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Research Article

Cervical Cancer Screening Program in Hyderabad and Surrounding Peri-urban Areas, South India: Prevalence of High Risk HPV Subtypes

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Abstract

Our work was aimed at (I) identifying women with abnormal cervix upon visual inspection and pap cytology testing (II) determining the presence of HPV infection in the individual, along with sub-typing in severe pathologies and cancer, which is an established risk factor for cervical malignancy reported world-over.

A total of 530 eligible women were screened. Our program witnessed an incremental increase in the number of women accessing screening after counselling for awareness of the importance of testing. 1.8% of our subjects showed the presence of high-risk HPV subtypes; all of them were associated with an abnormal Pap cytology. HPV was shown to be associated with an infectious pap, RCC, ASCUS, HSIL, SCC ($p<0.05$) when compared to normal cytology ($p=0.05$). Awareness and the importance of cervical examination is low. Health camps need to focus on counselling subjects about its benefits to improve their participation and ensure success of screening programs. The significant association of HPV infection with abnormal pathologies ($p=0.05$) and the presence of hr-HPV subtypes other than 16 and 18 draws attention to the need to evaluate the subtypes prevalent in our population and apply this information to cervical vaccination schemes.

Keywords: Cervical; Pap cytology; HPV; High-risk subtypes

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Introduction

Cervical cancer is a potentially preventable gynecological malignancy. It is the second most frequent cancer affecting women with reports of rise in prevalence from different parts of the country. With 5,28,000 new cases every year, cervical cancer is the fourth most common cancer affecting women worldwide (2,66,000 deaths in 2012). Almost 70% of the global burden falls in developing countries; more than one-fifth of all new cases are diagnosed in India (WHO, 2013) [1,2]. The disease consumes resources in the way of medical spending, lost productivity and like with other cancers, disturbing the family structure and dependents [3]. The execution of systematic cytology-based screening programs along with efficient follow-ups can greatly reduce the incidence and mortality of cervical cancer [4].

The conventional method of cervical cancer screening, Papanicolaou test (Pap test-strongly recommended for cervical cancer screening) led to significant reduction in mortality and morbidity in developed countries, where proportion of women who are screened by Pap test vary from 68 to 84 per cent. The initiation and sustenance of cervical cytology programs involving the screening of sexually active women annually, or once in every 2–5 years, have resulted in a large decline in cervical cancer incidence and mortality [5-7]. On the other hand, the screening coverage in Asian countries is low and varies from 50 per cent in Singapore with an existing Cancer Screening Program, to 2.6-5 per cent in India [8, 9]. As part of the screening program conducted way back in 1995 in Maharashtra, Western India, person-to-person and group health education on cervical cancer was provided to 97,000 women in Madha Tehsil, Solapur district and results showed a higher proportion of women presented with cervical cancer in earlier stages giving scope for intervention to significantly reduce case fatality [10].

Cytology screening (Pap test) is the standard method used for the screening for cervical cancer, however organized screening programs and follow-up are few. The failure of cytological testing in rural India is likely due to a number of factors which include (a) poor infrastructure, (b) lack of trained health professionals (c) absence of organized community based screening programs and (d) inadequate follow-up of patients with abnormal cervical cytology [11].

Some risk factors for cervical cancer are related to lifestyle habits like smoking, obesity, drugs taken for reproductive health, oral contraceptive use, etc. It is said that women who have had more than three full-term pregnancies or were younger than 17 at the time of their first pregnancy are at higher risk for cervical cancer. Having a family history of cervical cancer is a risk factor, especially if a direct relative such as the mother or sister has had cervical cancer [12]. Men who have multiple sexual partners or who are carriers of HPV DNA are vectors of high-risk HPV types and place their partner at high risk of developing cervical cancer. Sex workers are an important reservoir of high-risk HPVs. The role of seminal fluid in the pathogenesis of cervical cancer needs more investigation [13, 14].

In the past decade, a strong etiologic association between infection with high-risk HPV types and development of cervical cancer has been established [15]. Several workers now report that a vast majority of cervical cancer cases are caused by infection with certain sub-types of HPV, a sexually transmitted virus that infects cells and may result in precancerous lesions and invasive cancer [16].

