Proportion of Genital HPV Infection in Females of Reproductive Age Group in a Tertiary Care Hospital

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Abstract

The present observational study is aimed at calculating the proportion of females aged 21-50 years, in a sample of 200, infected with HPV, by analysing their pap smears. The pathological diagnosis for these samples consisted of inflammatory changes, bacterial vaginosis, fungal infection, low and high grade squamous intraepithelial lesions and koilocytosis. Objectives: To screen the cervical smears for HPV infection and calculate its proportion in the sample. Methods: Observational, Cross sectional study (n=200) of Pap smears sent to the Department of Pathology, from outpatients of Department of Gynaecology of a tertiary care hospital in a rural area. Results: 18 out of 200 (9%) of the samples were positive for HPV infection, while the others were associated with benign changes, such as inflammation, bacterial vaginosis, fungal infection such as candida, LSIL and HSIL. Conclusions: Pap testing is an excellent tool for detection of HPV infection. It is economic and easily accessible to most of the population. The high prevalence of HPV in the population signals the need for awareness and regular screening to aid in early intervention and subsequent prevention of cervical malignancy.

Keywords: HPV infection, Pap smear, Screening test.

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Introduction

Worldwide, HPV is estimated to infect about 12% of women at any given time [1]. HPV infection is the most frequently sexually transmitted disease in the world [2]. Risk factors for persistent genital HPV infections include early age of first sexual intercourse, multiple partners, smoking, and immunosuppression [3]. Genital HPV is typically spread by sustained direct skin-to-skin contact, with vaginal and anal sex being the most common method though transmission from oral sex can occur.

The peak prevalence of HPV infection in women occurs in their early 20s [4]. After women reach 20 years of age, the prevalence steadily declines, although a smaller second peak occurs in postmenopausal women in some geographic areas. The overall prevalence of high risk HPV infection decreases sharply with age [5]. The lower prevalence of HPV infection in older women compared to younger women is found to be independent of sexual behaviour. An explanation for the decline in HPV prevalence with age, even after adjustment for sexual behavior, may be the presence of increased immunity to HPV in some older women [6].

The relationship between cervical cancer and human papillomavirus (HPV) infection has been well established. HPV infection may be latent, subclinical, or clinical. The disease may manifest as genital warts or as low- or high-grade intraepithelial lesions [7]. Although cervical cancer is the primary concern in women with persistent HPV infection, other rarer HPV-induced cancers include those of the vagina, vulva, anus, and penis [8]. Sexually transmitted HPV is divided into 2 categories: low-risk and high-risk. Low-risk HPVs cause warts on or around the genitals. Type 6 and 11 cause 90% of all genital warts and recurrent respiratory papillomatosis that causes benign tumors in the air passages. Type 16 and 18 are two that are responsible for causing most of HPV-caused cancers. These high-risk HPVs cause 5% of the cancers in the world. In 2012, about 528,000 new cases and 266,000 deaths occurred from cervical cancer worldwide. Around 85% of these occurred in the developing world.
In developing countries, there is lack of effective screening programs for cervical cancer. In most cases cervical cancer can be prevented through early detection and treatment of abnormal cell changes that occur in the cervix years before cervical cancer develops. The traditional test for early detection has been the Pap test. The pap test is widely accepted due to its non-invasive nature, cost-effectiveness, simplicity and moderately high sensitivity. It is a primary method of HPV detection especially in regions with relatively low access to technological advancements.

A long recognized, pathognomic feature of HPV infection, is the appearance of koilocytic cells in the differentiated layers of squamous epithelium in the cervical smear. Koilocytes are superficial or intermediate squamous cells with large and irregular, well defined perinuclear halos with a cookie cutter border and cytoplasmic thickening; nuclei are enlarged (two to three times normal size) with undulating (raisin-like) nuclear membrane and rope like chromatin. Often bi or multinucleated with variation in nuclear size.

The American Cancer Society recommends that women should start screening with the Pap test at age 21; starting at age 30, women have three options available for screening: 1. A Pap test alone every three years, 2. Co-testing with a Pap and HPV test, every five years, 3. An HPV test alone, every five years. After age 65, women older than 65 who have had adequate prior screening and are not otherwise at high risk can stop screening. Women who have had a hysterectomy (with removal of the cervix) also do not need to be screened, unless they have a have a history of a high-grade precancerous lesions [9].

**REVIEW OF LITERATURE**

Most studies that pertain to calculating the prevalence of HPV in a population concluded that HPV prevalence and genotype distribution in women with or without clinical findings is an important predictor of cervical cancer.

