

RESEARCH ARTICLE

Hybrid Capture 2 Assay Based Evaluation of High-Risk HPV Status in Healthy Women of North-East India

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Abstract

Background: High risk HPV (HR-HPV) testing has been recommended as an effective tool along with cytology screening in identification of cervical intraepithelial lesions (CINs) and prevention of their progress towards invasive cervical cancer. The aim of this study was to assess the HR-HPV DNA status by Hybrid Capture 2 (HC2) assay in healthy asymptomatic women of North-East India. **Materials and Methods:** This study examined cervical cell samples of forty three (n=43) healthy women by HC2 assay. A High Risk HPV DNA kit (Qiagen) was used which can detect 13 high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. **Results:** The mean relative light units (RLU) for samples was in the range of 141-5, 94, 619. HR-HPV DNA was confirmed in 16% (7/43) of participant women samples. Among demographic and clinical parameters, menstrual irregularity (p=0.039) and infection history (p=0.028) has shown statistically significant differences between the HR-HPV-positive and negative groups. In the HR-HPV positive group, two women were confirmed for CINs after colposcopy and histopathologic examination. **Conclusions:** We suggest that there may be an association between irregular menstruation and infection history of the urogenital tract with HR-HPV DNA prevalence in North-East Indian asymptomatic women. HC2 assay can be a valuable tool for HR-HPV screening.

Keywords: HC2 assay - high risk HPV - healthy women of North East India

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Introduction

Cervical cancer burden in India has significant proposition for women health. In India, this cancer at present accounts for approximately 130,000 new diagnosed cases and 75,000 deaths annually (Ferlay et al., 2010). In United States of America and some western European countries, cervical cytology (Papanicolaou test i.e. Pap test) screening programs have led to the significant reduction of cervical cancer incidence mainly due to the early detection of cervical intraepithelial neoplasias (CINs). Data from a range of population-based studies now explicitly support for the incorporation of high-risk human papillomavirus (HR-HPV) DNA testing as an adjunct part of the existing screening program (Sarian et al, 2010; del Pino et al, 2011; Bhatla et al, 2012; Giorgi-Rossi et al, 2012; Arbyn et al, 2013; Brun-Micaleff et al, 2013). It has also been confirmed by the work of multiple research groups in large population samples that cytological screening is less sensitive compared to High risk HPV (HR-HPV) DNA testing in detection of histological cervical intraepithelial lesions (CIN2/3)

(Arbyn et al, 2012). Recently updated cervical screening guidelines have proposed a 5-year screening interval for women aged 30 years and older with “double-negative” Papanicolaou (Pap) and high-risk human papillomavirus (HR-HPV) results (Zhao et al, 2013). HR-HPV DNA negative women are unlikely to develop CIN 2 or 3 and invasive cervical cancer over a period of 5-10 years. High negative predictive value (NPV) permits safe and cost-effective lengthening of the cervical screening interval when HPV testing is used (Schiffman et al., 2011).

By the currently available technological advances, HR-HPV DNA can be detected early in quantitative mode. Much is left desired when looking for the cytological and histological changes which may also harbor carcinogenic HPV infection. Highly sensitive and specific tests have been established to identify the human papillomavirus (HPV) infections that are associated with detectable CIN (Gillio-Tos et al., 2013). Currently the two extensively worked out methods in published literature for detection of High risk HPV types (who have shown proven carcinogenic ability), are hybridization with signal amplification and genomic amplification using polymerase chain reaction

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(PCR). The first reliable, quality standardized HPV DNA test was developed by the Digene Corporation (Gaithersburg, MD, USA) and gained United States Food and Drug Administration (FDA) approval in 1999 for reflex testing of patients with atypical squamous cells of undetermined significance (ASC-US) cytology. In 2003, FDA approval was extended to the use of Hybrid Capture 2 alongside routine Papanicolaou (Pap) testing for women over the age of 30 years. A second-generation Hybrid Capture 2 High-Risk HPV DNA Test (hc2) remains widely applied and is associated with the largest evidence base for application (Cubie and Cuschieri, 2013). Consistent evidence is available indicating that HPV-triage with the Hybrid Capture 2 assay (HC2) is more accurate (higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. After conservative treatment of cervical pre-cancer, HPV testing picks up more quickly, with higher sensitivity and not lower specificity, residual or recurrent high-grade CIN than follow-up cytology (Arbyn et al., 2012).