HPV is a relatively small, non-enveloped virus; its genome consists of a single molecule of double-stranded, circular DNA containing approximately 7,900 bp size. About 100 different sub-types of HPV with distinguished variations in its genetic and oncogenic potential are known. The sub-types which precisely affect the anogenital tract are reported to be HPV sub-types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66 and 69 [16, 17]. Vaccines targeting HPV 16 and 18 have been shown to prevent cervical cancer in cases with persistent HPV infections in clinical trials [18]. However, the spectrum of HPV sub types targeted in current vaccine trials is based largely on the prevalence of HPV sub types in cancers from the developed world.

Cervical pathologies and screening programs worldwide

To date, cervical cancer prevention efforts world-wide have focused on screening sexually active women using cytology smears and treating precancerous lesions. It has been widely believed that invasive cervical cancer develops

from dysplastic precursor lesions, progressing steadily from mild to moderate, to severe dysplasia (CIN, ASCUS, LSIL,HSIL), then to carcinoma in situ, and finally to cancer [19]. It now appears that the direct precursor of cervical cancer is high-grade dysplasia, which, in about a third of instances, may progress to cervical cancer over a period of 10–15 years, while most low-grade dysplasia may regress spontaneously [20].

In most developed countries, women are advised to have their first smear test soon after becoming sexually active and subsequently once every 1–5 years. Many national guidelines are currently moving towards less frequent smear tests (once every 3–5 years) because it is recognized that cervical lesions develop slowly over several years. Women with low-grade lesions are generally advised to return for routine follow-up smears. Women with high-grade precursor lesions are further evaluated via colposcopy, biopsy, and subsequent treatment of confirmed lesions. Organized programs with systematic call, recall, follow-up and surveillance systems have had the greatest effect in cancer prevention [21]. Currently there are several on-going, cross-sectional studies being carried out on other screening approaches such as VIA, VIA with magnification (VIAM), and VILI, as well as HPV testing as alternative screening approaches [22].

The objectives of this study included increasing the awareness of women regarding cervical screening to prevent malignancy of the cervix, to establish organized screening practices, to identify factors for non-screening in future and to know the prevalence of various HPV types in our population. Cytological and molecular testing of Pap smears was performed; demographic details of the patient like socio-economic status, medical history, lifestyle of the woman and her spouse including sexual partners, parity, menstrual history etc. These strategies were aimed at finding out the effectiveness of systematic cytology-based screening programs on reducing the number of incidences and mortality rate of cervical cancer.

Materials and Methods

A community-based sampling of women, 3 years after first sexual activity, was done from the areas in and around Hyderabad during the period January 2012–March 2014. A total of 530 eligible women (based on designed criteria) who took part in our camps conducted in collaboration with, Andhra Mahila Sabha (AMS), Shaktishifa Foundation, Family Planning Association of India (FPAI), Lions Clubs, and Vasavi Hospitals, participated in our study. Criteria for participation: women 19 yrs and above who have been sexually active for more than 3 years, not undergone hysterectomy, not pregnant, those who were not menstruating at the time of sampling and those suspected of carrying fatal infectious conditions like AIDS or Hepatitis.

Consent from participants: Informed consent was collected from the subjects. The consent form was read aloud / translated to the participants in their native language. Personal and medical history of each of the eligible participants was then recorded in a well-designed proforma.

Counselling: Women were adequately counseled on the health and monetary benefits of availing the screening being offered to them as part of the camps.

Educating nursing staff/medical officers on smear sampling: We trained volunteers, attending nurses at health centers during camps who explained the procedure and testing that would be done with the sample. Emphasis was made to the medical / para-medical personnel, about the appropriate method of collecting the Pap smear, the regions that have to be covered while collecting the smears (ecto and endo-cervical cells), in addition to recording the visual impression of the cervix.

Collection of cervical smears: Of the 720 women who attended our camps, 530 consented to participate and were enrolled. The remaining 190 women could not be screened due to non-compliance / non-eligibility for screening. Cervical smears were taken by gynecologists with the help of butterfly spatulas on a slide, cells were fixed immediately after collecting and processed for cytological evaluation.