A Turkish study aimed to identify HPV prevalence and genotype distribution in women with or without cervical lesions admitted to Gynaecology and Obstetrics Clinics of one of the largest private hospitals in Istanbul between 2013 and 2017. Results were evaluated based on the HPV positivity in different cytological findings, and ages. The results showed that 649 out of 2464 patients (26.3%) were positive, and 1815 (73.7%) were negative for the presence of HPV [10].

A Chinese study in which women in Sichuan older than 20 years were screened for cervical cancer between January 2015 and December 2016 using liquid-based cytology testing and a flow cytometry-fluorescence hybridization test for HPV-DNA. Of the 17,319 women aged 20-85 years who participated in the study, Overall prevalence of HR-HPV infection was 12.6% [11].

Another Chinese study conducted between February 2013 and May 2016 explored the prevalence and genotype distribution of HPV infection among Han, Yi, and Bai women in various regions of Dali Bai Autonomous Prefecture, Yunnan Province, China. This cross-sectional study included 2779 women (20-76 years old). Overall HPV prevalence in the study population was 7.6% and HPV-16 were the most prevalent genotypes in the study area [12].

In a study carried out in suburban region of Trivandrum in 2000, prevalence of all types of HPV was studied and the testing employed PCR. The results revealed a steady frequency of infection across all age groups with an average of 6.1% with an increased incidence (7.3%) observed in the population of women belonging to the lower economic status [13].

A 2018 study on cervical cancer screening in asymptomatic women, 1100 women in the age group of 21–65 years underwent Pap testing, at a tertiary care hospital in an urban area of Mumbai, India. Overall sensitivity and specificity for the detection of LSIL were 75.8% and 94.6% and those for the detection of HSIL were 68.9% and 98.6%. And hence, it was concluded that Pap smear test is a very easy, noninvasive, useful, simple, safe, and very economical tool to detect preinvasive cervical epithelial lesions [14].

**OBJECTIVES**

- To screen the cervical smears for HPV infection.
- To calculate its proportion in reproductive age group of females

**MATERIAL AND METHODS**

The present analysis is an observational, cross-sectional study, conducted in the Pathology department of a tertiary care hospital in a rural area, between May 2017 and June 2018. The study was undertaken after the approval from the institutional ethical committee.

A sample of 200 women (n=200), all outpatients of the Gynaecology department, was selected to perform HPV detection by Pap testing. The study included all women between 21 to 50 years old.

For Pap testing, a speculum is inserted into the vaginal area, a small brush or swab is inserted into the opening of the cervix and twirled around to collect a sample of cells. The samples are placed in a solution from which cells are isolated and used to prepare slides for laboratory evaluation.
The fixed smears are taken and hydrated through 80%, 70%, 50% alcohol to distilled water. They are then placed in Harris’ Hematoxylin solution for 4 minutes and then washed in running tap water. The smears are then dehydrated with 95% ethyl alcohol and then stained with Orange 6 solution for 1-4 minutes, followed by a staining with Eosin A for 2-4 minutes. The slide is rinsed in 3 jars containing 95% alcohol, dehydrated and mounted.

Papanicolaou stain includes both acidic and basic dyes. Acidic dye stains the basic components of the cell and basic dye stain the acidic components of the cell. The polychromatic PAP stain involves five dyes in three solutions.

- Hematoxylin: stains cell nuclei blue.
- Orange Green 6: This is the first acidic cytoplasmic counterstain which stains matured and keratinized cells.
- Eosin Azure: This is the second counterstain.
- Eosin Y gives a pink colour to cytoplasm of mature squamous cells, nucleoli, cilia and red blood cells.
- Light green SF stains blue to cytoplasm of metabolically active cells like parabasal squamous cells, intermediate squamous cells and columnar cells.

The analysis was carried out in the Pathology department of the tertiary care hospital. The number of cases positive for HPV infection was noted and their total fraction was calculated and expressed in the form of percentages.

**Observations**

**Table-1: Age wise distribution of patients**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age Group</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21-30</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>31-40</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>41-50</td>
<td>89</td>
</tr>
</tbody>
</table>

**Table-2: Distribution according to clinical diagnosis**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Clinical Diagnosis</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Menstrual Irregularities</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Cervical Polyps</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>Abnormal Uterine Bleeding</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Abdominal Pain</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Routine Screening</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>Others</td>
<td>27</td>
</tr>
</tbody>
</table>

**Table-3: Distribution according to pathological diagnosis**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pathological Diagnosis</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inflammatory changes</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>Bacterial vaginosis</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Fungal infection</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>LSIL</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>HSIL</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>HPV Infection</td>
<td>18</td>
</tr>
</tbody>
</table>

**Table-4: Age wise distribution of HPV positive patients**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age Group</th>
<th>HPV positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21-30</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>31-40</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>41-50</td>
<td>10</td>
</tr>
</tbody>
</table>
RESULTS

44.5% of the patients in the sample were from the age group of 41-50 years, followed by those in the age group of 31-40 years which constituted 39% of the population; while only 16.5% of the patients belonged to the age group of 21-30 years.

