

**A STUDY OF HUMAN PAPILLOMAVIRUS SCREENING FOR
DETECTION OF CERVICAL CANCER AT EARLY STAGE OF
CANCER DETECTION IN MIDDLE EAST**

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Corresponding Author Email: pujabanerjee20@gmail.comReceived : 01.10.2020Revised : 03.11.2020Accepted : 04.12.2020**Abstract:**

The purpose of this study is to compare the efficacy of the PCR HPV test for detecting changes in cervical human papillomavirus to that of the Thinprep cytology test. Seventy female participants were analysed. Patients were examined using a speculum and then submitted to a thin-prep cytology test and multiplex PCR HPV analysis. There were 12 patients (17.1%) with NILM on the ThinPrep cytology test, 36 patients (51.4%) with ASC-US, 20 patients (28.6%) with LSIL, and 2 patients (2.9%) with HSIL. Twenty-six (37.2%) individuals tested negative for HPV by PCR, whereas 34.3% had low-risk HPV strains, 14.3% had HPV 16, and 7.1% had HPV 18. The results of ThinPrep cytology were correlated with those of conventional PAP smears ($P = 0.030$) and PCR HPV tests ($P = 0.000$). It was shown that the ThinPrep test was more sensitive than the PCR HPV test but not as specific. When used in tandem, the ThinPrep cytology test and the PCR HPV test can make a substantial contribution toward achieving a correct diagnosis and screening for cervical cancer.

Key words: Cervical cancer screening, prevention, and epidemiology; human papillomavirus

1. INTRODUCTION

Cancers of the cervix are much less common and much less deadly in nations where the Papanicolaou test is used routinely to screen women for them. However, in areas where screening was not regularly conducted, especially in developing nations, cytology-based cervical cancer screening programmes had much inferior results. Subpar results in cytology-based screening have been seen, which may be attributable to a combination of insufficient coverage, low patient compliance, subjective interpretation of data, and public health and legal difficulties. Recent decades have seen widespread agreement on the causal role of human papillomavirus (HPV) infection in the development of cervical cancer, and research has shown that HPV infection is a prerequisite for both preinvasive and invasive stages of the disease. As of now, more than 200 different types of HPV have been identified, and those that infect the cervix have been categorised according to their oncogenic potential as high-risk (HR), (i.e., with sufficient evidence for the causation of cervical cancer) (16 being the most potent type), possible high-risk (i.e., with limited evidence for the causation of cervical cancer) (26, 53, 66, 67, 68, 70, 73, 82, 70, 73, 82, 70, 73, 73, 82, 73, 83). More than 99 percent of invasive cancer cases and the vast majority of high-

grade pre-invasive cases involve HR-HPV types, implying that HPV detection may be a suitable option as a screening test for the detection of precursor lesions that, if left untreated, may progress to cancer. Actually, nowadays, many people think that detecting HR-HPV types is a more effective screening strategy than cytology. HPV screening has been shown to reduce mortality rates and is more sensitive than the Papanicolaou test at detecting high-grade transformed lesions and cancer. Further, in comparison to a normal Papanicolaou test result, a negative result in HPV testing allows for a longer time interval before repeat screening becomes necessary, which in turn allows for less frequent testing and higher patient compliance with screening programmes.

1.1 Cervical Cancer

Globally, cervical cancer ranks fourth most frequently among women, behind breast, colorectal, and lung cancer. About 604,000 cases of cervical cancer and 342,000 deaths were predicted by GLOBOCAN 2020. It is the third most frequently diagnosed cancer in females worldwide, with the vast majority of new cases and fatalities happening in low and middle-income countries (LMICs). Cervical cancer is the second most common gynaecological malignancy worldwide, affecting nearly 1 in 12 women. Cervical cancer is more common in underdeveloped countries, where it accounts for 15% of all cases of female cancer, compared to 3.6% in industrialised countries. Latin America Sub-Saharan Africa, and the Caribbean, Melanesia, India, and other regions of Asia have the world's highest incidence rates, with 20–40 new cases per 100,000 women according to age-standardized incidence rates (ASR). In India, where there are an estimated 126,000 new cases and 71,000 deaths annually, it is the most frequently occurring cancer in females. The risk is significant, especially in rural regions, and the absolute number of cases is on the rise due to population growth, even though the incidence rate has been steadily falling in some metropolitan populations. Over 40% of cervical cancers in India occur in urban regions, whereas 65% of them occur in rural areas. The highest incidence age is between 55 and 65 years old, with a rising incidence rate between the ages of 30 and 34. Women diagnosed with cervical cancer had a median age of 38 (age range 21-67 yrs). According to the MMTR (Madras Metropolitan Tumor Registry), between 2004 and 2008, the crude incidence rate (CIR) in Chennai was 19.8 per 100,000 people, while the age-standardized rate (ASR) was 22.0 per 100,000 people.

