COLPOSCOPY AS A TOOL FOR DETECTION OF HUMAN PAPILLOMAVIRUS INFECTION OF UTERINE CERVIX IN THE SETTING OF HIGH PREVALENCE OF GYNAECOLOGIC INFECTIONS

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ABSTRACT

A cross sectional study involving 257 women from the Maternal and Child Health Centre (MCH) in Delhi was initiated for screening clinically, cytologically, colposcopically, and microbiologically for several gynaecologic infections. Eighty percent of the women had one or more gynaecologic infections and 31.1% had three or more infections. Cytology revealed changes suggestive of condyloma in 3 (1.2%) women only, while colposcopic examination suggested HPV changes in 117 (45.5%) women. A very high proportion of colposcopically detected lesions (78.6%) had evidence of HPV related changes in histology. The specificity of these lesions were further confirmed by Pan HPV DNA in-situ hybridisation, when 84% of the colposcopy is a valuable tool for detecting subclinical HPV lesions in a setting with high prevalence of gynaecologic infections.

Keywords: colposcopy human papillomavirus (HPV), gynaecologic infections, DNA in-situ hybridisation

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INTRODUCTION

Genital human papillomavirus (HPV) infection may be clinically overt, subclinical or latent [1]. While latent infections have been described to be quite frequent even among healthy women [2], clinicians are usually confronted with the problems of clinically manifested and subclinical lesions. Clinically manifested infection, however, forms a small proportion of the total load of HPV infection [3]. Subclinical infection, on the other hand, may be a more significant problem.

We have been screening cytologically women attending major hospitals in New Delhi. Of the 95,716 women screened, only 499 (0.5%) had evidence of human papillomavirus (HPV) infection. Considering a very high incidence of uterine cervical cancer among Indian women (over 90,000 new cancers annually) [4], it seems that a large proportion of women with HPV infection, a putative agent for uterine cervical cancer, was being missed by cytologic examination. Thus, on a subsample, we planned to screen consecutive women attending outpatient department of a major women hospital by colposcopy for the detection of uterine cervical human papillomavirus infection.

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MATERIAL AND METHODS

Two hundred and fifty-seven women attending Outpatient Department (OPD) of one of the major women's hospitals in New Delhi were studied from July 1992-Dec 1993. A comprehensive pelvic examination was performed and clinical signs, symptoms and other relevant information pertaining to contraceptive practices including sexual and reproductive activity of the women were recorded after obtaining the informed consent. The terminology [5] for the clinical findings most frequently encountered are as follows: 1) Cervical ectopia is the bright red appearance of the cervix around the external os, with a clearly defined edge, the colour indicating the underlying vascular tissue through a thin epithelium; 2) bleeding ectopias are those that bleed on touch because of fragile epithelial tissue; 3) a hypertrophied cervix is an enlargement of the entire cervix with firm-to-hard consistency and an irregular surface contour; 4) an unhealthy cervix is a hypertrophied, elongated cervix with an irregular contour, with or without a nabothian cyst, and with abnormal discharge; 5) cervicitis is described as inflammatory condition of the cervix with the presence of discharge varying in colour and consistency and is associated with eversion presented as velvety to granular perioral redness or as patchy erythema. There may be an associated tenderness and/or thickening in the fornics. Pelvic inflammatory disease was diagnosed clinically.

Cervical scrapes were collected for Pap's staining using wooden Ayer's spatula. Pap's smear slides were transported to the labortory in 95% ethyl alcohol. Papanicolaou stained smears were examined under light microscopy (400x) by a cytopathologist and smears were categorised according to the WHO reporting system [6]. The presence of various infective agents like *Trichomonas vaginalis*, yeast vaginitis and morphologic changes suggestive of Herpes Simplex Virus (HSV) and human papillomavirus infection (HPV) were also looked for.

Patients were examined colposcopically using Zeiss

Colposcope (West Germany). A standard predefined terminology[7] was used to describe the colposcopic changes. The cervix was evaluated for normal colposcopic changes, metaplasia (immature and mature) and atypical transformation zone (ATZ). The sub-clinical HPV infection was suspected colposcopically as ATZ with acetowhite epithelium, with or without punctation, mosaicism and atypical vascular pattern. Colposcopic directed biopsies were taken from any suspected lesion and transported to the laboratory in 10% buffered formalin solution. Human papillomavirus infection was diagnosed on smears and haemotoxilin and eosin stained biopsy sections using standard criteria[8]. Immunohistochemical staining of paraffin sections were done to detect HPV inclusion bodies in the cervical epithelial cells, using polyclonal HPV antibodies and peroxidase antiperoxidase conjugates (Dakopatts, Denmark)[9]. Biopsy sections were also hybridised with biotin labeled Pan-HPV probes[10] (Kreatech Biotech, Amsterdam, Netherland).

These women were also screened for other gynaecologic infections. In brief, endocervical secretions were collected to detect chlamydial and gonococcal antigens using commercial ELISA kits (Abbott, USA). The bacterial vaginosis (BV) was diagnosed using standard criteria[11] ie, positivity of vaginal secretions for amine test, clue cells and *gardnerella* type organisms in Gram stained smears. Under wet smear microscopy, *T vaginalis*, yeast and clue cells were screened. Serology was done for syphilis (TPHA antibodies), HSV (IgA antibodies)[12] and for human immunodeficiency virus (HIV - I & II)(ELISA).