There had been various Indian studies which employed Hybrid Capture 2(HC2) assay for HR-HPV DNA detection in asymptomatic and symptomatic women population. The geographic regions represented in these studies were northern, eastern and southern India. The HR-HPV DNA positivity was found in the frequency range of 6% to 18% which may be attributed to the difference in study design and also to the geographical and ethnic diversity of India (Bhatla et al., 2009; Sowjanya et al., 2009; Datta et al., 2010; Piyathilake et al., 2010; Katyal et al., 2011; Pandey et al., 2012; Basu et al., 2013; Bhatla et al., 2013).

The Population based Cancer Registry, PBCR Report (2009-11), of Indian Council of Medical Research (ICMR) India (2013) had shown that Aizawl district of North-East India had the highest age adjusted incidence rates (AAR) of 24.3 for cervix uteri cancer among all the PBCRs. The AARs of other North-eastern region PBCRs are as: Mizoram state (17.1), Kamrup urban (14.0), Nagaland (11.0), Cachar (10.9), Imphal west (10.6), Tripura state (10.1), East Khasi hills (9.9), Manipur state(8.1) and Dibrugarh (5.6). Cervix uteri cancer feature in the top five leading sites in North-East viz. second in Cachar and Sikkim, third in Kamrup (urban), Manipur State, Imphal West District, Mizoram state and Aizwal and fifth in Dibrugarh District. In our earlier study, we confirmed the presence of HPV-16, -18, -31, -45, -59, -58, HPV-6/11 in cervical cancer patients of North East India by using nested multiplex PCR along with HC2 assay (Das et al., 2013). At present there is no HC2 assay related data about HR-HPV DNA available from asymptomatic healthy women population of North-East India. With the prospect of clinical applicability of HC2 assay, primary data from this region is warranted. This pilot study has been undertaken to assess the high-risk HPV DNA prevalence in healthy women.

Materials and Methods

Selection of participant women

Women of North- East Indian region residing in Guwahati and neighboring areas in state of Assam were

selected for this study. During personal interview the socio-demographic and risk factor associated information was collected in a proforma. Informed consent was acquired from all the participants. The participants were sensitized about Human papillomavirus and its role in the development of cervical cancer. This study was approved by the Medical Ethics Committee of Dr B. Borooah Cancer Institute. The cervical exfoliate cell samples from healthy women were collected at the Dr B. Borooah Cancer Institute and Hayat super specialty Hospital, Guwahati, India from April 2013 through June 2013.

Specimen collection

The healthy women were asked to lie in the dorsal position and a Cusco bivalve vaginal speculum was introduced. A sterile disposable brush for HC2 specimen collection was used which was provided in DNAPap cervical sampler kit (Qiagen, Gaithersburg, MD). The cervical brush sampler was introduced inside the endocervix with the lowermost bristles touching the ectocervix, rotated three–five times full turns in a counter-clockwise direction, exerting gentle pressure, and immediately transferred to a tube with 1 ml specimen transport medium (STM; Qiagen) for HPV DNA detection by HC2. All samples were stored at 2-8°C and tested within two week.

