RESEARCH ARTICLE

HUMAN PAPILLOMA VIRUS IN CERVICAL NEOPLASIA MANIPAL UNIVERSITY

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ABSTRACT

The prospective study was conducted on women in a tertiary care centre to determine the incidence of Human papilloma virus genome in women with cervical neoplasia and to correlate the available risk factors for HPV infection and cervical cancer. Cervical smears were taken and graded according to Bethesda system. Women who had Pap smear suggestive of CIN either underwent colposcopy and guided biopsy or 4 quadrant biopsy. Women who had a frank growth underwent cervical biopsy. A cervical swab was also taken from the ectocervix and endocervix which were kept in a sterile container and stored at -4°C. Patients with histological confirmation of CIN (Cervical intraepithelial neoplasia) / ICC (Invasive cervical cancer) were taken as cases and subjected to HPV DNA study. Incidence of HPV positivity among cases was 38.23% with an odds ratio of 21.66. All HPV positive women were infected with a single virus. The most common virus identified was HPV 16 with incidence of 32.35%. High parity, low economic status and illiteracy were a significant risk factor in causing infection & cervical neoplasm.

INTRODUCTION

Cancer of the uterine cervix is the second-most common malignant tumour in the world, but is the number one cancer in Indian women, posing a major public health problem. In India, about 100,000 women develop this cancer every year, constituting about 16% of the world’s annual incidence. For over a century, it was believed that cervical cancer is associated with ‘sexual behaviour’, indicating involvement of a sexually transmissible infectious agent. However, it took more than a decade before the causal role of specific types of HPVs in cancer of the cervix and their precursorlesions was accepted. Human papillomaviruses (HPVs) have emerged as the principal sexually transmitted causal agents in the development of cancer of the uterine cervix in women. Presently, more than 100 HPV genotypes have been identified in humans, and about one-third of them have been sequenced. Of these, while HPV types 16 and 18 are considered to be the high-risk types, HPV 6 and 11 are the low – risk types in the development of cervical cancer. Evidence for causal role of HPV in the development of cervical neoplasiascomes from the etiological and epidemiological observations together with the experimental findings of the molecular pathways elicited by HPV-transforming genes. Further evidence in favour of papillomavirus as the carcinoma virus comes from the findings of presence of HPV infections in cancers of oral, oesophageal, larynx and non-melanoma skin cancers. The oncogenic potentials of the virus have been attributed to its E6 and E7 genes. The products of these two genes stimulate cell proliferation by activating the cell – cyclespecific proteins and interfere with the functions of cellular growth – regulatory proteins, p53 and Rb. Identification and characterization of several human pathogenic HPV types warrant prevention of viral infection through vaccination or therapeutic intervention which could eventually control infection and expression of human pathogenic papillomaviruses.

MATERIALS AND METHODS

Location and duration of study

The study was conducted on women in Government Lady Goschen Hospital, Mangalore and KMC Attavar Hospital Mangalore from January 2005 to September 2006.

Experimental design

The study conducted was a hospital based prospective study in which a total number of 176 women were screened. The study population included women who presented to the outpatient department with complaints of discharge per vagina, abnormal bleeding per vagina or cachexia. Patients also went through thorough physical & pelvic examination. The index test was based on the health of the cervix. 75 women were found to have a normal looking cervix & 101 women with frank growth of the cervix. The women with normal looking cervices underwent a cervical smear with Ayer’s spatula which was fixed with 95% alcohol and ether. The smears were graded

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according to Bethesda system. 20 smears were reported to be suggestive of CIN and 55 cases were found to have normal Pap smear. The 20 women who had pap smear suggestive of CIN either underwent colposcopy & guided biopsy or a 4 quadrant biopsy. 15 women had a histological confirmation of CIN and were taken into cases group. The 101 women who had a frank growth underwent cervical biopsy, and 33 women had histological confirmation of invasive carcinoma which were included into the cases. Out of which 13 women declined to continue in the study leaving the number of cases to be 40.

Hence among the 40 cases, 15 cases were of cervical intraepithelial neoplasia and 25 cases were of Invasive carcinoma of the cervix.

Each participant also was administered a standardized questionnaire on socio economic status, sexual behaviour, reproductive history, contraceptive practices, history of STD, demographic profile etc. The questionnaire was interpreted into the local language by the observer when required.

