schiller, J. T. , & Lowy, D. R. (2012). Understanding and learning from the success of prophylactic human papillomavirus vaccines. *Nature Reviews Microbiology, 10*(10), 681–692. https://doi. org/10. 1038/nrmicro2872

171. Schiller, J. T. , Castellsagué, X. , & Garland, S. M. (2012). A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine, 30,* F123–F138. https://doi. org/10. 1016/j. vaccine. 2012. 04. 108

172. Scott, M. , Nakagawa, M. , & Moscicki, A. B. (2001). Cell-mediated immune response to human papillomavirus infection. *Clinical and Diagnostic Laboratory Immunology, 8*(2), 209–220. https://doi. org/10. 1128/CDLI. 8. 2. 209-220. 2001

173. Shah, V. , Vyas, S. , Singh, A. , & Shrivastava, M. (2012). Awareness and knowledge of cervical cancer and its prevention among the nursing staff of a tertiary health institute in Ahmedabad, Gujarat, India. *Ecancermedicalscience, 6*(1), 1–6. https://doi. org/10. 3332/ecancer. 2012. 270

174. Sharma, A. , & Singh, S. (2019). Pathology spectrum of cervical lesions and cytohistological correlation: A study in a tertiary care center. *International Journal of Medical Research Professionals, 6*(6), 5–10.

175. Sherman, M. E. , Schiffman, M. , & Cox, J. T. (2008). Erratum: Effects of age and human papilloma viral load on colposcopy triage: Data from the randomized atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion triage study (ALTS). *Journal of Lower Genital Tract Disease, 12*(3), 255. https://doi. org/10. 1097/01. lgt. 0000305249. 08060. 7a

176. Shields, T. S. , Brinton, L. A. , Burk, R. D. , Wang, S. S. , Weinstein, S. J. , Ziegler, R. G. , Studentsov, Y. Y. , McAdams, M. , & Schiffman, M. (2004). A case-control study of

risk factors for invasive cervical cancer among U. S. women exposed to oncogenic types of human papillomavirus. *Cancer Epidemiology, Biomarkers & Prevention, 13*(10), 1574–1582.

177. Shope, B. R. E. , & Hurst, B. E. W. (1933). Infectious papillomatosis of rabbits. *The Journal of Experimental Medicine, 58*(5), 561–568. https://doi. org/10. 1084/jem. 58. 5. 561
178. Sinchana, K. M. , Jadhav, M. N. , & Kittur, S. K. (2022). Cytomorphological study of cervical Pap smear based on the Bethesda system 2014. *Indian Journal of Pathology and Oncology, 9*(3), 232–237. https://doi. org/10. 18231/j. ijpo. 2022. 055
179. Singh, M. , Jha, R. P. , Shri, N. , Bhattacharyya, K. , Patel, P. , & Dhamnetiya, D. (2022). Secular trends in incidence and mortality of cervical cancer in India and its states, 1990-2019: Data from the Global Burden of Disease 2019 Study. *BMC Cancer, 22*(1), 1–12. https://doi. org/10. 1186/s12885-022-09232-w
180. Skinner, S. R. , Wheeler, C. M. , Romanowski, B. , Castellsagué, X. , Lazcano-Ponce, E. , Del Rosario-Raymundo, M. R. , Vallejos, C. , Minkina, G. , Pereira Da Silva, D. , McNeil, S. , Prilepskaya, V. , Gogotadze, I. , Money, D. , Garland, S. M. , Romanenko, V. , Harper, D. M. , Levin, M. J. , Chatterjee, A. , Geeraerts, B. , … Baril, L. (2016). Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study. *International Journal of Cancer, 138*(10), 2428–2438. https://doi. org/10. 1002/ijc. 29971
181. Smalley Rumfield, C. , Pellom, S. T. , Morillon, Y. M. , Schlom, J. , & Jochems, C. (2020). Immunomodulation to enhance the efficacy of an HPV therapeutic vaccine. *Journal for ImmunoTherapy of Cancer, 8*(1). https://doi. org/10. 1136/jitc-2020-000612
182. Souho, T. , Benlemlih, M. , & Bennani, B. (2015). Human papillomavirus infection and fertility alteration: A systematic review. *PLoS ONE, 10*(5), 1–9. https://doi. org/10. 1371/journal. pone. 0126936
183. Sowjanaya, A. P. , Jain, M. , Poli, U. R. , Padma, S. , Das, M. , Shah, K. V. , Rao, B. N. , Devi, R. R. , Gravitt, P. E. , & Ramakrishna, G. (2005). Prevalence and distribution of high-risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in andhra Pradesh, India. *BMC Infectious Diseases, 5*, 1–7. https://doi. org/10. 1186/1471-2334-5-116