HPV-DNA testing by Hybrid Capture 2 Assay

Hybrid Capture 2 testing was performed using the HC2 High- Risk HPV DNA Test kit (Qiagen) in the automated hybrid capture system (Qiagen, Gaithersburg, MD) according to the manufacturer's instructions to detect the presence of 13 high-risk HPV (HR-HPV) types. Thirteen types of HR-HPVs detectable by HC2 assay are: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; however, specific HPV types could not be determined. The HC2 assay uses in vitro nucleic acid hybridization for qualitative detection of 13 types of high-risk HPV by signal amplification rather than DNA amplification. Target DNA in specimens was hybridized to a specific HPV RNA probe and RNA–DNA hybrids were captured and detected in microplate chemiluminescence by Luminometer. Light signals were measured as relative light units (RLUs) with light intensity indicating the presence or absence of target DNA in the tested specimen. RLU measurements equal to or higher than the cutoff (CO) value ($RLU/CO \geq 1$), indicated the presence of HPV DNA in the sample. $RLU/CO < 1$ indicated the absence of specific HPV DNA or HPV DNA below the detection limit. When RLU is equal to CO it confers the presence of approximately 5,000 virus copies in the specimen. The high-risk HPV quality control was 5 pg/ml cloned HPV 16 and carrier DNA. The high-risk HPV calibrator was 1 pg/ml cloned HPV 16 and carrier DNA. The negative calibrator was carrier DNA. Two confirmed cases of cervical cancer with High risk HPV positivity were also used as control samples. All the samples have been tested twice and samples with positivity in both the test run were only scored as positive. The test reports were generated as per the format of the Hybrid Capture™ software ver. 2.0

Colposcopy

All HR-HPV positive women underwent a colposcopic examination by an experienced gynaecologist. Biopsies were taken from all acetowhite lesions. Women were considered to be free of disease if CIN or invasive cancer were ruled out after biopsy or if colposcopy was normal, thereby obviating the need for taking a biopsy.

Statistical analysis

Statistical analysis of data was done in Graphpad prism software ver 5.0 for Windows. The prevalence of high-risk HPV infection in test groups and relationship between HPV status and risk factors were tested using a Fisher's exact test. Statistical significance was considered for P-value of ≤ 0.05 for all hypothesis testing.

Results

The mean RLU for High risk HPV calibrator (positive cutoff; CO) was 516 and Negative calibrator mean value was 166. The mean RLU for samples was found in the range of 141-5,94,619. The comparison between HPV positive and negative groups by age, ethnicity, education, age at first pregnancy, menstrual regularity, Infection history and safe sexual conduct was shown in Table 1. A total of forty three healthy asymptomatic women cervical exfoliated cell specimens were analyzed by HC2 assay. The occurrence of high-risk HPVs was confirmed in 16.2% (7/43) of the women. The mean age of HR- HPV-positive group was 37.5 years, with a range of 32-49 years. Mean age in HPV-positive group was not significantly different than HPV negative group (mean age: 38.8 years; range 22-65 years) ($p=0.652$). There was no statistically significant difference between HR- HPV-positive and negative groups observed for Ethnicity ($p=1.00$), education status

($p=1.000$), age at first pregnancy ($p=0.664$), safe sexual conduct ($p=0.656$). Among the HR-HPV positive women, 85% were in the age group >35 years whereas 14% were in ≤ 35 years. Among the HR-HPV negative group, 30% of ≤ 35 years and 70% were >35 years.

However, menstrual irregularity ($p=0.039$) and infection history ($p=0.028$) had shown statistically significant difference between the HR-HPV-positive and negative groups. Between the HR-HPV positive and negative group, 67% of the positive group women and 10% of the negative group women had the irregular menstrual pattern. Infection history was found in 55% of the HPV positive group whereas it was only 7% in HPV negative group. The HPV positive women were subjected for Colposcopic examination. One of the HR-HPV positive women in which RLU/ CO ratio was 1152, has been confirmed for CINII in colposcopic biopsy examination. Another woman with RLU/CO ratio of 3.54 was also confirmed for CINI. The remaining of the HR-HPV positive cases were found normal in colposcopic examination.

Discussion

In our present study, significant association was found in the occurrence of HR- HPVs in the positive and negative group of women especially with infection history ($p=0.02$) and menstrual irregularity ($p=0.03$).

