**Study of epidemiology of HPV infection in the Uterine Cervix of Women’s in Delhi /NCR regions, India**

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**Abstract**

One of the most common cancers among Indian women is the cancer of cervix. Hence, the Present study is undertaken to determine the prevalence of HR-HPV DNA in women in Delhi / NCR regions. The significance of detection of HPV provides the base to be used as a tool to identify women, at the risk of subsequent development of cervical cancer. It is the utmost necessity to identify the prevalence of high risk- Human Papilloma Virus (HR-HPV) in the women with cervical cytology for early treatment. Total of 1931 samples (cervical samples) from different hospitals in Delhi / NCR regions of India were collected in between January 2006 to December 2009. The cervical cytobrush was used for collection of samples from cervix and then the samples were transported in virus transport media (Digene Diag, Md). Hybrid capture assay II (HCA II) for HPV DNA detection from Digene Diagnostics (Silver Spring, Md.) was used for the detection of High risk Human Papilloma Virus. High risk Human Papilloma Virus was detected in 232 cases (12.01%). As analyzed it was observed that positivity rate has increased for last few years, 9.49 %, 11.66 %, 11.94 %, 14.7 %, in the year 2006, 2007, 2008, and 2009 respectively.

**Key words:**

Hybrid Capture Assay, Human Papilloma Virus, retinoblastoma.

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**INTRODUCTION**
Cancer of the cervix is the second most common cancer among women worldwide [1]. In India it is the major cause of cancer in women and a leading cause of deaths due to cancer. Every year, more than 130,000 new cases and about 70,000 deaths were recorded. The persistent infection by specific high-risk human papillomaviruses (HR-HPVs) is essential for the progression of cervical lesion and women who are infected with HR-HPVs are likely to develop cancer. Cancerous HPV types are associated with cervical cancer, and non-cancerous HPV types are associated with warts of the genital areas and low grade disease of the cervix [2]. Various studies have demonstrated that more than 98% of invasive cervical cancers harbor HPV type 16 (HPV-16) and HPV-18 [3]. The human papillomaviruses and DNA viruses infect the epithelial cells. More than 100 genotypes within the family of HPV are known which vary in their tissue tropism and oncogenic potential. HPV types are defined on the basis of homology of the viral genome [4]. Different types have more than 10% difference in their DNA sequence, including the L1 gene. HPV subtypes 16 and 18 introduce the gene E6 and E7 which codes for proteins that inhibit p53 and retinoblastoma protein (Rb) which are two important tumor suppressor genes in humans. The p53 gene product is involved in regulation of apoptosis (cell suicide), and Rb is responsible for halting the cell cycle at the G1 phase. When the Rb function is impaired the cell is allowed to progress to S phase and complete mitosis, resulting in proliferation and hence neoplastic transformation.

The risk of acquiring anogenital human papillomavirus (HPV) infection is associated mainly with early sexual experience, number of lifetime sexual partners, and sexual contact with highly promiscuous partners [4, 5]. Persistent infection with HPV has been identified as the most important cause of cervical cancer [6]. Supervision by cytological observation has raised worry that some cases of high grade disease might escape finding because of absence or inadequate understanding and that women might practice anxiety over a prolonged period [7].

Diagnosis of cervical disease, indicating the presence of abnormal cervical epithelial cells, is usually obtained by colposcopic examination by panningulatio stained smears [8]. This has been the method of choice since the 1950’s, proving valuable for mass screenings and enabling detecting lesion early enough to be treated effectively treated. The Pap smear, however, has limited sensitivity detecting cancer precursors, giving a false – negative rate ranging from 20 to 30 %. Abiding concerns with Pap smear based screening, counting the deprived reproducibility of outcome, inadequate sensitivity and, as a result, necessitate for screening on a comparatively common source in order to accomplish satisfactory program sensitivity [9]. Hence, complementary methods that will provide the improvement of cervical disease diagnosis have been studied for the past two decades. Recently develop, the generation of hybrid capture assay HCA II) for HPV DNA detection from Digene diagnostics (silver Spring, Md) is a nonradioactive, relatively rapid, liquid hybridization assay designed to detect eighteen HPV types compared with other available HPV test kits, the hybrid capture test is also designed to provide quantitative estimates of viral load, which may correlate with the grade and natural history of cervical pathology.

**MATERIAL AND METHODS**

**Chemicals and Reagents**

All materials and chemicals used in the study were of analytical reagent grade and of highest quality available, and are purchased from reliable firms and institutes (SRL, MERCK, RANBAXY, HIMEDIA, SIGMA and SUYOG).

**Sample Collection**
Total of 1931 samples were collected from women’s at different hospitals in Delhi / NCR region, India, from January 2006 to December, 2009. Women were referred to hybrid capture, after clinical suspicion of HPV infection during a routine exam. The cervical smears were collected using a cervical cytobrush and transported in specimen transport media (Digene Diag, Md).