Pap smears Staining: A nuclear stain, Haematoxylin is used to stain cell nuclei. OG-6 counterstain to stain

the small cells of keratinizing squamous cell carcinoma. Second counterstain, EA-36, stains the superficial epithelial squamous cells [23]. Different cytologies were obtained according to Bethesda system of classification for Reporting Cervical Cytological Diagnoses. The different cytologies obtained were normal, inflammatory, infection, RCC, atrophic, ASCUS, LSIL, HSIL and SCC.

DNA isolation from cervical smear: Cytobrush was used to collect the cells from both ecto and endo-cervical regions. DNA was isolated from the cells collected from the cervix region by our routine salting out method [24].

HPV Detection

DNA from 530 patients was screened for the presence HPV by PGMY09/11 primers [25]. PCR products were checked on 3% ethidium bromide stained agarose gel. Presence of a 480bp amplicon indicates HPV positivity (Fig 1). Known HPV positive control and a negative control were used to rule out the cross contamination and to assess satisfactory experimentation.

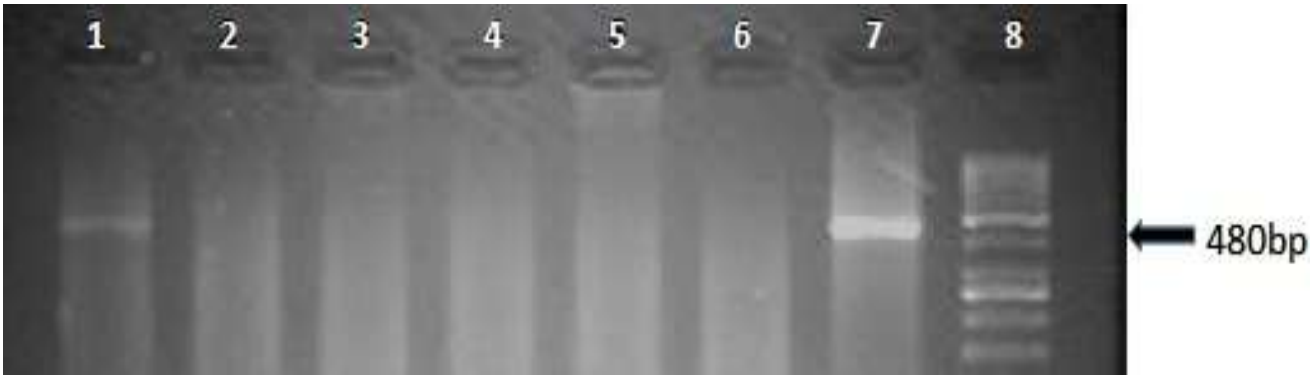

Fig. 1 *Lane* 1- Sample1 (showing 480bp product), *Lane* 2-5: Sample2-5 (Negative for HPV), *Lane* 6- Negative control, *Lane* 7- Positive control (480bp), *Lane* 8- 100bp Marker

High-risk HPV subtyping

HPV positive samples (detected by PGMYO9/11) were subjected to high-risk HPV subtyping by high-risk subtyping kit (HPV High Risk Typing Sacace Biotechnologies, Catalog # V-25-50F) (Fig 2). Results were interpreted according to the manufacturer's protocol.