According to the World Health Organization estimates more than 300 million new infections of *Trichomonas vaginalis*, *Chlamydia trachomatis* or *Neisseria gonorrhoea* of female genitalia occur annually throughout the world. Individuals infected with one microorganism are often more susceptible to infection with a second sexually transmitted pathogen (Fahey et al., 2006). We observed that significant proportion of women in HR-HPV positive group had a history of infection in their urogenital tract as compared to the negative group.

Generally, adolescents and young adults are the demographic age groups most frequently affected with sexually transmitted infection, and women are more likely than men to suffer the consequences of these serious infections (Fahey et al., 2006). Among the HR-HPV positive and negative group of women in our study, 72% were in the age group of >35 years.

Endocrine-induced changes during the menstrual cycle control uterine cell growth and differentiation, as well as the composition and function of immune cells in the uterus. The human uterus undergoes dynamic changes during the menstrual cycle; these changes are largely driven by the sex steroid hormones estradiol and progesterone. Human uterine epithelial cells act as sentinels of protection against potentially pathogenic microorganisms, promote normal reproductive tract physiology including during pregnancy, and play an essential role in regulating immunity (Fahey et al., 2006). We found that prolonged period of irregular menstrual event was predominant among 67% of the women with HR-HPV positivity. Whereas only 10% women in the HR-HPV negative group had problem of irregular menstruation. An irregular menstruation for prolonged period may make the women more susceptible

Table 1. Characteristics of Asymptomatic Healthy Women Participants (n=43)

Characteristics	HR HPV positive	HR-HPV Negative	p-value [§]
Age			
≤ 35	1	11	
> 35	6	25	0.65
Ethnicity			
Native*	4	19	
Non-Native	3	17	1
Education			
Upto Secondary	4	18	
Graduate and above	3	18	1
Age at First Pregnancy			
< 18 Years	3	11	
≥ 18 Years	4	25	0.66
Menstrual Regularity			
Yes	3	30	
No	4	6	0.03 [#]
Safe Sexual Conduct			
Yes	3	10	
No	4	26	0.65
Infection History			
Yes	5	9	
No	2	27	0.02 [#]

*Indigenous community of North East India, [#]statistically significant, [§]Fisher's test

to harbor sexually transmitted infection like Human papillomavirus.

For a developing country like India with huge population, currently adopted cervical screening techniques (cytology) established in the developed countries, cannot be implemented as such. Following of the few factors can better explain the Indian scenario: (a) Lack of appropriate logistical infrastructure, (b) Critical shortage of sufficiently trained clinicians and cytotechnicians to perform these screenings, (c) dearth of systematic organization of community oriented screening programs and (d) poor procedure for follow-up.

As cervical cancer prevention strategy, which is in practice across India, the current recommendation is one time cytology based screen at the age of 35-40 years. Due to low sensitivity of Pap test, annual cytology screening is predominantly followed as standard protocol in the clinics across western European and North American countries as a part and parcel of national health care policy. In India, more than two third of the population live in rural areas and approximately similar proportion of population falls under the category of poverty. They are entirely dependent on the government sponsored social welfare programmes such as National Rural Health Mission (NRHM) for their basic health requirements.

The HPV DNA test by HC2 is automated, sensitive and rapid. Due to automation it reduces the chances of inter-observer differences which are common in cytology based screening. Its minimum threshold detection limit of 5000 copies of viral load in a clinical specimen offers suitably high sensitivity. This test can be completed in less than 4 hours. There have been a few Indian studies which provided supporting evidence that self-collected HC2 samples may have fairly similar sensitivity in comparison to the physician collected specimen when analyzed by automated HC2 system (Bhatla et al, 2009; Sowjanya et al, 2009).

Self-collection of cervical samples for human papillomavirus (HPV) testing can help to raise the participation rate in cervical cancer screening among non-participating women for cervical cytology screening (Jentschke et al., 2013). With visual presentation the self collection can be explained to the participant women. The simplicity of self-collection feature of HC2 assay for HPV DNA testing can be a suitable option because it obviates the need for a speculum examination and also the need to visit a physician or hospital for this purpose. It may be especially suitable for remote geographical locations, such as in North East India, where these facilities may not be in appropriate order. Self collection also offers a privilege of privacy to the women. This privacy factor may be of significance with reference to those communities in our country of diverse ethnic cultural traditions, where women still have sadly little access to qualified physicians and clinics for their urino-genital tract related clinical issues.

