Molecular genetic studies on HPV related cervical cancer in Indian women

Human Genetics Unit
Indian Statistical Institute, Kolkata, India

Informed consent:

I am about to enter a study conducted by the Indian Statistical Institute, 203 B.T. Road, Kolkata, on cervical cancer. The study aims at improving the diagnosis and treatment of HPV-associated cervical lesions and cancer. Hence, it involves medically examining the status of the neck of my womb with a smear test (Pap Test), virus (Human Papilloma Virus, HPV) test, a vinegar (4% acetic acid) application test if necessary, and tissue biopsy of my cervix. All the possible risks and benefits have been explained to me and I have understood them.

I have been told that (a) A woman may have a condition in the neck of her womb, called dysplasia. Dysplasia can be evaluated and safely followed with a routine (3-4 month) Pap test and vinegar tests. However, left untreated, dysplasia may turn into cancer over time. (b) These tests are painless and harmless, and can be performed in an outdoor clinic or Doctor's office or in a hospital. I understand that a small scraping from the neck of my womb will be taken with a flat wooden spatula; a small piece of tissue will be collected from my cervix which will be used for molecular genetic analysis. (c) No discomforts or any infections from these procedures are anticipated; (d) I shall be free to leave the study at any time.

I am hereby giving consent at my own free will and free from any pressure to (a) collection of cell or tissue materials from my cervix and (b) all types of analysis of the materials for non-profit research purpose towards prevention of cancer by the Indian Statistical Institute or their direct collaborators of this study.

I am however, NOT giving consent for disclosure of any personal information either direct or derived from the analysis of my biological samples to anyone without my further consent.

Witness Date Signature

Name: Address:

Mother's/Parent's/Guardian's name:
List of publication


  (^these authors have contributed equally)

Conferences attended and awards received during the course of PhD:

- **Oral presentation** in the 30th annual convention of Indian Association for cancer research and International symposium on “Signaling Network and Cancer” organized by Indian Institute of Chemical Biology (CSIR-IICB), Kolkata, India (6th-9th February, 2011).

- Received “**Best Poster Award from an Emerging Country Participant**” in Basic Science and **Travel Award** from International Papillomavirus Society (IPVS), in the 26th International Papillomavirus Conference, organized by IPVS, at Montreal, Canada (3-8 July, 2010).

- Awarded **1st prize in poster presentation** from the hands of Nobel laureate Dr. Harald zur Hausen, at the “Symposium on Cervical Cancer Control in India” organized by Cancer Foundation of India (CFI), Kolkata, India (December 4, 2009).
• **Young scientist award** (First Prize) in the Biennial Conference (Interim) of Asia Oceania Research Organization on Genital Infection and Neoplasia (AOGIN) organized by AOGIN and Chittaranjan National Cancer Institute, Kolkata, India (April 25-26, 2009).

• **Young investigator award** (First Prize) in the “International symposium on Human Papillomavirus-associated cancers: translating research into cancer prevention and medicine” organized by Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India (November 1-3, 2008).

• **Best Poster Award** in the XXXIII Annual Conference of the Indian Society of Human Genetics and International Symposium on “Genetics Revisited: the Genomics and Proteomics Advantage” organized by Andhra University, Visakhapatnam, Andhra Pradesh, India (February 11-13, 2008).

• Received **Travel Award** in the 13th **Human Genome Meeting** organized by Human Genome Organization (HUGO) and Council for Scientific and Industrial Research (CSIR), India at Hyderabad, India (September 27-30, 2008).
Association of viral load with HPV16 positive cervical cancer pathogenesis: Causal relevance in isolates harboring intact viral E2 gene

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ABSTRACT

We tested the hypothesis that cervical cancers (CaCx) harbor high HPV16 viral load compared to controls and this is influenced by E2 status and age of subjects. Viral load (natural log transformed values) per 100 ng genomic DNA was estimated (152 cases and 87 controls) by Taqman assay. Median viral load was significantly higher (Mann-Whitney U test) among cases (17.21) compared to controls (9.86), irrespective of E2 status or upon considering E2 status as a covariate in logistic regression model (p < 0.001). Viral load of E2 intact cases (17.80) was significantly higher (p < 0.001) compared to those with disrupted E2 (9.78). At equivalent probability of being a case, viral load was higher among individuals (i) of lower age, irrespective of E2 status, and (ii) with intact E2 but of similar age as those with disrupted E2. Thus viral load in association with E2 status and/or age might be of causal relevance in CaCx pathogenesis.

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Introduction

Cervical cancer (CaCx) is one of the leading causes of death among Indian women, contributing to one-fourth of the global total of deaths incurred due to this. Recent reports (http://www-dep.iarc.fr/) depict that 132,082 new cases and 74,118 deaths are recorded every year (age-standardized rates: incidence = 30.7; mortality = 17.8) in India. While HPV infections appear to be the major etiological factors for carcinogenesis, after a long latent period. This points to the need of HPV testing and the identification of HPV-related cofactors that might be relevant for detecting those at risk of developing CaCx in the long run.

It is known that viral genome in episomal form replicates along with the differentiating epithelial cells from basal membrane to the superficial zone and is shed off along with the sloughed-off epithelial cells resulting in transient infection (zur Hausen, 2002). Persistent infection and viral genome integration into the host genome is known to mediate oncogenicity (Woodman et al., 2007). E1 and E2 are the early viral proteins needed for viral replication and translation, while E6 and E7 are the oncoproteins responsible for cellular transformation by inactivation of p53 and pRb proteins, respectively. E2 protein represses E6 and E7 expression. Integration of viral genome into the host genome, chiefly at fragile sites (Kalantari et al., 2001; Wentzensen et al., 2004), not only affects various cellular pathways of the host cell-cycle machinery, but also disrupts the viral E2 gene most commonly in the hinge region of the HPV16 E2 protein. In absence of E2-driven repression, E6 and E7 are overexpressed driving infected cells toward transformation.

On the contrary, our study (Bhattacharjee and Sengupta, 2006a) as well as a few others (Narayan et al., 2004) has identified that a substantial proportion of individuals with CaCx harbor intact E2, which could be either purely intact or concomitant, i.e., a mixture of intact and disrupted forms. Such observations, point toward the biological plausibility of cervical carcinogenesis under the impact of HPV16 intact E2 gene or intact viral genomes, in addition to E2 disruption due to viral genome integration into the host genome. Many study groups have proposed viral load estimates per cell or per unit amount of genomic DNA, as a potential HPV-related biomarker, which could be used for predicting those at risk of CaCx development (Franco and Coutlée, 2009). However, there are several reports, which have failed to relate high HPV16 DNA copy number with CaCx development (Swan et al., 1999; Josefsson et al., 2000; van Duin et al., 2002; Hernandez-Hernandez et al., 2003; Abba et al., 2003; de Boer et al., 2007).

We undertook the present study to investigate the association of HPV16 viral load, if any, with CaCx by comparing HPV16 positive cytologically normal women with those diagnosed with CaCx (squamous cell carcinomas). Apart from considering the potential of viral load...