1.2 Cervical Cancer: Diagnosis and Prevention

The "required" cause of cervical cancer is a chronic infection of the lower genital tract by one of roughly 15 high-risk HPV (hr HPV) strains. When the same HPV DNA is detected in samples taken 6–12 months apart, this indicates a persistent infection. In long-term studies, more than 80% of women develop at least one hrHPV infection, demonstrating the widespread nature and ease of transmission of this virus. One tenth of all infections become persistent, however, and these women are at risk of developing cervical precancerous lesions. About 71% of the 604,000 annual new cases of cervical cancer are caused by HPV types 18 and 16. Another 19% are caused by HPV types 31, 33, 45, 52, and 58. Nearly 90% of incident HPV infections are eliminated within a period of 2 years after infection, and just about 10% persist in

around 10% of women. Some researchers believe that the virus may remain latent in basal cells and could reactivate at a later time, while others believe that it has been eradicated entirely. As a result of our understanding of HPV epidemiology and the role it plays in cancer development, two important techniques for prevention and early diagnosis have emerged.

(1) HPV vaccination; and

(2) screening for precancerous lesions.

Tragically, many low- and middle-income countries (LMICs) still lack effective intervention programmes, despite the fact that eliminating cervical cancer is possible.

1.3 HPV vaccine for primary cervical cancer prevention

In a cross-sectional study conducted on women over the age of 30, the estimated global prevalence of HPV was 11.7%, with the highest incidence found in Sub-Saharan Africa at roughly 24.7%. However, the prevalence varied widely by nation, ranging from 2.7% to 42.7%. Women under the age of 25 have the highest age-specific cross-sectional HPV prevalence, suggesting that sexual transmission is the primary mode of transmission after sexual debut. In light of this, females between the ages of 10 and 14 should be the primary focus of any preventative HPV vaccination programme. The first HPV vaccine was introduced in 2006. There are currently three prophylactic HPV vaccines available for use in females and males starting at the age of 9 to prevent hrHPV-related premalignant lesions and cancers of the cervix, vulva, vagina, and anus. These vaccines include a bivalent vaccine targeting HPV 18 and 16, a quadrivalent vaccine targeting HPV 11, 16, 6, and 18, and a nonavalent vaccine targeting HPV 31, 33, 35, 39, 45, 52, 58, and 66. The anogenital warts caused by HPV 11 and 6 are also the focus of the most recent two vaccinations. Recently, China licenced a bivalent HPV vaccine (Cecolin; Xiamen Innovax Biotech Co., Ltd), which is now going through the WHO prequalification process. Since none of the vaccinations contain actual viral DNA, even though they are made up of virus-like particles, they are all safe for use. Girls and boys between the ages of 9 and 14 years old should receive two doses (0.5 mL at 0 and 6–12 months apart). Three doses (0.5 mL at 0, 1, and 6 months) are recommended by the manufacturer for individuals aged 15 and over who are immunocompromised. The most up-to-date statistics were evaluated by WHO, and they found no cause for alarm regarding HPV vaccines.

1.4 Treatment of precancerous cervical lesions as a secondary method of preventing cervical cancer

Cervical cancer screenings are a vital part of any plan to eradicate this disease worldwide. Screening is conducted to detect high-grade CIN and adenocarcinoma in situ, two of the most common precancerous lesions in the cervix, in order to treat them efficiently, hence delaying the progression to invasive cancer and reducing cervical cancer mortality rates. In light of this, preventing cervical cancer will continue to be a top priority for the foreseeable future. Conventional cytology (Pap smear), and human papillomavirus (HPV) testing, liquid-based cytology (LBC), and visual inspection with acetic acid in low- and middle-income countries (LMICs) have all been used successfully for cervical screening (VIA). Screening with the Pap smear at regular intervals has led to a significant decrease in cervical cancer risk in high-

