Cervical cancer and human papillomavirus among slum dwellers in India

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ABSTRACT

Almost half a million new cases of cervical cancer are diagnosed each year worldwide. Human papillomavirus is recognised as one of the leading causes and is associated with 90% of cases. However, other risk factors (e.g., age of first sexual contact, number of sexual partners, multiparity, diet, genetic predisposition and environment) are also associated with cervical cancer. The present retrospective study is performed on a cohort of women from the slums of a major Indian city. The patients are aged between 38 and 68 years (mean: 49.3 years) and are multiparous (mean number of children: 3.4). In this group, 61% have a history of miscarriages. Histological sections from cone biopsy are tested for the presence of high-grade human papillomavirus (HPV) using GP5+/GP6+ and MY09/MY11 primers and a set of β-globin primers. Only 33% of the cancer patients studied were positive for high-grade HPV DNA, suggesting that predisposition to cervical cancer in this cohort is not highly associated with HPV, and that other risk factors may increase the risk of cervical cancer.

KEY WORDS: Cervical intraepithelial neoplasia.
Cervical neoplasms.
Human papillomavirus.
Parity.

Introduction

Cervical cancer is the second most common cancer among women worldwide,[1] especially in developing countries,[2] with half a million new cases reported each year. The progression of cervical cancer is a multi-step process. Initially, normal cells undergo precancerous changes and ultimately develop into cancer cells. These precancerous conditions include cervical intraepithelial neoplasia (CIN), squamous intraepithelial lesion (SIL) and dysplasia.

The development of cancer from precancerous conditions, which are largely symptomless, is a continuous process that may take several years. There are two main types of cervical cancer: squamous cell carcinoma and adenocarcinoma. A mixture of types is called adenosquamous carcinoma or mixed carcinoma.[3]

Baseman and Koutsky[4] pointed out that a number of researchers thought differently about the progression of cervical cancer, taking the view that low- and high-grade cervical lesions are distinct human papillomavirus (HPV) infectious processes.

In low-grade squamous intraepithelial lesions there is transient manifestation of productive viral infection, during which the HPV-infected epithelium undergoes differentiation and maturation, producing few abnormal cells.

In high-grade squamous intraepithelial lesions, HPV infection of replicating immature cells prevents epithelial maturation and differentiation, leading to continued replication of immature cells and the accumulation of genetic abnormalities, leading to a clone of cancer cells.

Cervical cancer is more common in sexually active women aged over 40. Symptoms include vaginal discharges, light bleeding between menstrual periods, painful intercourse and bleeding thereafter.

In addition to HPV, several other risk factors have been associated with cervical cancer. These include smoking, sexual intercourse at an early age, intercourse with uncircumcised males, multiple partners, diet, use of oral contraceptives, multiple pregnancies, poverty and a family history of cervical cancer.[5]

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Cervical cancer is most common in developing countries. In 1990 it was estimated that almost 61,000 (20%) cancer-associated deaths in females were due to cervical cancer. This figure is projected to increase to 79,000 by 2010.

Each year, 500,000 cervical cancer cases are diagnosed worldwide, of which 100,000 are in India. Diagnosis and treatment is imperative and should be available easily and effectively. In almost 100 villages in India, screening programmes using Papanicolaou (Pap) smear examination are running.[6]

However, cytological screening is not organised properly. The testing is of poor quality and is carried out by unqualified personnel; thus, it has little impact on the problem, despite the large numbers of cytology smears taken. Against this background, India has limited healthcare resources and funds; hence, cervical screening cannot always be afforded.[7]

The most important factor responsible for cervical cancer is HPV, which is a small DNA virus containing double-stranded DNA in a circular genome. This virus infects mucosa and skin.

Many women have HPV infections but not all develop into cervical cancer. One possible reason is that HPV infection alone is not responsible for cervical cancer, but that environmental factors also predispose to cancer

development. Moreover, if viral DNA does not integrated in the host genome and is only present in episomal form then it does not cause cancer.

To date, more than 100 HPV types have been identified. With regard to cervical cancer development, low-risk HPVs include types 6,11,42 and 43, intermediate risk HPVs include types 35, 44, 45, 51, 52 and 58, and high-risk HPVs include types 16, 18, 31 and 33. Types 16 and 18 are mostly found in cervical cancer and are transmitted by sexual contact.[8]

Material and methods

Histological sections were obtained after ethical approval from 17 patients diagnosed with cervical cancer who had undergone surgery at the M. P. Shah Cancer Hospital, Ahmedabad, India.

Participants were chosen on the basis of their socio-economic status. All were living in slums and were regarded as living below the urban poverty line, as defined by the Indian government. In India, urban poverty is defined in terms of minimum calorie intake (2100 calories per capita per day).[9]

All patients studied claimed to be monogamous and only had sexual relations with their husbands. None of the patients smoked or took the contraceptive pill.