184. Spronk, I. , Korevaar, J. C. , Poos, R. , Davids, R. , Hilderink, H. , Schellevis, F. G. , Verheij, R. A. , & Nielen, M. M. J. (2019). Calculating incidence rates and prevalence proportions: Not as simple as it seems. *BMC Public Health*, *19*(1), 1–9. https://doi. org/10. 1186/s12889-019-6820-3

185. Sreedevi, A. , Javed, R. , & Dinesh, A. (2015). Epidemiology of cervical cancer with special focus on India. *International Journal of Women's Health, 7*, 405–414. https://doi. org/10. 2147/IJWH. S50001

186. Sri, S. , Ramani, P. , Premkumar, P. , Ramshankar, V. , Ramasubramanian, A. , & Krishnan, R. (2021). Prevalence of Human Papillomavirus (HPV) 16 and 18 in oral malignant and potentially malignant disorders: A polymerase chain reaction analysis - A comparative study. *Annals of Maxillofacial Surgery*, *11*(1), 6–11. https://doi. org/10. 4103/ams. ams_376_20

187. Srivastava, S. , Gupta, S. , & Roy, J. K. (2012). High prevalence of oncogenic HPV-16 in cervical smears of asymptomatic women of eastern Uttar Pradesh, India: A population-based study. *Journal of Biosciences*, *37*(1), 63–72. https://doi. org/10. 1007/s12038-012-9181-y

188. Stamataki, P. , Papazafiropoulou, A. , Elefsiniotis, I. , Giannakopoulou, M. , Brokalaki, H. , Apostolopoulou, E. , Sarafis, P. , & Saroglou, G. (2010). Prevalence of HPV infection among Greek women attending a gynecological outpatient clinic. *BMC infectious diseases*, *10*, 27. https://doi. org/10. 1186/1471-2334-10-27

189. Stanley, M. A. (2001). Human papillomavirus and cervical carcinogenesis. *Best Practice and Research: Clinical Obstetrics and Gynaecology*, *15*(5), 663–676. https://doi. org/10. 1053/beog. 2001. 0213

190. Stanley, M. , Pinto, L. A. , & Trimble, C. (2012). Human papillomavirus vaccines - immune responses. In *Vaccine*, 30(5). https://doi. org/10. 1016/j. vaccine. 2012. 04. 106

191. Stelzle, D. , Tanaka, L. F. , Lee, K. K. , Khalil, A. I. , Baussano, I. , Shah, A. S. V. , Minozzi, S. , & Bray, F. (2021). Estimates of the global burden of cervical cancer associated with HIV. *The Lancet Global Health*, *9*(2), e161–e169. https://doi. org/10. 1016/S2214-109X(20)30459-9

192. Strauss, J. , Gatti-Mays, M. E. , Cho, B. C. , Hill, A. , Salas, S. , McClay, E. , Redman, J. M. , Sater, H. A. , Donahue, R. N. , Jochems, C. , Lamping, E. , Burmeister, A. , Marté, J.