A recent randomized trial has recommended that RLU/CO ratio >2 can be a suitable diagnostic threshold for assessment of HPV DNA positive women at risk of developing CIN lesions concomitantly and reducing the probability of over-diagnosis (Gillio-Tos et al., 2013). Our present study has shown that four number of HR-HPV

positive cases have the RLU/CO ratio within the range of 1-2 whereas it was >3 for remainder of the three cases. The two cases which were confirmed for CIN had RLU/CO ratio more than three.

The HC2 assay can assist in identification of HR-HPV positive women who need immediate cytological assessment and further course of treatment. The currently available updates for cervical cancer screening guidelines from the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology recommend cytology and HPV co-testing as the preferred primary screening strategy for women aged 30-65 years. It is advocated that the interval for repeat screening can be extended to 5 years due to the increased sensitivity with this co-testing. The HR-HPV testing may be useful for management of patients with abnormal cervical smears (Tao et al., 2013). It has been shown that a single HPV test at around 35 years of age is the most cost-effective intervention in reducing the burden of cervical cancer in countries with low or no screening coverage (Goldie et al., 2005). Katyal and Mehrotra (2011) suggested that women with atypical cells of undetermined significance (ASCUS) and Low-grade Squamous Intraepithelial Lesion (LSIL) on cervical cytology should be subjected to HPV DNA test and only if found positive should be referred for colposcopy.

In conclusion, in many low and middle income countries validated mass HPV screening assays are still out of reach for government sponsored health care policy. Though HC2 assay offers a quantity of advantage over cervical cytology but the price tag associated with this assay has been a major obstacle in its realization across the clinics of India. It may be too early to arrive at a definitive conclusion about the immediate clinical application of HC2 assay in North-East India but this assay hold some promising features for high risk HPV DNA detection which may need careful consideration. Our data indicates that there may be an interrelation between clinical symptoms of irregular menstruation and infection history of urino-genital tract with HR-HPV DNA occurrence. Further study in carefully selected larger samples from North-East Indian healthy women will be required to ascertain the validity of the present observations.

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References

- Arbyn M, Roelens J, Simoens C, et al (2013). Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. *Cochrane Database Syst Rev*, **3**, 8054.
- Basu P, Mittal S, Bhaumik S, et al (2013). Prevalence of high-risk human papillomavirus and cervical intraepithelial neoplasias in a previously unscreened population—a pooled analysis from three studies. *Int J Cancer*, **132**, 1693-9
- Bhatla N, Dar L, Patro AR, et al (2009). Can human