income nations, but it is not practicable in low-resource settings due to inadequate organisation, coverage, and lack of quality assurance, leading to inferior outcomes. HPV-based screening provides lower variability, higher accuracy, and sensitivity and better replicability compared with traditional or LBC. In the context of decreased HPV infections in vaccinated populations, several healthcare systems are converting to primary HPV screening, whose stronger negative predictive value allows extended screening intervals or even a single lifetime test in low-resource settings. Recently published European guidelines emphasise the importance of primary HPV-based screening above conventional cytology-based screening. HPV screening is currently being implemented on a national or regional level in the Netherlands, Turkey, Finland, , United Kingdom Sweden, and theItaly. Australia, Chile, Argentina, and Mexico are just a few of the countries that have started using HPV screening programmes. Colposcopy referrals have grown as a result, but so have the rates at which CIN3+ lesions and cervical malignancies are found.

1.5 Pregnancy-related cervical cancer rates

A multidisciplinary group is needed to properly care for these patients. In order to honour the patient and her partner's wishes, it is necessary to discuss the strategy with both parties. In general, pregnant women with cervical cancer are treated in the same way as those who are not pregnant. Patients are treated as soon as possible before the 16th to 20th week of pregnancy. Depending on the severity of the condition, surgery, chemotherapy, or radiation may be used as a treatment option. In many cases, the embryo will abort itself due to radiation exposure. From the late second trimester onward, surgery and chemotherapy can be performed in selected patients while sustaining the pregnancy. After 20 weeks of pregnancy, individuals with Stages IA2, IB2, and 1B1 have the option of deferring definitive therapy without a worse prognosis than in nonpregnant patients. The timing of delivery requires balancing the interests of the mother and the foetal health. When performed at a tertiary centre with appropriate newborn care, birth by classical caesarean section and radical hysterectomy at the same time is undertaken no later than 34 weeks of gestation.

2. REVIEW OF LITERATURE

Turki Jalil Abduladheem (2020) Only about 15%–20% of all human malignancies may be attributed to viruses. Infection with an oncogenic virus has been linked to a variety of carcinogenic processes. About 15 different types of HPV have been linked to cancer. Despite the progress made in testing, cervical cancer remains a major health concern. The prevalence and mortality per geographic area of cervical cancer were dramatically diverse. (CC). Cervical cancer is the fourth leading cause of cancer death among women. The major risk factor for developing cervical cancer is a history of human papillomavirus (HPV) infection in the cervix. Inflammation is a fast host defence mechanism that helps the body deal with diseases like viruses by activating the innate immune system. Inflammation has benefits if it is short-lived and under tight control, but it might backfire if it persists for too long. HPV proteins contribute to the generation of chronic inflammation in a roundabout way. The incidence of HPV also varies greatly by age group. Two peaks of HPV positive in younger and older

adults have been seen in various groups. A variety of studies have been undertaken internationally on the epidemiology of HPV infection and carcinogenic qualities related to particular HPV genotypes. There are, however, a few nations where population-dependent occurrences have not been established. Furthermore, cervical cancer screening practises vary from country to country.

Tony L. Brown (2018) In terms of cancer incidence, cervical cancer ranks fourth among women worldwide. The most recent data available (2012) estimates that there were 528,000 new instances of cervical cancer and over 266,000 deaths from the disease. More than 75% of sexually active adults worldwide have had some form of HPV infection. A causal link between this virus and the progression of precancerous lesions (which can lead to cervical cancer) has been identified. Cervical cancer is a worldwide problem for these reasons. Early detection is the cornerstone of the HPV-based strategy for preventing cervical cancer. The earlier a woman discovers she has HPV and begins treatment, the better her prognosis will be. To this day, cervical cancer remains the leading cause of cancer-related death among women in low- and middle-income nations. The early detection of precancerous lesions is largely responsible for the lower incidence rates observed in developed nations. Several methods, including HPV DNA testing, cytologic screening, visual inspection with acetic acid (VIA), and HPV vaccinations, have been shown to be helpful in lowering the occurrence of cervical cancer in at-risk women. Many studies have demonstrated that there is a persistent gap between women's knowledge of the proven connection between HPV and cervical cancer and the necessity of cervical screening. Women's knowledge of cervical cancer, its risk factors, and screening methods directly correlates to the disease's early detection rates (test)

Shin-je Ghim (2002) Human papillomaviruses (HPVs) are the causative agents of cervical cancer, and HPVs 18 and 16 in particular are particularly responsible for this STD. Twenty percent of the world's 500,000 annual cases of cervical cancer are diagnosed in India. Due to limited funding, India and many other developing nations lack access to mass cancer screening programmes that can diagnose and cure cervical cancer and its precursor diseases. The outcome of curative and palliative therapy for cervical cancer varies from patient to patient, in part due to the individuality of each patient's immune system. This article provides the practising gynaecological oncologist with a description of the natural history of cervical carcinogenesis as well as an explanation of the rationale behind various preventative and therapeutic strategies. Screening, diagnosing, and treating cervical cancer and its precursor lesions should be more manageable if a prophylactic vaccination against HPV-18 and-16 were available, as well as a therapeutic vaccine against cervical malignancies.