L. , Cordes, L. M. , Bilusic, M. , Karzai, F. , Ojalvo, L. S. , Jehl, G. , Rolfe, P. A. , & Gulley, J. L. (2020). Bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with human papillomavirus-associated malignancies. *Journal for ImmunoTherapy of Cancer, 8*(2), 1–10. https://doi. org/10. 1136/jitc-2020-001395

193. Sung, H. , Ferlay, J. , Siegel, R. L. , Laversanne, M. , Soerjomataram, I. , Jemal, A. , & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians, 71*(3), 209–249. https://doi. org/10. 3322/caac. 21660
194. Syrjänen, K. J. (2017). Two landmark studies published in 1976/1977 paved the way for the recognition of human papillomavirus as the major cause of the global cancer burden. *Acta Cytologica, 61*(4–5), 316–337. https://doi. org/10. 1159/000477372
195. Syrjänen, S. , Shabalova, I. , Petrovichev, N. , Kozachenko, V. , Zakharova, T. , Pajanidi, J. , Podistov, J. , Chemeris, G. , Sozaeva, L. , Lipova, E. , Tsidaeva, I. , Ivanchenko, O. , Pshepurko, A. , Zakharenko, S. , Nerovjna, R. , Kljukina, L. , Erokhina, O. , Branovskaja, M. , Nikitina, M. , & Syrjänen, K. (2004). Acquisition of High-Risk Human Papillomavirus Infections and Pap Smear Abnormalities among Women in the New Independent States of the Former Soviet Union. *Journal of Clinical Microbiology, 42*(2), 505–511. https://doi. org/10. 1128/JCM. 42. 2. 505-511. 2004
196. Tao, X. , Austin, R. M. , Yu, T. , Zhong, F. , Zhou, X. , Cong, Q. , Sui, L. , & Zhao, C. (2022). Risk stratification for cervical neoplasia using extended high-risk HPV genotyping in women with ASC-US cytology: A large retrospective study from China. *Cancer Cytopathology, 130*(4), 248–258. https://doi. org/10. 1002/cncy. 22536
197. Tarkowski, T. A. , Koumans, E. H. , Sawyer, M. , Pierce, A. , Black, C. M. , Papp, J. R. , Markowitz, L. , & Unger, E. R. (2004). Epidemiology of Human Papillomavirus Infection and Abnormal Cytologic Test Results in an Urban Adolescent Population. *Journal of Infectious Diseases, 189*(1), 46–50. https://doi. org/10. 1086/380466
198. Tawe, L. , Grover, S. , Narasimhamurthy, M. , Moyo, S. , Gaseitsiwe, S. , Kasvosve, I. , & Paganotti, G. M. (2018). Molecular detection of human papillomavirus (HPV) in highly fragmented DNA from cervical cancer biopsies using double-nested PCR. *MethodsX, 5*, 569–578. https://doi. org/10. 1016/j. mex. 2018. 05. 018

199. Tekalegn, Y. , Sahiledengle, B. , Woldeyohannes, D. , Atlaw, D. , Degno, S. , Desta, F. , Bekele, K. , Aseffa, T. , Gezahegn, H. , Kene, C. , Kops, N. L. N. L. , Bessel, M. , Horvath, J. D. C. D. C. , Domingues, C. , De Souza, F. M. A. , Benzaken, A. S. , Mendes Pereira, G. F. , Maranhão, A. G. K. , Villa, L. L. , … & Fonseca-Moutinho, J. A. (2019). High parity is associated with increased risk of cervical cancer: Systematic review and meta-analysis of case–control studies. *Women's Health, 9*(6), 1–7. https://doi. org/10. 1136/bmjopen-2018-027438

200. Toh, Z. Q. , Kosasih, J. , Russell, F. M. , Garland, S. M. , Mulholland, E. K. , & Licciardi, P. V. (2019). Recombinant human papillomavirus nonavalent vaccine in the prevention of cancers caused by human papillomavirus. In *Infection and Drug Resistance* (Vol. 12, pp. 1951–1967). Dove Medical Press Ltd. https://doi. org/10. 2147/IDR. S178381