A vast majority, about 29.5% of patients in the sample visited the hospital for routine screening, while 17% patients presented with cervical polyps upon clinical examination. Patients with menstrual irregularities and abnormal uterine bleeding, each comprise 15% of the sample, while those with abdominal pain formed 10%.

Upon cytological examination, 65% of all the patients in the sample only had inflammatory changes without any associated complications. 13.5% of the samples revealed low grade squamous intraepithelial lesions and only 2% showed high grade squamous intraepithelial lesions. Bacterial vaginosis and fungal infections, were detected in 5 and 5.5% of the samples respectively. 9% of the samples showed koilocytic changes, i.e., positivity for HPV infection.

In those samples showing positivity for HPV infection, 55.55% belonged to patients of age group 41-50 years, 27.78% were from patients of age group 31-40 years and 16.67% from the age group of 21-30 years.

In all the HPV positive cases, 22.22% of patients presented with an abnormal uterine bleeding, cervical polyps and abdominal pain, each, on clinical examination. 16.67% of HPV positive patients complained of menstrual irregularities while the remaining, came for routine screening and other complaints.

DISCUSSION

A pap smear is the method of choice for screening of cervical cancer. As it relies on microscopic evaluation of cytology, its sensitivity as well as specificity is expected to be lower than the far more advanced PCR based methods.

A broad comparison of similar studies is evident of the fact that HPV infection has a significant prevalence within the general population. An Indian study dated 2000, based at suburban regions of Trivandrum, Kerela, revealed the overall HPV prevalence at 6.1%. The prevalence was almost steady across the age groups [13]. However, in the present study, prevalence not only differs numerically, but also discloses a steady raise with increasing age.

The majority of studies that concern themselves with assessing the fraction of HPV infection in the population, try to provide a basis for the development of a cervical cancer screening strategy and vaccine implementation. The results of meta-analysis of a Chinese study carried out between January 2000 and June 2018, showed that the overall infection rate of high-risk HPVs in mainland Chinese women was 19.0% [15]. And hence, the epidemiology of high-risk HPVs in mainland Chinese women is basically consistent with that for the rest of the world.

A recent pilot study in Romania aimed to estimate the prevalence of abnormal cytology and positive high-risk human papillomavirus (hr-HPV) test. The population prevalence of positive screening results was found out to be 12% [16]. The prevalence of abnormal cytology increased with increasing age, whereas the prevalence of positive hr-HPV test showed a bimodal age pattern; as opposed to the present study wherein, prevalence of HPV is shown to increase with age.
Finally, a study showing near equivalence with the aforementioned Romanian research was another China based study, carried out in the Nanhai area of Foshan. Screening for genotypes of HPV was carried out using rapid flow-through hybridization of nucleic acid molecules and the resultant overall prevalence of HPV was found out to be 13.5% [17].

The differences in results pertain to the differences in the method of testing employed. Usage of liquid based cytology, PCR based testing, significantly raises sensitivity to the detection of HPV when compared to Pap testing.

CONCLUSION

On an average, more than one tenth of women all over the world are found to have HPV infection. Its relationship with cervical cancer makes the study of its prevalence a point of interest. There are various methods for screening women for HPV infection, ranging from simple methods such as visual inspection, Pap testing, to the more advanced DNA PCR, Immunohistochemistry and ELISA. Owing to the low cost, simplicity as well as great accessibility to the susceptible population, Pap testing is the method of choice for preliminary screening of women of all age groups.

Pap testing is based upon the study of altered cell morphology of squamocolumnar cells of cervix, which are collected non invasively, by gentle scraping with a spatula and are stained. The characteristic finding in an HPV infected patient is the presence of koilocytic atypia (i.e. nuclear enlargement, hyperchromasia and a perinuclear halo) in the pap smear. In most cases, koilocytosis is followed by cervical intraepithelial neoplasia, which if left untreated, can progress to malignancy.

The implication of this study is in early detection and intervention in positive cases to prevent cervical cancer and the morbidity associated with it. Furthermore, it intends to create awareness about the Human Papillomavirus and provides a basic idea of its prevalence within the general female population and can be helpful to gynaecologists and physicians for advising their patients and to steer them towards regular screening.

REFERENCES