Sections were dewaxed and hydrated. The tissue was scraped from each slide with a sterile swab and transferred to a sterile Eppendorf tube containing 1 mL lysis buffer (0.01 mol/L Tris buffer, 0.005 mol/L EDTA and 1% sodium dodecyl sulphate [pH 8.0]) and 20 µL proteinase K (10 mg/mL). Tubes were incubated at 37°C overnight and DNA was extracted using a phenol/chloroform method.[10] The DNA was reconstituted in 20 µL sterile, nuclease-free distilled water.

Table 1. Details of the patients studied.

Patient	Age (years)	Number of children	Number of miscarriages
1	56	3	2
2	67	4	2
3	68	2	1
4	56	4	2
5	48	4	1
6	41	4	–
7	45	3	1
8	38	3	2
9	42	2	–
10	51	5	2
11	45	3	–
12	59	4	–
13	40	2	1
14	42	3	2
15	51	5	–
16	47	3	1
17	46	2	–

Polymerase chain reaction

Human DNA integrity was checked using a set of primers that amplified the β-globin gene, and HPV was detected using two sets of primers (MY09/ MY11 and GP5+/6+). The sequences were as follows: MY11 (positive strand primer) 5′ GCM GGW CAT AAY AAT GG 3′; MY09 (negative strand primer) 5′ CGT CCM ARR GGA WAC TGA TC, where M=A+C, R=A+G, W=A+T, Y=C+T. The GP5+/6+ primer sequences were: GP5+ 5′ TTTGTTACTGTGGTAGATAC 3′; GP6+ 5′ GAAAAATAAACTGTAAATCA 3′

The reaction mix comprised 25 µL Mastermix (Promega, UK), 0.5 µmol/L each forward and reverse primer, 2 µL template DNA and 13 µL nuclease-free water. Thermocycling conditions included initial denaturation at 94°C for 5 min, 40 cycles of denaturing at 94°C, annealing at 55°C, and an extension at 72°C, each for 1 min, followed by a final extension at 72°C for 5 min.

Gel electrophoresis

After PCR amplification, 10 µL amplicons were mixed with 2 µL 6x loading dye (Promega) and loaded on a 1% agarose gel submerged in 1xTBE (Trizma base: 10.8 g boric acid, 5.5 g 0.5 mol/L EDTA [pH 8.0] in 1 L distilled water). Electrophoresis was for 1 h at 100 V. The gel was stained in 0.01% ethidium bromide for 20 min and visualised under an ultraviolet (UV) transilluminator.

Results

The mean age of the patients was 49.3 years. Only one woman in the cohort was unmarried and did not have any children (not shown in table). Average number of children was 3.2. Twelve (67%) women had at least one miscarriage (Table 1).

Figure 1 shows the results using the MY09/11 primers. The expected size of the amplicons was approximately 450 bp. Figure 2 shows the results using the GP5+ and GP6+ primers. The expected size of the amplicons was approximately 150 bp.

Initial PCR results showed that only two samples were positive. On re-amplification of the primary amplicon, the MY09/11 primers gave four positive results, while the GP5+/6+ primers gave six positive results. This suggests that the initial amount of viral material was very low, and re-amplification helped to identify samples with low viral copy number.

Four samples were positive with both MY09/11 and GP5+/6+ primers (i.e., all samples positive with MY09/11 primers were positive with GP5+/6+ primers), but GP5+/6+ primers gave two additional positive results that were not detected with the MY09/11 primers.

In the cohort of patients studied, prevalence of high-grade HPV was 33%.

Discussion

According to 1995 estimates, female life expectancy in India is 59.6 years,[9] India has 500,000 new cases of cancer annually

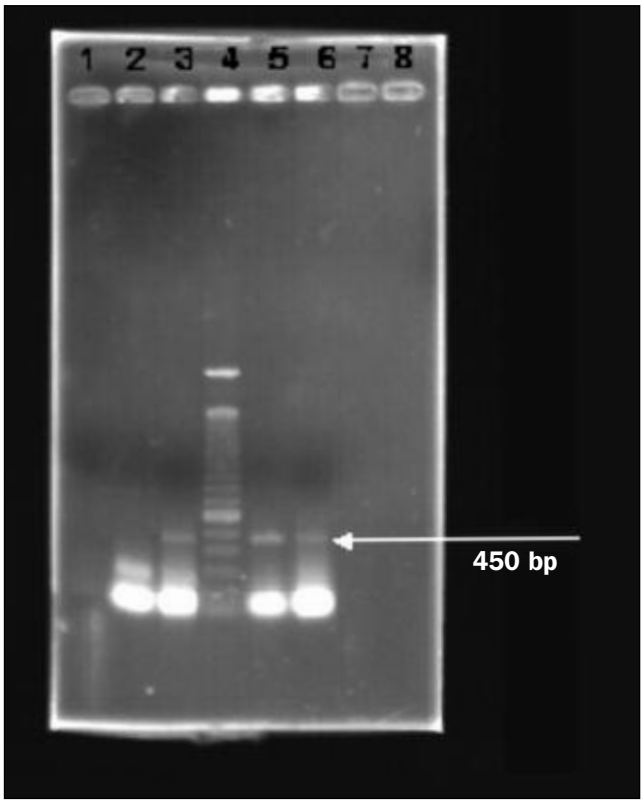

Fig. 1. Amplicons obtained using MY09/11 primers were separated on 1% agarose gel. Lane 1: negative control. Lane 4: 100 bp DNA ladder. Lanes 3,5 and 6: positive results. Lanes 2, 7 and 8: negative results. Expected size of amplicons is approximately 450 bp.

