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Association between high risk human papillomavirus infection and co-infection with *Candida spp*. and *Trichomonas vaginalis* in women with cervical premalignant and malignant lesions

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ABSTRACT

Background: Human papillomavirus (HPV) is the necessary cause of cervical cancer. Cervico-vaginal infection with pathogens like *Chlamydia* is a likely cofactor. The interactions between HPV, *Trichomonas vaginalis* (TV) and *Candida* spp. are less understood, though inflammation induced by these pathogens has been demonstrated to facilitate oncogenesis.
Objective: Our study aimed to evaluate the association between *Candida spp*. and TV co-infection with HPV in cervical oncogenesis.
Study design: Women with normal cervix who were high-risk HPV-negative (N = 104) and HPV-positive (N = 105); women with CIN 1 (N = 106) and CIN 2/CIN 3 (N = 62) were recruited from a community based cervical cancer screening program. Cervical cancer patients (N = 106) were recruited from a tertiary care oncology clinic. High-risk HPV was detected by Hybrid Capture II technique; *Candida* spp. and TV were detected by culturing the high vaginal swabs followed by microscopic examination in all. The disease status was established by histopathology in all the women.
Result: HPV-positive women had significantly higher risk of having precursor lesions (of any grade) and cancer compared to HPV-negative women. *Candida* spp. or TV infection did not alter the risk of low grade or high grade lesions among HPV- positive women. HPV positive women co-infected with TV had higher risk of cervical cancer but not those co-infected with *Candida* spp.
Conclusion: The higher risk of cancer observed in the women co-infected with HPV and TV without any enhanced risk of CIN 3 suggests secondary infection of the malignant growth by TV rather than any causal role. Co-infection with *Candida* spp. and/or TV infection did not increase the carcinogenic effect of HPV on cervix.

1. Background

Human papillomavirus is a DNA virus of family *Papillomaviridae* and has been implicated in several cancers including cervical cancer. The fact that high risk human papillomavirus (HPV) infection is the 'necessary' but not 'sufficient' cause of cervical cancer was the basis for the evaluation of several potential co-factors as cervical carcinogens. Included in the list are the infections of lower genital tract caused by different pathogens. Human immunodeficiency virus (HIV) is known to predispose the affected women to acquire HPV infections and also promote development of cervical neoplasias in them through the up-regulation of HPV oncogene expression [1]. Robust epidemiological evidences exist to suggest that *Chlamydia* spp. and *Herpes simplex virus – 2* (HSV-2) could be the possible co-factors in the cervical carcinogenesis [2–5].

The role of the other two common sexually transmitted infections, *Candida* spp. and *Trichomonas vaginalis* (TV), as co-factors in cervical carcinogenesis is less clear. The fungus *Candida* spp. is a part of the human commensal flora causing superficial and systemic infections while *Trichomonas vaginalis* is a flagellated protozoa and a common cause of sexually transmitted infections. It has been suggested that these infections could elevate the risk of cervical cancer in HPV positive women due to an inflammatory response associated with the free radical generation and the development of genetic

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instability [6]. The disruption of the inflamed cervical epithelium by these microorganisms has been hypothesized to increase the risk of HPV infection by allowing the virus to penetrate the basal cells of the epithelium. In spite of these theoretical possibilities the interaction between HPV, *Candida* spp. and/or TV in causing cervical malignancies is still less understood. The existing studies were extremely heterogeneous in their design, the infection was diagnosed on the basis of the morphological changes on cytology, which is inherently inaccurate and the disease outcome was established in many of the studies on the basis of cervical cytology rather than histopathology [7–9].

2. Objectives

The objective of the present study was to evaluate the association between high risk HPV infection and co-infections with *Candida* spp. and/or TV in histopathology proved low and high grade intraepithelial lesions and invasive cancers of cervix.