201. Tolcher, A. , Papadopoulos, K. , Cole, Y. , Rivas, K. , Chandana, S. , Sinclair, S. , Wood, D. , Nadler, P. I. , & Lakhani, N. (2018). A Phase 1a/2a trial of AVID100, an anti-EGFR antibody-drug conjugate. *Annals of Oncology, 29*(March), iii8. https://doi. org/10. 1093/annonc/mdy048. 004

202. Tyagi, A. , Vishnoi, K. , Mahata, S. , Verma, G. , Srivastava, Y. , Masaldan, S. , Roy, B. G. , Bharti, A. C. , & Das, B. C. (2016). Cervical cancer stem cells selectively overexpress HPV oncoprotein E6 that controls stemness and self-renewal through upregulation of HES1. *Clinical Cancer Research, 22*(16), 4170–4184. https://doi. org/10. 1158/1078-0432. CCR-15-2574

203. Venezia, S. , Refolo, V. , Polissidis, A. , Stefanis, L. , Wenning, G. K. , & Stefanova, N. (2017). Toll-like receptor 4 stimulation with monophosphoryl lipid A ameliorates motor deficits and nigral neurodegeneration triggered by extraneuronal α-synucleinopathy. *BMC Neuroscience*, *18*, 1–13. https://doi. org/10. 1186/s13024-017-0195-7

204. Venuti, A. , & Paolini, F. (2012). HPV detection methods in head and neck cancer. *Head and Neck Pathology, 6*(Suppl. 1), 63–74. https://doi. org/10. 1007/s12105-012-0372-5

205. Villa, L. L. (2006). Prophylactic HPV vaccines: Reducing the burden of HPV-related diseases. *Vaccine, 24*(Suppl. 1), S23. https://doi. org/10. 1016/j. vaccine. 2005. 09. 001

206. Villa, L. L. , & Denny, L. (2006). Chapter 7 Methods for detection of HPV infection and its clinical utility. *International Journal of Gynecology and Obstetrics, 94*(Suppl. 1). https://doi. org/10. 1016/S0020-7292(07)60013-7

207. Vinoda, J. , Malila, N. , Swaminathan, R. , & Okuru, P. (2015). Survival of patients with cervical cancer in rural India. *Journal of Clinical Oncology, 4*(4), 290–296.
208. Vinodhini, K. , Shanmughapriya, S. , Sanmugham, S. , Senthikumar, G. , Das, B. C. , & Natarajaseenivasan, K. (2012). Prevalence of high-risk HPV and associated risk factors in cases of cervical carcinoma in Tamil Nadu, India. *International Journal of Gynecology & Obstetrics, 119*(3), 253–256. https://doi. org/10. 1016/J. IJGO. 2012. 06. 019
209. Wang, C. , Wright, T. C. , Denny, L. , & Kuhn, L. (2011). Rapid rise in detection of human papillomavirus (HPV) infection soon after incident HIV infection among South African women. *Journal of Infectious Diseases, 203*(4), 479–486. https://doi. org/10. 1093/infdis/jiq083
210. Wang, R. , Pan, W. , Jin, L. , Huang, W. , Li, Y. , Wu, D. , Gao, C. , Ma, D. , & Liao, S. (2020). Human papillomavirus vaccine against cervical cancer: Opportunity and challenge. *Cancer Letters, 471*, 88–102. https://doi. org/10. 1016/j. canlet. 2019. 11. 039
211. Westra, W. H. (2014). Detection of human papillomavirus (HPV) in clinical samples: Evolving methods and strategies for the accurate determination of HPV status of head and neck carcinomas. *Oral Oncology, 50*(9), 771–779. https://doi. org/10. 1016/J. ORALONCOLOGY. 2014. 05. 004
212. Wilting, S. M. , & Steenbergen, R. D. M. (2016). Molecular events leading to HPV-induced high grade neoplasia. *Papillomavirus Research, 2*, 85–88. https://doi. org/10. 1016/j. pvr. 2016. 04. 003
213. Winer, R. L. , Lee, S. K. , Hughes, J. P. , Adam, D. E. , Kiviat, N. B. , & Koutsky, L. A. (2003). Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. *American Journal of Epidemiology, 157*(3), 218–226. https://doi. org/10. 1093/aje/kwf180
214. Wolday, D. , Derese, M. , Gebressellassie, S. , Tsegaye, B. , Ergete, W. , Gebrehiwot, Y. , Caplan, O. , Wolf, D. G. , & Maayan, S. (2018). HPV genotype distribution among women with normal and abnormal cervical cytology presenting in a tertiary gynecology referral clinic in Ethiopia. *Infectious Agents and Cancer, 13*(1), 4–11. https://doi. org/10. 1186/s13027-018-0201-x
215. Wright, T. C. , Stoler, M. H. , Behrens, C. M. , Sharma, A. , Zhang, G. , & Wright, T. L. (2015). Primary cervical cancer screening with human papillomavirus: End of study