Neerja Bhatla, (2021) As a direct result of the 2018 FIGO Cancer Report, the World Health Organization (WHO) has developed a global strategy for eliminating cervical cancer, with the goal of implementing it by 2030. Vaccination against human papillomavirus (HPV) has been adopted as part of the national programme in more than 130 countries. As HPV testing has become more common, significant progress has been made in the screening process. The effects of these actions will not be visible for several years. Meanwhile, each year, over 500,000 new cases are added.

Numerous retrospective assessments of data based on FIGO's updated cervical cancer staging (2018) have been published. Surgery with a smaller incision is more risky for women with cervical cancer. In this chapter, we will examine the different approaches to treating cervical cancer based on the stage of the disease, paying special emphasis to palliation and quality of life concerns.

3.METHODOLOGY

- **Materials and Methods**

Seventy participants were included in this retrospective analysis. Patients having positive conventional PAP smear results, thin prep-cytology results, or HPV DNA positive results from the gynaecology outpatient department at King Faisal Hospital, KSA were found using a computerised search between January 2017 and January 2018. The research protocol followed the guidelines set forth by the Human Subjects Research Committee and the 2008 revisions to the Helsinki Declaration. The participants' ages were recorded (from 23 to 53 years old), and other clinical information was gathered as well. Patients were examined with a speculum and then submitted to a traditional PAP smear, a Thin-Cytology test, and a Multiplex PCR HPV analysis.

- **Inclusion Criteria**

The current study included patients with comprehensive medical records, patients over the age of 22, new patients, and patients with abnormal past results in conventional PAP smear, thincytology test, or PCR HPV analysis.

- **Cancer Detection Through Cytology**

All samples were processed through a ThinPrep 2000 system in preparation for liquid-based cytology. It has been determined by the Food and Drug Administration that the ThinPrep system is suitable for processing Pap smears. The endocervical canal was brushed with a cytobrush to acquire PreservCyt samples. A bottle of PreservCyt transport medium was quickly inserted into the cytobrush's packaging. After the contents of the PreservCyt vial have been removed, the vial is sealed, tagged, and shipped to a lab with a ThinPrep processor. These samples were kept at room temperature (15–20 degrees Celsius) for up to 24 hours before being shipped to the lab. By rotating the vial (T3000), the ThinPrep processor homogenises the sample by generating shear forces in the fluid that are strong enough to disaggregate randomly connected material, break up blood and mucus, and preserve cell clusters. The cells are then collected on the TransCyt filter membrane, transported to a glass slide, and allowed to form a monolayer deposit around 20 mm in diameter. Slides are automatically ejected into a fixative bath of 95% ethanol before being stained with Papanicolaou stain. Cellularity can be easily evaluated in a liquid-based preparation by comparing the sample to reference pictures or by counting well-preserved squamous cells in a predetermined number of high-power shots. The Bethesda System was used to create cytological reports, which included an assessment of whether or not the transformation zone was properly sampled (based on the presence or absence of metaplastic and/or columnar cells). In case a second analysis was required,

PreservCyt samples were kept in a refrigerator between 15 and 20 degrees Celsius for up to six weeks.

• **Polymerase Chain Reaction HPV Testing**

Within a week of collection, PCR analysis was performed on PreservCyt samples. Endocervical cells were vortexed aggressively after being suspended in a PreservCyt transport medium. 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, and 68 DNA sequence data were downloaded from Genbank. The specificity of each primer pair was validated by BLAST analysis, and an appositive control consisting of a pair of primers targeting globin was provided.