- papillomavirus DNA testing of self-collected vaginal samples compare with physician-collected cervical samples and cytology for cervical cancer screening in developing countries? *Cancer Epidemiol*, **33**, 446-50
- Bhatla N, Puri K, Joseph E, et al (2013). Association of Chlamydia trachomatis infection with human papillomavirus (HPV) & cervical intraepithelial neoplasia-a pilot study. *Indian J Med Res*, **137**, 533-9
- Bhatla N, Puri K, Kriplani A, et al (2012). Adjunctive testing for cervical cancer screening in low resource settings. *Aust N Z J Obstet Gynaecol*, **52**, 133-9
- Brun-Micaleff E, Coffy A, Rey V, et al (2013). Cervical cancer screening by cytology and human papillomavirus testing during pregnancy in French women with poor adhesion to regular cervical screening. *J Med Virol*, **86**, 536-45.
- Cubie HA, Cuschieri K (2013). Understanding HPV tests and their appropriate applications. *Cytopathology*, **24**, 289-308
- Das D, Rai AK, Katakki AC, et al (2013). Nested multiplex PCR based detection of human papillomavirus in cervical carcinoma patients of North- East India. *Asian Pac J Cancer Prev*, **14**, 785-90
- Datta P, Bhatla N, Dar L, et al (2010). Prevalence of human papillomavirus infection among young women in North India. *Cancer Epidemiol*, **34**, 157-61
- Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.
- Ferreccio C, Barriga MI, Lagos M, et al (2012). Screening trial of human papillomavirus for early detection of cervical cancer in Santiago. *Int J Cancer*, **132**, 916-23
- Gillio-Tos A, De Marco L, Carozzi F M, et al (2013). New Technologies for Cervical Cancer of the Hybrid Capture 2 Test: Data from the (NTCC) Study. *J Clin Microbiol*, **51**, 2901-7
- Giorgi-Rossi P, Franceschi S, Ronco G (2012). HPV prevalence and accuracy of HPV testing to detect high-grade cervical intraepithelial neoplasia. *Int J Cancer*, **130**, 1387-94
- Goldie SJ, Gaffikin L, Goldhaber-Fiebert JD, et al (2005). Cost effectiveness of cervical cancer screening in five developing countries. *N Engl J Med*, **353**, 2158-68
- Jentschke M, Soergel P, Hillemanns P (2013). Evaluation of a multiplex real time PCR assay for the detection of human papillomavirus infections on self-collected cervicovaginal lavage samples. *J Virol Methods*, **193**, 131-4
- Katyal S, Mehrotra R (2011). Complementary procedures in cervical cancer screening in low resource settings. *J Obstet Gynaecol India*, **61**, 436-8
- Kitchener HC, Almonte M, Thomson C, et al (2009). HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. *Lancet Oncol*, **10**, 672-82
- Leinonen M, Nieminen P, Kotaniemi-Talonen L, et al (2009). Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. *J Natl Cancer Inst*, **101**, 1612-23
- Meijer CJ, Berkhof J, Castle PE, et al (2009). Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int J Cancer*, **124**, 516-20
- Pandey S, Mishra M, Chandrawati (2012). Human papillomavirus screening in north Indian women. *Asian Pac J Cancer Prev*, **13**, 2643-6
- Piyathilake CJ, Badiga S, Paul P, et al (2010). Indian women with higher serum concentrations of folate and vitamin B12 are significantly less likely to be infected with carcinogenic or high-risk (HR) types of human papillomaviruses (HPVs). *Int J Womens Health*, **2**, 7-12
- Ronco G, Giorgi-Rossi P, Carozzi F, et al (2008). Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test. *J Natl Cancer Inst*, **100**, 492-501
- Sarian LO, Derchain S, Shabalova I, et al (2010). Optional screening strategies for cervical cancer using standalone tests and their combinations among low- and medium-income populations in Latin America and Eastern Europe. *J Med Screen*, **17**, 195-203
- Schiffman M, Wentzensen N, Wacholder S, et al (2011). Human papillomavirus testing in the prevention of cervical cancer. *J Natl Cancer Inst*, **103**, 368-83
- Sowjanya AP, Jain M, Poli UR, et al (2005). Prevalence and distribution of high-risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India. *BMC Infect Dis*, **5**, 116
- Sowjanya AP, Paul P, Vedantham H, et al (2009). Suitability of self-collected vaginal samples for cervical cancer screening in periurban villages in Andhra Pradesh, India. *Cancer Epidemiol Biomarkers Prev*, **18**, 1373-8
- Tao K, Yan J, Yang H, et al (2013). Comparative study of the Cervista and Hybrid Capture 2 methods in detecting high-risk human papillomavirus in cervical lesions. *Diagn. Cytopathol*. [Epub ahead of print??]
- Three-Year Report of Population Based Cancer Registries 2009-2011: Incidence and Distribution of Cancer. (2013). NCDIR-NCRP (ICMR), Bangalore.
- Zhao C, Weng B, Li Z, Yang H, Austin RM (2013). Follow-up outcomes of a large cohort of low-risk women with negative imaged liquid-based cytology and negative HPV test results. *Am J Clin Pathol*, **139**, 32-8