results from the ATHENA study using HPV as the first-line screening test. *Gynecologic Oncology, 136*(2), 189–197. https://doi. org/10. 1016/j. ygyno. 2014. 11. 076

216. Xi, L. F. , Koutsky, L. A. , Castle, P. E. , Edelstein, Z. R. , Meyers, C. , Ho, J. , & Schiffman, M. (2009). Relationship between cigarette smoking and human papillomavirus types 16 and 18 DNA load. *Cancer Epidemiology, Biomarkers & Prevention, 18*(12), 3490–3497. https://doi. org/10. 1158/1055-9965. EPI-09-0763
217. Yang, A. , Farmer, E. , Wu, T. C. , & Hung, C. (2016). Perspectives for therapeutic HPV vaccine development. *Journal of Translational Medicine, 14*(1), 28. https://doi. org/10. 1186/s12929-016-0293-9
218. Yaqoob, F. , & Kaul, I. (2018). Cervical screening in a tertiary care centre in Jammu. *JK Science, 20*(3), 132–135. http://www. jkscience. org/archives/volume203/7-Orignal Article. pdf
219. Yeoh, G. P. S. , & Chan, K. W. (1997). The accuracy of Papanicolaou smear predictions: Cytohistological correlation of 283 cases. *Asian Pacific Journal of Cancer Prevention, 3*(4), 373–376.
220. Yim, E. , Ph, D. , Park, J. , & Ph, D. (2005). The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis. *Cancer Research, 37*(6), 319–324.
221. Zhang, H. , Li, C. , Lai, P. F. H. , Chen, J. , Xie, F. , Xia, Y. , & Ai, L. (2021). Fractionation, chemical characterization and immunostimulatory activity of β-glucan and galactoglucan from *Russula vinosa* Lindblad. *Carbohydrate Polymers, 256*, 117559. https://doi. org/10. 1016/j. carbpol. 2020. 117559
222. Zhang, S. , Xu, H. , Zhang, L. , & Qiao, Y. (n. d.). Cervical cancer: Epidemiology, risk factors and screening. *Journal of Pathology, 40*(1). https://doi. org/10. 21147/j. issn. 1000-9604. 2020. 06. 05
223. Zur Hausen, H. (2002). Papillomaviruses and cancer: From basic studies to clinical application. *Nature Reviews Cancer, 2*(5), 342–350. https://doi. org/10. 1038/nrc798
224. Zur Hausen, H. (2009). Human papillomavirus & cervical cancer. *Indian Journal of Medical Research, 130*(3), 209.