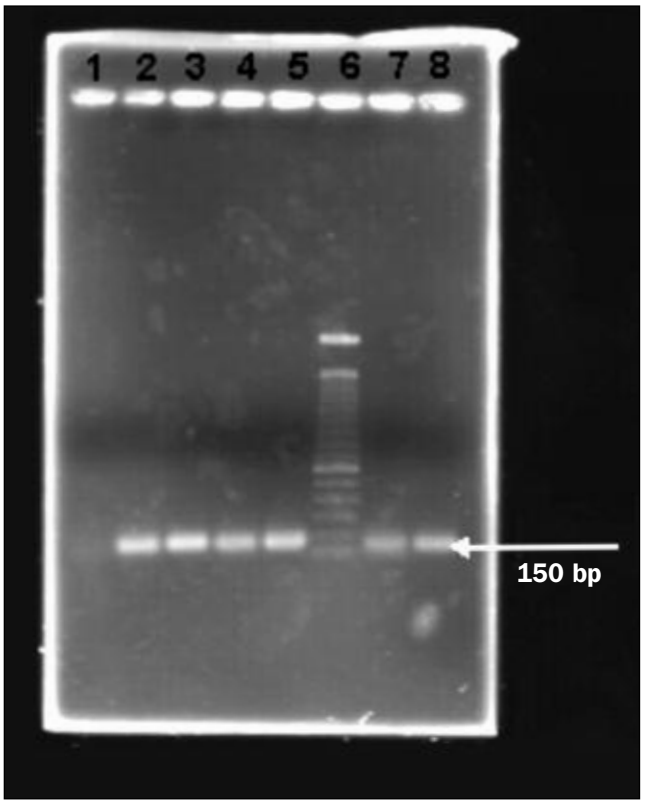

Fig. 2. Amplicons obtained using the GP5+ and GP6+ primers separated on 1% agarose gel. Lane 1: negative control. Lanes 2–5, 7 and 8: positive results. Lane 6: 100 bp DNA ladder. Expected size of amplicon is approximately 150 bp.

and deaths from cancer total around 300,000. The most common malignancies are cancer of the oral cavity (about 35% of all cases relating to tobacco use and pan chewing), cervix, and breast.

Despite the very low standard of living enjoyed by the patients in the present study, family size on average was 3.2 children per household and contraception was not practised. This may be due to religious beliefs or poor access to family planning advice. The rate of miscarriage is also high in this group.

In the present study, prevalence of high-grade HPV was 33%. In a recent study by Franceschi *et al*.[11] in a rural area in the Dindigul district of Tamil Nadu, India, the prevalence of HPV was 21.9%. In the Franceschi study group, 14% of the participants did not have any cervical abnormality. In a study carried out by Sankaranarayanan and colleagues[12] on women aged 30–59 years in Osmanabad, 10.3% tested positive for HPV.

These studies suggest that prevalence of HPV is very low in India. Similar results have been reported by Rughooputh and colleagues,[13] who carried out a retrospective study in Mauritius. In their study, a high-grade HPV prevalence of 19% was found. There may be several explanations for this low rate of positivity, of which poor DNA quality may be one.

In the present study, however, PCR on the β-globin gene was positive for all samples tested. The amount of viral DNA present in the tissue sections may have been low, but re-amplification solved the problem, even if the initial section contained only a single copy of viral DNA.

Clearly, other factors are responsible for the cervical cancer in this cohort. Genetic predisposition to cancer, multiparity, poor diet, living in squalid conditions with poor hygiene facilities, age at first sexual intercourse, for example, all may influence cancer development.

Although the presence of HPV is transient and clinically non-significant in the majority of infections, about 10% of infected patients maintain the infection. In the cohort studied here, 33% who developed high-grade precancerous lesions or cervical cancer were positive for HPV.

Ideally, sexually active women should be encouraged to have regular cervical screening. In an emerging economy (e.g., India), however, this may be of low priority. As a high proportion of women living in slum areas are illiterate, conveying the message about cervical cancer is likely to be difficult and have little impact.

Although a high association between HPV and cervical cancer exists, other factors influence the progression of the disease, More detailed studies of ethnic distribution, lifestyle, diet, genetic predisposition or mutations in cells leading to metastasis of tumours affecting the cervix may help to shed more light on the aetiology of cervical cancer in this population. Better hygiene, diet and education may be the key to the problem, rather than use of expensive interventions such as vaccination.

The cohort size in the present study was small, as it was difficult to obtain samples specifically from slum dwellers. However, the authors hope to expand this study in the future. □

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