RESULTS

The average age of the women was 32.1 years with a standard deviation of 8.1 years. Two hundred and seventeen (84.4%) women had gynaecologic symptoms whereas 40 women (15.6%) had no such symptoms and came for family planning advice. Clinical examination revealed hypertrophied cervix in 79 (30.7%), cervical ectopy in 64 (24.9%), bleeding ectopy in 66 (25.7%), cervicitis in 50 (19.4%), vaginitis in 60 (23.3%) and vulvitis in 18 (7.0%). Pelvic inflammatory disease was detected in 10 (3.9%) women. Clinically overt genital human papillomavirus lesions were detected in only seven women (2.7%).

High prevalence of gynaecologic infections was observed in this population. Fifty-one women (19.8%) had no detectable infection while 206 (80.2%) had evidence of one or more infections. Out of these 206 women, 64 (24.9%) were infected with one aetiologic agent, 62 (24.1%) with two infections and 80 (31.1%) had three or more different infections.

Cytology revealed changes suggestive of condyloma in 3 women (1.2%), inflammation in 204 women (79.4%), dysplasia in 5 women (1.9%) and atypias in 3 women (1.2%). Forty-one women (15.9%) had normal smears while one smear was inadequate for evaluation. All the 3 patients with cytologically detected condylomatous lesions were confirmed by biopsy. Colposcopic directed biopsies were performed in 117 women (45.5%) where changes suggestive of HPV infections were observed. Ninety-two women (78.6%) with colposcopically detected HPV lesions also showed HPV-related changes in histology. Eighty of these lesions (86.9%) had associated chronic cervicitis, 8 (8.7%) had associated dysplasia of different grades and 4 (1.6%) had no associated lesions. Twenty-five women (21.4%) could not be confirmed histologically as having HPV-related changes: 3 of them had dysplasia and 22 had chronic cervicitis. The majority of colposcopically detected HPV lesions comprised of flat condyloma (72%). Papillary condyloma (11%), inverted condyloma (10%) and spiked condyloma (7%) formed small proportions of the total HPV-related lesions.

Table I – Frequency of gynaecologic infections

Gynaecologic infection	Frequency	Percentage (%)
Human papillomavirus (HPV)	117	45.5
Bacterial vaginosis	86	33.5
Herpes simplex virus (IgA antibodies)	62	24.1
Chlamydia	60	23.3
T Vaginalis	40	15.6
Gonorrhea	14	5.4
Syphilis (TPHA)	11	4.3
HIV - I & II (antibodies)	0	0.0

Immunohistochemical staining of biopsy sections from 50 women with colposcopic evidence of HPV related lesions revealed positive nuclear staining in 32 (64.0%) women. Likewise, when Pan-HPV-DNA in-situ hybridisation was done on these biopsies, 42 (84%) tissues were found positive for HPV-DNA.

DISCUSSION

This paper deals with the sub-clinical human papillomavirus infections of lower genital tract. It is clear that about one half of the women had evidence of sub-clinical human papillomavirus infection as evidenced by colposcopic examination. This is in sharp contrast to the detection rate of only 1.2% of koilocytolic changes in cytology smears. Thus, it is clear that cytology has a rather poor sensitivity for detection of HPV lesions. Extremely poor sensitivity for detection of HPV lesions. Extremely poor sensitivity of cytology may be due to heavy (80.2%) load of gynaecologic infections among these women with 31.1% of them having 3 or more different infections. Such a heavy load of infection might have had a masking effect on the cytodiagnosis of HPV infections. Indeed, a poor sensitivity of cytological detection of flat HPV lesions has been documented[2]. A high specificity of HPV lesion as revealed through histologic confirmation suggests that colposcopy is a useful tool for evaluation of women for HPV, especially in the setting of high prevalence of gynaecologic infections. In the present study, 78.6% of colposcopically detected HPV lesions could be proven through biopsy. It has been suggested elsewhere[13] that the specific HPV-DNA sequences by in-situ hybridisation, preferably using non-radio-isotope method. The current study shows that the concurrent rate between colposcopy and HPV-DNA sequence was as high as 84.0%. Thus, the majority of the HPV lesions detected through colposcopy were specific lesions and not non-specific papillomatous changes.

The colposcopically detected HPV lesions are clinically significant in the sense that they have the potential to develop precancerous/cancerous lesions. In the present ongoing study, 14 women (11.9%) with HPV sub-clinical lesions have already developed dysplasias during the first follow-up year. The follow-up is still continuing. It has been already reported that after long term follow-up, one-third of the women with histologically proven HPV lesions would eventually develop precancerous lesions[14]. Extrapolating this finding to our data shows that one-third of the 92 (ie 31) colposcopically detected and hiopsy proved HPV lesions would eventually develop precancerous lesions. On the other hand, only one of the three cases of cytologically detected and biopsy, proved lesions would progress. Though the unit cost in Indian Rupees (IR) of colposcopic examination

(IR=500) is approximately five times higher than that of cytology (IR=100) the yield of HPV-lesions with a potential to progress is 31-fold. Thus, in the long-run, use of colposcope in this setup would not only have a higher yield of significant lesions but also would be cost-effective compared to cytology screening.

In short, colposcopy seems to be a valuable tool in this setting of high rate of gynaecologic infections for the detection of subclinical human papillomavirus infection.

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