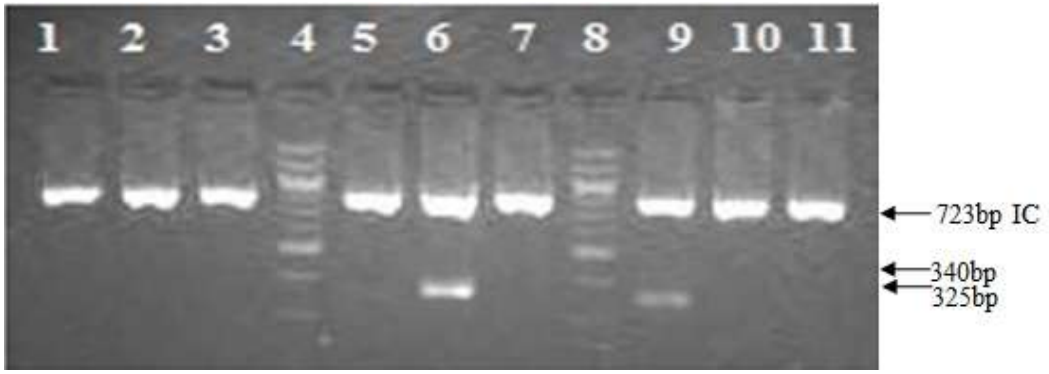

Fig. 2 *Lane* 1-3: Sample 1 (Negative for HR-HPV); *Lane* 5-7: Sample 2 (340bp-HR-HPV 39) *Lane* 9-11: Sample 3 (325bp-HR-HPV 16); *Lane* 4 & 8: 100bp Marker
IC: 723bp internal control of β-globin gene

Statistical analysis: Statistical analysis was performed using MedCalc software (12.2.1 version), for comparison of proportions, to determine significant differences in frequency of cytological types (normal vs other cytologies). Odds Ratio was done for HPV, hr-HPV vs Pap cytologies to estimate the strength of association or level of significance ($p<0.05$).

Results

The mean age of women in the study population was 38.73yrs ($\pm$10.82) with the age range from 19-65 years (Table 1). Majority of the women belonged to low and middle (49.6% and 46.2%) socio-economic strata, according to socio-economic class. A total of 530 women were screened for Pap smear cytology. Of them, 16.41% cases showed a normal cytology with the mean age 31.43yrs ($\pm$7.47), 43.39% had an inflammatory smear with the mean age 37.95yrs ($\pm$ 9.32), 26.22% showed infection with the mean age 39.01yrs ($\pm$10.42), 4.15% showed reactive cellular changes with the mean age 44.59yrs ($\pm$ 11.05), 6.41% showed an atrophic smear with the mean age 56.82yrs ($\pm$ 7.10), 0.75% showed ASCUS with the mean age 40.5yrs ($\pm$8.38), 1.88% showed LSIL with the mean age 39.4yrs ($\pm$10.42), 0.37% showed HSIL with the mean age 46.5yrs ($\pm$2.12), 0.37% showed squamous cell carcinoma with the mean age 41.5yrs ($\pm$12.02). Figure 3 shows the distribution of different pap cytologies in our study group.

Table 1 Demographic details of the subjects

No.	Variables	Range			Mean+ SD		
		Normal (n=87)	**Inflammatory (n=230)**	**Abnormal cytology (n=213) excl. inflammation**	**Normal (n=87)**	**Inflammatory (n=213)**	**Abnormal cytology (n=213) excl. inflammation**
1	Age (Years)	19-55	19-65	20-65	31.43+7.47	37.95+9.32	42.6+11.8
2	Ageat Menarche (Years)	10-17	10-18	10-18	12.82+1.322	12.73+1.33	12.93+1.44
3	Age at Marriage (Years)	6-35	7-31	5-33	17.95+4.12	17.83+4.31	17.36+3.93
4	Age at first intercourse (Years)	13-35	12-31	12-33	17.95+4.12	18.16+3.86	17.59+3.54
5	Age at the first child birth (Yrs)	15-36	15-33	14-35	19.65+4.23	19.75+3.84	18.69+3.58
6	Parity (Number)	0-6	0-9	0-10	3.195+1.590	2.913+1.457	2.553+1.350

Note: excl: Excluding

Table 2 Representing high-risk HPV subtype in different cytologies

Cytology	No of cases *n* (%)	HPV *n* (%)	No of hr-HPV positive *n* (%)	hr-HPV subtypes
NORMAL	87 (16.4)	6 (6.8)	0	-
INFLAMMATORY	230 (43.3)	17(7.39)	0	-
INFECTION	139 (26.2)	29 (20.8)	5 (3.5)	16,35,39, 31,45,59,66
RCC	22 (4.15)	10 (45.4)	2 (9.9)	16
ATROPHIC	34 (6.41)	6 (17.6)	0	-
ASCUS	4 (0.75)	3 (75)	0	-
LSIL	10 (1.88)	3 (30)	0	-
HSIL	2 (0.3)	2 (100)	1 (50)	52
SCC	2 (0.3)	2 (100)	2 (100)	16,35
TOTAL	530	78 (14.7)	10 (12.8)	