Detection or testing of Human Papillomavirus

The assay kit detects high-risk HPV genomes. The high-risk types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. All the, specimens were treated with denaturing solution containing NaOH to denature the ds circular DNA of Human Papilloma Virus. The liberated single stranded DNA was hybridized in solution with a RNA probe cocktail for thirteen high risk groups of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Each reaction mixture, containing any number of RNA: DNA hybrids that formed, was transferred to a microtitre plate containing capture tube coated with anti RNA: DNA hybrid antibodies; consequently, immobilizing them. Nonreactive material was removed by washing, and a dioxetane – based chemiluminescent compound, Lumi–phos 530, was added as a substrate for alkaline phosphatase. The light produced by the ensuing reaction was measured by Luminometer. Light measurements were expressed as Relative light units (RLU). As a negative control, sonicated herring sperm DNA in Digene transport media (100 mg/ml) was used. Triplicate specimens of HPV 16 or HPV 11 DNA at 10 pg / ml served as a positive control for high – risk probes. All RLU measurements for specimens were divided by the mean RLU of the three appropriate positive controls (PCs) to give a ratio of specimen RLU/PC. A ratio of 1.0 was regarded as negative Since the amount of light produced by the hybrid capture assay is theoretically proportional to the amount of target HPV DNA, HCA II can be analyzed as a quantitative method.

RESULTS

The results shows age specific HR-HPV prevalence were highest in women of 15-25 year group and decreased in 26-35 year and 36-45 year age groups. The increased prevalence was observed in older age group i.e. more than 46 years. These results are clearly depicted in table 1.

Table 1: Table depicting age wise distribution of Positivity Rate in percentage for HR-HPV DNA in females

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Age distribution</th>
<th>Total number of samples (HR-HPV DNA)</th>
<th>Samples found Positive for HR-HPV DNA</th>
<th>Positivity Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15-25</td>
<td>86</td>
<td>16</td>
<td>18.6</td>
</tr>
<tr>
<td>2</td>
<td>26-35</td>
<td>815</td>
<td>99</td>
<td>12.2</td>
</tr>
<tr>
<td>3</td>
<td>36-45</td>
<td>652</td>
<td>66</td>
<td>10.1</td>
</tr>
<tr>
<td>4</td>
<td>46-55</td>
<td>268</td>
<td>37</td>
<td>13.8</td>
</tr>
<tr>
<td>5</td>
<td>56-65</td>
<td>82</td>
<td>11</td>
<td>13.42</td>
</tr>
<tr>
<td>6</td>
<td>66-75</td>
<td>28</td>
<td>3</td>
<td>10.72</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1931</strong></td>
<td><strong>232</strong></td>
<td></td>
<td><strong>12.01</strong></td>
</tr>
</tbody>
</table>

In the study, HR HPV DNA was detected in 232 (12.01%). This shows that positivity is increasing in recent years, in 2006 (9.49%), in 2007 (11.66%), in 2008 (11.94%), and in 2009 (14.7%). Fig 1 depicts a comparison between total no. of samples (HR-HPV DNA), samples found positive for HR-HPV DNA and Positivity Rate (%) for HR-HPV DNA in females.
DISCUSSION

Two different observations were clear from above results. First, the incidence of HPV infection is increasing every year. Second, there is clear age group related infection. HPV infection is increasing every year which can said that sexual uninhibited sexual encounters are increasing which are resulting in the increased incidence of HPV infection. This is also evident from the second observation that it is very age specific as incidence at young age is far greater than middle and old ages. The age group with most incidence of HPV infection is 15 to 25 years. This is the age which is most sexually active. Then we see a gradual decrease in incidence of HPV infection up to the age of 45 years. The number of infection is increasing due to many factors and most prominent is of ignorance. Even though the number of educated population is increasing day by day in country like India but the awareness level of HPV is nonexistent. People are aware of many sexually transmitted diseases like AIDS of which there are various programmes promoted by different agencies around the country. But there are no coherent programmes to spread awareness about HPV which leave women totally unaware of this infection. As a result there is no timely diagnosis of the infection which become persistence and develops in cancer. As it is evident that early detection can greatly reduce death by cervical cancer as it can be treated if infection is found in early stages. So a coherent policy about the spread of awareness about HPV infection is absolutely necessary which can stop the spread the cervical cancer by detecting the infection at early stage help physicians better patient management.

CONCLUSION

Our results support that HR-HPV infection mainly associated with early sexual experience, for high prevalence at older age indicated to multiple sexual partner or virus express symptoms at this age group. In India many findings from rural areas of southern parts showed very high prevalence than our findings. Our subjects were from urban area, as they take prevention and get vaccine. So, our suggestions are to vaccinate the women for HR – HPV at the age of 10-12 year. The development of a reliable, accurate and cost effective HPV test method is needed in order to move HPV testing into routine clinical practice. The hybrid capture test has good reliability and accuracy, although room for improvement remains. Available techniques are very costly that’s why rural women in developing nation like India cannot screen for it and they spread the virus in community.

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References