Then a cervical swab was taken from the ectocervix and endocervix & kept in a sterile container. This was later stored at -4°C & then samples were shifted for testing to Reliance Life Sciences at Mumbai where they were processed & tested.

In the ICC group when a biopsy specimen was procured, it was kept in a sterile jar containing distilled water & stored in the refrigerator at -4°C & later shifted to Reliance Life Sciences by cold transport.

Inclusion criteria

1. Histologic confirmation of cervical neoplasia (CIN/ICC)
2. No prior treatment for cancer

Detection of HPV DNA

The samples were analysed by Reliance LifeScience lab situated in Mumbai for adequacy of DNA & inadequate samples were excluded from the study. 6 samples for cases (1 for CIN and 5 for ICC) were considered inadequate for DNA mostly due to necrosis. Hence only 34 cases were available for DNA analysis.

The presence of HPV DNA in cervical cells was assessed by polymerase chain reaction using general primer MY 09/11 which was directed towards L1 region of HPV genome.

Statistical Analysis

Done by Chi square test and Fisher exact test. p - value of ≤0.05 was considered significant.

RESULTS

A total of 40 cases were included of which 15 cases were of cervical intraepithelial neoplasia (CIN) and 25 cases of Invasive carcinoma of the cervix (ICC). From our study 6 samples for cases (1 for CIN and 5 for ICC) were considered inadequate for DNA mostly due to necrosis and hence were omitted. So we finally had 34 cases (14 CIN and 20 ICC).

The CIN group had 9 cases of CIN 2 and 5 cases of CIN 3. The ICC group had 19 large cell non keratinizing squamous cell carcinoma and 1 case of large cell non keratinizing carcinoma of which, there were 3 cases of stage Ia, 1 case of stage IIa, 4 cases of stage IIb, and 12 cases of stage IIIb.

Table 1 Stages Of Invasive Carcinoma Cervix

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Ib</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IIa</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>IIb</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>IIIa</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IIIb</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

From the 34 cases, 13 cases were positive for HPV DNA making the incidence of HPV to be 38.23% with an odds ratio of 21.66 for our study. 2/14 (14.2%) were positive for CIN and 11/20 (55%) were positive for ICC.

Table 2 HPV Positivity among cases

<table>
<thead>
<tr>
<th></th>
<th>CIN</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV+</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>HPV-</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

The most common virus type was HPV 16 with incidence of 32.35% (11/34 cases). HPV 18 which is also one of the commonest high risk HPV types was not identified amongst women in our study. The other HPV type was HPV 56 in one case. In all cases only single viral genome was identified. No multiple infections were seen.

Table 3 HPV genotypes

<table>
<thead>
<tr>
<th></th>
<th>CIN</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 16</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>HPV 56</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HPV 58</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Women who were HPV positive were of age 31-40 in CIN and 41-70 in ICC group. 11 of these women were multiparous with parity more than 3. 70% of the women were illiterate and were from low socio-economic status (8/13). All of these women had first intercourse before the age of 18 years and 9/13 had their first pregnancy before 18 years. None of these women used any barrier contraception.

DISCUSSION

Worldwide, HPV 16 is the commonest type followed by HPV 18. Geographical variations in non – HPV 16 distributions have been noted. In Asian countries, the predominant HPV types after 16 and 18 were 58 and 53. HPV 18 was found more commonly. Several Indian studies have looked at the distribution of HPV types in cervical neoplasia. Though the majority of studies have used polymerase chain reaction, techniques like In situ hybridization and Southern Blot have also been used. Although considered the gold standard for HPV
diagnosis. Southern Blot is laborious to perform and specific probes against certain types allow for the detection of only these types.

In our study (38.23%) was much lower than other studies done in Mangalore, which is in neighboring district to Mangalore.

The most commonest virus type identified in our study was HPV 16 with incidence of 32.35%. HPV 18 which is one of the commonest high risk HPV types was not identified amongst women in our study. The types found in the cases are consistent with the type distribution shown in cervical cancer worldwide. 16,16 In relation to HPV type, the association of HPV DNA in premalignant and malignant conditions of the cervix. In our study all HPV positive women were infected with a single virus. No multiple viral genomes were identified. The most common virus type identified in our study was HPV 16. HPV 18 which is considered one of the commonest high risk HPV types was not identified.

In our study incidence of illiterate and low economic status women who were positive for HPV genome was high. Mostly due to lack of awareness and unhygienic habits among these women.

High parity was a significant risk factor for infection & cervical neoplasm.

### Bibliography


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