APPENDIX 1

GOVERNMENT MEDICAL COLLEGE, JAMMU
INSTITUTIONAL ETHICS COMMITTEE

No: IEC/GMC/2020/85 Dated: 29/05/2020

CERTIFICATE

This is to certify that the proposal of thesis/research/project submitted by Ridhima Jasrotia Department of Pathology entitled "Clinical Spectrum of Cervical Cancer and Co-relation with Human Papilloma Virus in Jammu region" belonging to CAT-C (as per ICMR guideline) study in medical education, was discussed & deliberated in IEC by circulation on 26-05-2020. The committee/ Sub Committee approved the said project and permitted the same on ethical grounds as per the requirement of MCI/Jammu University/ as per protocol presented and described in the proposal submitted.

Copy for the concerned

Vishal Tandon.

Dr. Vishal Tandon
Professor
Deptt of Pharmacology
Member Secretary
IEC ,GMC Jammu

Copy to:-
1 PMC Jammu for information please.
2 Professor and Head Department of Pathology GMC Jammu for information.
3 Office copy

APPENDIX 2

SWASTIK™ DIAGNOSTIC LABORATORY

SWASTIK DIAGNOSTIC LABORATORY
Dr.Modi Market, DograHall, NewSecretariate Road
Jammu (Tawi)-180001
Phones: 0191-2548509,2575984,2520170
CONSENT FORM LBC/HPV

DATE:

SID No

Name Age Tel.No.

Marital Status Address

PATIENT CONSENT

I_____S/o,D/o,W/o_____give my consent for PAP/LBC/HPV& I have been explained the procedure for the tests. I have been given full opportunity to ask relevant questions & refuse consent.

Undertaking: I fully understand that any incorrect statement or concealment may be detrimental to my health or recipient's health. All above information is true to the best of my knowledge & belief.

Signature of Patient

SWASTIK™ DIAGNOSTIC LABORATORY

Questionnaire

Title of the Study: Clinical Spectrum of Cervical Cancer and its Correlation with HPV in Jammu Region.

Principal Investigator
Ridhima
PhD Scholar
Department of Microbiology (Lovely Professional University, Phagwara).

Question related to risk factors.

1. Early Marriage	Yes/No
2. History of any STD	Yes/No
3. Early Pregnancy	Yes/No
4. Vaginal Bleeding	Yes/No
5. Smoking	Yes/No
6. Oral contraceptives at an early stage	Yes/No
7. Socioeconomic factors	Yes/No
8. Multiparity	Yes/No

SIGNATURE

HOD LAB

Approved by

LIST OF PUBLICATIONS:

1. Jasrotia, R. , Dhanjal, D. S. , Bhardwaj, S. , Sharma, P. , Chopra, C. , Singh, R. , & Goyal, A. (2022). Nanotechnology-based vaccines: Cervical cancer management and perspectives. *Journal of Drug Delivery Science and Technology, 71*, 103351. https://doi. org/10. 1016/j. jddst. 2022. 103351

2. Jasrotia, R. , Kashyap, I. , Suri, J. , Chopra, C. , Wani, A. K. , Tizro, N. , Goyal, A. , & Singh, R. (2024). Assessing knowledge and awareness levels regarding cervical cancer and HPV vaccination in the Jammu region. *Iranian Journal of Microbiology, 16*(4), 515–523.

LIST OF CONFERENCES ATTENDED:

1. Participated in the oral presentation on the topic "To find the correlation of cervical cytology and HPV in Jammu region" at the"4th Indo Oncology Summit" Organized by the International Association of Oncology (IAO) held on 11th, 12th & 13th November 2022 Bhuvneshwar, India.

2. Participated in an Oral Presentation on the topic entitled "Detection of cervical cancer along with a screening of HR-HPV in the Jammu region" at the International Conference on Bioengineering and Biosciences (ICBB-2022) held on 18-19 November 2022 organized by the Department of Biotechnology, School of Bio-engineering and Biosciences.