Is epithelial cell abnormality in cervical samples along with E6/7 mRNA expression of human Papilloma virus is correlated with socioeconomic status

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Introduction
Cervical cancer ranked second among most commonly diagnosed cancer and in less developed countries it is third leading cause of cancer related death among females. Cancer cervix is a multifactorial disease. Major risk factors are age, illiteracy, low socioeconomic status, early menarche, marital status, early marriage, early first childbirth, age at last child birth, multiparity, abortion, multiple sexual partners, late menopause, genital infection, poor genital hygiene, tobacco use, passive smoking and contraceptive use. Other risk factors includes co-infection with Chlamydia trachomatis, non-use of condoms by partners and nutritional factors. Human Papilloma virus (HPV) infection is the most important risk factor. It has been shown recently that cervical cancer is strongly associated with the presence of high risk or oncogenic Human Papilloma virus (HPV) types (up to 100%). It is the expression of viral oncogenes E6 /E7 which is prerequisite for progression toward malignancy and maintenance of the cancerous phenotype. As the severity of the lesion increases levels of E6/E7 also rises.

Aims and Objectives
This study is carried out to correlate impact of socioeconomic status on occurrence of epithelial cell abnormality reported on cervical Pap smear study using 2014 Bethesda System and flow cytometry detection of HPV mRNA expression in present scenario.

Materials and Methods
This study was conducted in Department of Pathology, Mahatma Gandhi Medical College and Maharaja Yeshwant Rao Hospital, Indore, Madhya Pradesh, India. It is a prospective study. The study duration was one year from July 2017 to June 2018 Sample size for the study was of 40 cases.

For all patients, different socioeconomic variables like level of income, level of education, parity and awareness about risk factors were noted. For this study purpose, level of income was divided in low, middle and high based on income, 5000/per month, 5000- 30,0000 per month and >30,000/per
month respectively. Education level was divided in to illiterate and literate based on whether patient has received any formal education or not. All 40 selected women were examined per vaginally and by speculum after acquiring a detailed history and verbal consent from them. The woman was placed in dorsal lithotomy position. After proper positioning of the woman, cervix was viewed by introducing Sims’ vaginal speculum and anterior vaginal retractor and external os was identified. Pap smears were made by introducing cervical brush/ cytobrush with a detachable head were inserted into the external os and rotating it through 360 degrees 8-10 times in clockwise direction near the squamo- columnar junction. The cellular material thus obtained was quickly, but gently smeared on a clean glass slide. The glass slide was then immediately put into the Coplin jar containing 95% ethanol which acted as a fixative. The prepared smears were then stained according to Papanicolaou's technique.

The white head of the cervical brush was detached and put into the Sure Path preservative vial .Vial was then shaken well and stored at room temperature till the samples were processed for the run in flow cytometer.

Results
All 40 Pap smears studied as per the Bethesda system 2014 for reporting of cervical Pap smears. Age specific analysis of data revealed 72.5% (29/40) were from 21-40 yrs age. Abnormalities Negative for intraepithelial lesion or Malignancy (NILM) was reported in 62.5% (25/40) smears. 37.5% (15/40) showed various epithelial abnormalities including ASCUS, ASC-H, Low grade Squamous Intraepithelial Lesion (LSIL) and High Grade Intraepithelial lesion (HSIL) and squamous cell carcinoma.

Table 1 Distribution of cases on Socio-economic status

<table>
<thead>
<tr>
<th>Socioeconomic variable</th>
<th>Cases show epithelial cell abnormality (n=15)</th>
<th>Cases show HPV mRNA expression (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Rural</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>2. Urban</td>
<td>05</td>
<td>03</td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Low</td>
<td>09</td>
<td>13</td>
</tr>
<tr>
<td>2. Middle</td>
<td>05</td>
<td>05</td>
</tr>
<tr>
<td>3. High</td>
<td>01</td>
<td>02</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nulliparous</td>
<td>0</td>
<td>01</td>
</tr>
<tr>
<td>2. Parity &lt;2</td>
<td>0</td>
<td>02</td>
</tr>
<tr>
<td>3. Parity &gt;2</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Illiterate</td>
<td>09</td>
<td>13</td>
</tr>
<tr>
<td>2. Literate</td>
<td>06</td>
<td>07</td>
</tr>
</tbody>
</table>

Discussion
In our study, maximum number of cases were seen in 21-40 years of age. In various other studies done by Sunita et al, Mandakani et al and P Vijaya Lakshmi et al, the maximum age of patients was 31-50 years of age and correlated with progressive rise in incidence of dysplasia and carcinoma with increasing age.

In our study, 100 % of abnormal smears and 85% of HPV mRNA expression were from women with more than 2 children. Misra et al and Goes et al also reported progressive rise in incidence of cervical cancer with increased parity. Multiparity causes repeated cervical trauma causing squamous metaplastic changes which is a strong risk factor for development of cervical cancer. Another possible mechanism is increased endogenous estrogen production during repeated pregnancy causing persistence of HPV infection by synergizing the effect of HPV oncoproteins and down-regulating cell mediated immune response progressing to dysplasia and carcinoma.

In our study, majority of the patients with epithelial cell abnormality 60 % and 65% cases with HPV mRNA expression were from low income category. Poverty has a cascading effect on their lives leading to malnourishment, poor hygiene and sanitation, low immunity, low female literacy and poor access to healthcare facility.
Studies conducted by Misra et al.\textsuperscript{27} and Zhang et al.\textsuperscript{29} and showed strong association between poor genital hygiene and cervical carcinoma. According to one study,\textsuperscript{17} there is 100% increase in incidence of cervical cancer and 60% increase in incidence of dysplasia and carcinoma in situ. Other studies that have found a preponderance of HPV in low socioeconomic groups\textsuperscript{30-32}.

**Conclusion**

Increasing incidence of cervical dysplasia and invasive cervical cancer can be prevented by spreading awareness about various socioeconomic and etiological contributory factors. All these factors causes development of dysplasia and progression to cervical cancer. In our study, cases with rural background with Low income, high parity and illiterate had higher number of abnormal smears along with high positive expression of HPV mRNA E6/7 which is most important risk factor for cervical carcinogenesis. This stresses the need for more social awareness specific to cervical cancer, literacy, provision of safe and adequate water for hygiene and effective family planning. Stressing the need for Improvement in these factors, especially in rural areas can go a long way in reducing burden of cervical cancer in developing countries.

**References**

27. Mishra JS, Srivastava S, Singh U, Srivastava AN. Risk factors and strategies
for control of Carcinoma Cervix: Hospital based cytological screening experience of 35 years. Indian Journal of Cancer 2009;46(2).