3. Study design

3.1. Selection of subjects

The case-control study was conducted among the women participating in a community based cervical cancer screening demonstration project in eastern India. The demonstration project was implemented by Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The research ethics committee of CNCI approved the study. Women between 30–60 years of age were screened with HPV DNA test and visual inspection with acetic acid (VIA) test. The detailed methodology of screening and the primary outcomes of the demonstration project have already been published [10]. In brief, the women eligible for cervical cancer screening had cervical scrapes collected for HPV test by trained health workers. The samples were tested for the presence of DNA of the thirteen oncogenic high risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) using the Hybrid Capture II (HC II; Digene Corporation, Gaithersburg, MD, USA) assay at the CNCI laboratory. A positive result was recorded for specimens with relative light unit/positive controls (RLU/PC) ratio of 1 or greater, which corresponded to 5000 or more viral copies. VIA was performed by the health workers after collecting the samples for the HPV test. All the women positive on either of the screening tests were further evaluated with colposcopy by trained colposcopists. Punch biopsies were obtained from all the colposcopically significant lesions. Women who were HPV positive but had apparently normal cervix on colposcopy had a punch biopsy obtained from the 12 O'clock position of the cervix. Hysterectomized or pregnant women and women with past history of cervical neoplasias were not screened in the project [11]. For the present study, 104 consecutive HPV negative women with normal cervix on colposcopy and/or histopathology, 105 consecutive HPV positive women with histopathology proved normal cervix, 106 consecutive women with histopathology proved CIN 1 (HPV negative or positive) and 62 consecutive women with histopathology proved CIN 2/CIN 3 (HPV negative or positive) were recruited from the screening project. As there were only few cases of invasive cervical cancer detected through screening, total 106 patients with histopathology proved invasive cervical cancer were recruited from the outpatients of the Department of Gynecological Oncology at CNCI. The cervical cancer patients were also tested for high risk HPV using the HC II test. The women were included in the study only if they provided written informed consent to participate.

3.2. Specimen collection & processing

All the women were recalled within one month of confirmation of diagnosis for the collection of cervico-vaginal samples to detect *Candida spp*. and TV in the lower genital tract.

A high vaginal specimen was taken from the posterior vaginal fornix with a sterile cotton swab soaked in normal saline for *Candida* detection. The materials from the swab were inoculated by streak method on Saboraud's dextrose agar (SDA) (HiMedia^{TM}, India) plates and sent to the laboratory immediately at room temperature. The SDA plates were incubated at 37 °C. The pasty, opaque, cream-colored colonies grew in 24–48 h if the samples were positive for *Candida*. The gram staining detected the gram positive budding yeast cells and hyphae.

Another sterile cotton swab soaked in normal saline was used to collect the discharge from the posterior vaginal fornix and the specimen was inoculated in Kupferberg's broth based media (HiMedia^{TM}, India) for isolation of TV.

For optimum growth a long tube was filled with the medium so as to provide anaerobic conditions at the bottom and the inoculum was placed at the bottom of the tube. The tube was transported at room temperature to the laboratory. The tube was incubated at 37 °C to provide optimum growth conditions for the organism. Wet-mount preparations were examined for TV once a day from the second day onwards to detect the motile organisms. Most of the isolates were positive within 2–4 days. The tube was incubated for 7 days before declaring the result as negative.

Women who bled excessively from cervix during specimen collection were excluded as excessive blood might interfere with the isolation of the infectious organisms. All the histology slides from cervix were reviewed by the study pathologist to confirm the diagnosis.

3.3. Statistical analysis

The socio-demographic and reproductive characteristics of the women were presented as proportions. Comparisons of these characteristics by HPV, *Candida* spp. and TV co-infection status were done using Pearson's chi-square test. The effect of co-infections on the outcomes of CIN 1, CIN 2, CIN 3 and invasive cancer was assessed using odds ratios (ORs) and their 95% confidence intervals (CIs) obtained from multinomial logistic regression models. The 'no cervical neoplasia' final diagnosis was taken as the base outcome, that is for the assessment of each of the outcome, only data of women with that particular outcome and those of the women with 'no cervical neoplasia' final diagnosis were used in the regression model. In the multivariate regression models, the estimates were adjusted for only the factors that were significantly associated with the co-infection status. Statistical significant associations were inferred at a 5% level. To test whether the OR estimates obtained for the HPV-co-infections strata were significantly different, a chi-square test of homogeneity was used. Heterogeneity was concluded at a p-value less than 10%. All analyses were carried out in Stata 13.0 (StataCorp LP, Texas, USA).

4. Results

HCII for high risk HPV was positive in 59.4% (63/106) women with CIN 1, 52.8% (19/36) women with CIN 2, 92.3% (24/26) women with CIN 3 and 96.2% (102/106) women with invasive cervical cancer diagnoses. TV was detected in 54.7% (93/170) HCII negative and in 72.8% (228/313) HCII positive women ($p < 0.001$). *Candida* spp. was detected comparatively less frequently; 23.5% (40/170) and 28.1% (88/313) of the HCII negative and HCII positive women