• **Interpretation of Cytological Results**

A cytopathologist examined the slides, made diagnoses, and categorised the findings using the most recent version of the Bethesda Classification System. Negative for intraepithelial lesion or malignancy, atypical squamous cells of undetermined significance, atypical squamous cells cannot exclude high grade squamous intraepithelial lesion, low grade squamous intraepithelial lesion, high grade squamous intraepithelial lesion, or squamous cell carcinoma, which were all terms used in the Bethesda system. All smear tests were read and diagnosed without access to HPV data.

4.RESULTS AND DISCUSSION

Table 1. Association between Thin Prep and HPV DNA results

HAP DNA	NILM (n=12)	ASCUS (n=36)	LSIL (n=20)	HSIL (n=2)	P Value
HPV18 (n=5)	0	2	2	1	0.000
HPV16(n=10)	4	1	4	1	
Low risk (n=24)	7	7	10	0	
Negative (n=26)	1	24	1	0	
Others (n=5)	0	2	3	0	

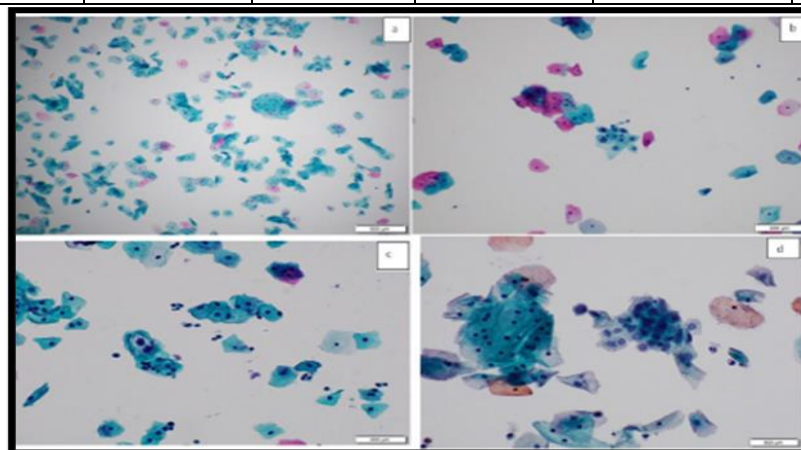


Figure 1: Thin Prep Pap smear

When it comes to the demographics of our patients, we find that the mean age is 34.610.923% and the median age is 32.577.67. The ages of the participants ranged from 23 to 53. Patients are divided into two groups based on their median age: those younger than 32 years (consisting of 36 people) and those older than 32 years (including all patients) (including 34 patients). Of the patients, 22 are single and 48 are married. Twenty-seven of the married patients are having more than two children, and ten of the married patients are using some form of birth control. Thirty-six patients (51.4%) had atypical squamous cells of undetermined significance (ASCUS); twenty patients (28.6%) had a low grade squamous intraepithelial lesion (LSIL); and two patients (2.9%) had a high grade squamous intraepithelial lesion . Twenty-six (37.2%) patients had negative PCR HPV results; 34% (34) had low risk HPV types; 14% (10 patients) had HPV 16, 7% (5 patients) had HPV 18, and 7% (5 patients) had other forms of HPV infection. Sixty-three patients (or 90%) had a single HPV genotype infection, while seven patients (10%) had two HPV genotype infections. Five patients with dual genotypes had both HPV16 and another form of HPV, with two patients having HPV16 and one each of low-risk and other types of HPV, and one patient having HPV18 infection. A mixed infection with HPV18 and low-risk strains was found in the other 2.

5. CONCLUSIONS

The majority of cervical malignancies are caused by the human papillomavirus (HPV). Because of this, many programmes that aim to prevent cervical cancer also include cytological screening, focusing particularly on dysplasia as a means of detecting HPV. More sensitive and specific cytological assays for HPV detection have been the subject of numerous clinical trials. This has led to the development of liquid-based gynecologic specimens. Improved fixation, consistency of cell transfer, and less obscuring effects make liquid-based preparations preferable to traditional smears. The residual specimen after liquid-based cytology can be kept and used for immunostaining and HPV DNA testing, which is a significant benefit. Because up to 90% of cervix scrapings are discarded following standard PAP smears, a large number of unsatisfactory smears result. The percentage of poor quality specimens could be cut in half using liquid-based cytology. More and more people are paying attention to HPV DNA because of its increased sensitivity and ability to detect "high-risk" HPV strains that most typically impact the cervix. A DNA test for HPV is the most reliable method for detecting dangerous strains of the virus. The FDA has cleared the HPV test for use in screening women over the age of 30 and in following up on women who have had inconclusive cytology findings.

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