HPV vs Cytology

A total of 530 Pap smear samples were screened for presence of HPV, overall 14.7% of HPV positivity was found in screened population. HPV was detected in 6.89% of the cases with normal cytology, 7.39% of inflammatory cervix cases (p=0.879), 20.86% of cases with cervical infection (OR=3.55, 95%CI, 1.41-8.97, p=0.0071), 45.45% of the cases that showed reactive cellular changes (OR=11.2, 95% CI, 3.45-36.60, p=0.0001), 17.64% of cases with atrophy (p=0.13), 75% of ASCUS cases (p=0.13), 30% of LSIL (OR=5.78, 95%CI, 1.18-28.27 p=0.03), 50% of HSIL (p=0.004), and 100% of cases with squamous cell carcinoma (p=0.001). Of these HPV positive cases, 12.82% (10 out of 78) had the presence of hr-HPV sub-types.

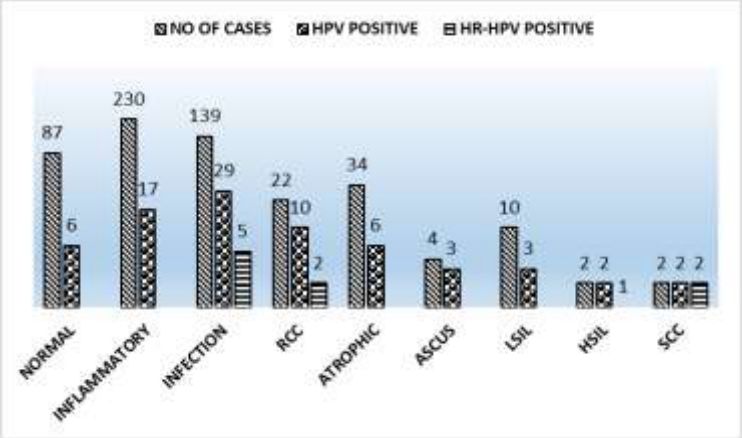

Fig. 3 Represents prevalance of HPV and hr-HPV in different pap cytologies

High-risk HPV Vs Cytology

High-risk subtypes were present in 5/139 cases (3.59%) with infectious cytology report, 2/22 (9.90%) reported with reactive cellular changes, 1/2 (50%) with HSIL, and 2/2 (100%) squamous cell carcinoma (p=0.001) (Table 2).

Of the hr-HPV subtypes, 50% had an infectious cytology, 20% with RCC, 10% with HSIL cytology and 20% were SCCs (Fig 3).

Parity was low in abnormal cases (mean±SD: 2.553±1.350) when compared to cases with normal cytology (mean±SD: 3.195±1.590). We did not find any significant difference between normal and abnormal cytologies with regard to age at menarche, marriage/age at first intercourse and age at first child birth. The mean age of women showing abnormal cytology 42.6 (±11.8) was significantly higher than the mean age of women with a normal pap cytology 31.43 (±7.47) (Table 1). It is notable that medical history of most of the patients with abnormal pap cytology (RCC, ASCUS, LSIL, HSIL and SCC) had diabetes, thyroid disorders and hypertension.

Discussion

Cervical cancer remains largely uncontrolled in developing countries such as India because of ineffective patient screening and follow-up. Owing to poor awareness and participation, the incidence of invasive cervical cancer remains high, especially in rural India, despite the availability of Pap testing on an opportunistic basis [26].

In India, there are few organized or high-level opportunistic screening programs for cervical cancer in some of the states. There have been a few success stories of effective screening and prevention of cervical cancer from some parts of the country. Efforts to improve awareness in the population have resulted in early detection and improved survival from cervical cancer in a backward rural region in western India [27, 28]. Data from population-based cancer registries in different regions indicate a slow, but steady, decline in the incidence of cervical cancer. However, the rates are still too high, particularly in the rural areas, and the absolute number of cases is on the rise.

We encountered initial high rates of non-compliance (5.86%) from the participants in our camps owing in part, to ignorance of the importance of cervical screening and due to the stigma associated with a pelvic examination, per se. After the few initial camps, we adopted the strategy of counselling the participants before the pap smear was collected, on how safe the procedure was, about usefulness of the test in avoiding future risk for cancer and that women should make use of the free service while it was being offered as a part of our project; maximum participation with hardly any drop-outs were seen after pre-test counselling was done. Through local medical officers, other para-medical volunteers, it was possible to reach remote areas with support from Family Planning Association India, Lions Club, Andhra Mahila Sabha and Vasavi Hospital.

Advanced / precancerous lesions of the cervix were seen mostly in patients over the age of 40 years; it is notable from our data that most of the women who showed a normal cervix upon examination were around the age of 30 years. Cervical tissue seems to heal well in the earlier age group but with increasing age becomes more prone to pathological tissue changes. The same could be said keeping in view HPV infection – only persistent infection with HPV, associated with later age was a risk factor for cervical malignancy. Unlike other subjects who showed increased parity as a risk factor, our results show that lower parity seems to be associated with a pathological cervix as compared to cases with higher parity. This suggested that hormonal factors associated with cervix, i.e. lesser exposure of cervix to pregnancy-related hormones was probably a risk factor. Patients who showed the presence of hr-HPV, all had the medical history like diabetes, thyroid dysfunction etc., making the cervical tissue more prone to infection and associated inflammation. Immunological response of cervical tissue is better in early age and hence low infection and lesser scope for persistence of HPV infection as seen from our results is associated with a normal cervical cytology.

Of the cases that tested positive for hr-HPV subtypes, 40% had diabetes, 20% cases had altered thyroid levels. A higher incidence of infections is seen in patients with diabetes due to the hyperglycemic environment which promotes

immune dysfunction. There is low production of interleukins and reduced antimicrobial activity [29]. These effects of hyperglycemia are seen in all tissues and organs; one of the common infection-prone regions is the vagina (fungal or bacterial or protozoan infections). These factors may contribute to persistence of HPV infection in the cervix, leading to transforming squamous epithelial cells to malignant forms.

Cervical cytology is considered to be a very specific test for high-grade precancerous lesions or cancer but, even if the quality of collection and spreading of cells, fixation, and staining of smears, and reporting by well-trained technicians and cytopathologists are good, its sensitivity is only moderate. So co-testing for the presence of HPV high-risk subtypes was done to improve the chances for detecting an abnormal cervix (given the etiological role for HPV in cervical malignancy). Routine cervical screening programs do not include HPV testing for reporting the risk and/or the need for intensive follow-up, in a patient. Further, because geographical variation in the sub-type distribution may exist, knowledge about the distribution of HPV types in cervical cancers and HPV types circulating in the communities in different regions of India would be useful in devising the optimal strategy for vaccination in our population [30].

High-risk subtypes 16, 31, 35, 39, 45, 52, 59, 66 have been seen in our cases; subtype 66 is not reported often from our population. While 16 was the most prevalent subtype either singly or existing as multiple infection with other subtypes, high-risk subtype 35 was seen to be next to subtype 16 (Table 2). HPV infection was seen mostly in cases where the Pap smear showed an infectious cytology, in addition to the cases with reactive cellular changes or other precancerous lesions (LSIL, HSIL). Among healthy women, a HPV infection is known to clear within 1–2 years. Our cases which showed presence of high-risk HPVs have been put on follow-up program to monitor the changes in the cervix by repeated pap and colposcopy where warranted.

Organizing camps and creating awareness by educating the volunteers and subjects who participate in these camps, educating the medical / para-medical staff in local health centers regarding the appropriate screening practices and suitable patient follow-up based on the pap cytology report and HPV testing can considerably reduce the burden of cervical cancer in our population.

Conclusion

Although cytological screening is being carried out, there are no organized programs and the testing is often of poor quality and performed inadequately and inefficiently among the population. The findings from research on various approaches to screening (in terms of accuracy and effectiveness) should be taken into account when considering reorganizing existing programs. Women should take active part and be concerned with issues dealing with their health and personal hygiene, awareness is the first step towards getting involved in cancer prevention. Information on the presence of hr-HPV subtypes other than 16 and 18 prevalent in our population should be used to device adequate vaccination strategies suitable for our population.

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