

**ABSENCE OF HUMAN PAPILLOMAVIRUS GENOME IN OCULAR SURFACE  
SQUAMOUS NEOPLASIA PATIENT FROM NORTH EAST INDIA**

Akshay Chandra Deka<sup>1\*</sup>, Ananta Madhab Dutta<sup>2</sup>, Vidhya S.<sup>1</sup> and Pratap Chandra Sarma<sup>1</sup>

<sup>1</sup>Department of Medical Laboratory Technology, Assam down town University, Guwahati, Assam.

<sup>2</sup>Department of Chemistry, Assam down town University, Guwahati, Assam.

**Corresponding Author: Dr. Akshay Chandra Deka**

Department of Medical Laboratory Technology, Assam Down Town University, Guwahati, Assam.

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**ABSTRACT**

**Aim** - To study the probable human papilloma virus infection in Ocular surface squamous neoplasia (OSSN from North eastern part of India. **Materials and method**- We have retrieved histologically proven cases of OSSN formalin-fixed, paraffin-embedded tissue blocks (n = 40) and fresh tissues (n = 5) for the study. The presence of HPV was detected by polymerase chain reaction using MY09/MY11 consensus primers. SiHa and HeLa cell lines were used as positive controls for HPV analysis. **Results**- This study includes 31 squamous cell carcinomas and 9 conjunctival intraepithelial neoplasias. All of the cases were found to be negative for HPV using polymerase chain reaction whereas the SiHa and HeLa cell lines were positive which harbor HPV DNA. **Conclusions**- Sensitive, polymerase chain reaction did not show the presence of HPV DNA in OSSN. Thus, HPV appears to play no significant role in the etiology of OSSN in northeastern India.

**KEYWORDS:** Human papilloma virus, CSSC, OSSN, Polymerase chain Reaction.

**INTRODUCTION**

Human papillomavirus (HPV) is belonging to the Papoviridae family and a double-stranded DNA virus.<sup>[1]</sup> Over 170 types of HPV have been identified, and they are designated by numbers.<sup>[2]</sup> HPV is the most common sexually transmitted infection globally. Most people are infected at some point in their lives.<sup>[3]</sup> The role of HPV in the origin of squamous cell carcinoma of the cervix, anogenital region, carcinoma of the head and neck, and oral mucosa is well established.<sup>[5]</sup> but its role in ocular surface squamous neoplasia (OSSN) is still vague. These tumors, most often seen in elderly patients, comprise a spectrum of intraepithelial and invasive tumors.<sup>[4]</sup> OSSN are the most common tumors of the ocular surface. They are slow growing, and they usually appear as mass like lesions associated with redness and conjunctivitis. Contributing factors implied in the pathogenesis of OSSN include high exposure to ultraviolet rays, irradiation, immunosuppression after human immunodeficiency virus infection, organ transplantation, chronic irritation and genetically predisposed states, such as xeroderma pigmentosum (XP).<sup>[5]</sup> Some studies have shown HPV to be associated with benign papillomas and Squamous cell carcinoma of human conjunctiva, whereas others have not found an association. In this study, we wanted to explicate the role of HPV in OSSN occurring in Northeastern India, using a sensitive type-specific polymerase chain reaction (PCR) assay.

**MATERIALS AND METHOD**

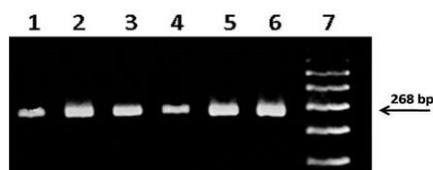
The study protocol was approved by the ethics committee of Assam down town University, Guwahati. Laboratory processing of the preserved blocks done by fixation with 10% neutral buffered formalin and embedding in paraffin wax. The block were stored in room temperature. Blocks with a large amount of tissue were sectioned and selected for DNA extraction. The medical records of the patients were reviewed for clinical details, chdemographics and other systemic diseases. 4-5-µm thickness sections were prepared from each paraffin block. HeLa cell lines were used as positive controls for HPV analysis.

**DETECTION OF HPV BY PCR**

DNA extraction was done by QIAamp DNA mini kit (Qiagen, Germany). Extracted DNA was subjected to PCR for β globin gene targeting of the 248 base pair fragment of the β globin gene for the quality of DNA. PCO4 (5'GAAGAGCCAAGGACAGGTAC-3') and GH20 (5'-CAACTTCATCCACGTTACC-3') primers were used as a forward and a reverse primer respectively. The reaction mixture contained 10mM of Taq buffer, 1.5mM MgCl<sub>2</sub>, 2.5U of Taq polymerase, 200µm each dNTP and 10 pmol of each primer. The conditions for the PCR were initial denaturation of 94°C for 4 min, followed by 35 cycles of 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 45 seconds and a final

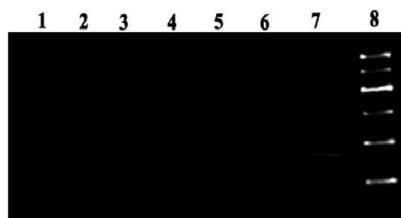
extension at 72°C for 5 min. PCR products were analyzed on a 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

The consensus primer (M09/My11) was used for amplifying HPV. The primer set (My09: 5'-GCACAGGGACATAACAATTGG-3' and My11: 5'-CGTCCAAAAGGAAACTG-3') was capable of amplifying a wide spectrum of HPV types to produce a PCR product of 450 bp.<sup>6</sup> The amplification mixture consisted of 1x PCR buffer, (10 mM Tris/HCl, pH 8.3, 50 mM KCl, and 1.5 mM MgCl<sub>2</sub>), 200 μM of each dNTP, 100 pmol of each primer, 2.5 units of Taq DNA polymerase (Bangalore Genei, cat-105926/Old No.MME5M and 500 ng of DNA in a final volume of 25 μl. Thirty five cycles were completed as follows: 45 s at 94°C, 45 s at 45°C, and 45 s at 72°C. The initial denaturation step was for 3 min at 94°C, and a final extension step was prolonged to 5 min at 72°C. Each batch of samples included negative controls containing water and positive control DNA from an HPV positive HeLa cell lines. The first round amplified product was used in the second round PCR. Thirty five amplification cycles were completed according to the following protocol: Initial denaturation was for 5 min at 94°C, followed by denaturation for 1 min at 94°C, annealing for 2 min at 45°C, extension for 1.5 min at 72°C, and a final extension step of 5 min at 72°C. Each batch of samples included first round negative control and first round positive control product. GP05/GP06 primer set, a nondegenerate primer (GP05: 5'-TTTGTTACTGTGGTAGATAC-3' and GP06: 5'-GAAAAATAAAGTGTAAATCA-3') was used for second round PCR, which detects a PCR product of approximately 145 bp.<sup>7</sup> PCR products were analyzed on a 2% agarose gel and visualized by ethidium bromide staining.



lane 1-6: OSSN tissue sample  
Lane 7: Molecular weight marker (100bp- 600bp)

**Figure 1**



Lane 1-6: OSSN tissue samples  
Lane 7: positive control(HeLa Cell lines)  
Lane 8: Molecular weight marker (100bp- 600bp)

**Figure 2**

## RESULT

Extracted DNA was subjected to Beta globin house keeping gene PCR to check the quality of extracted DNA. Sensitivity of the nested PCR was checked with positive control HeLa cell lines. PCR could amplify DNA of HeLa cells DNA upto 10<sup>-2</sup> dilution. Primers were not able to amplify other viral DNA such as HSV 1 and 2, CMV, VZV and Adeno virus and were found to be specific only for human papilloma virus DNA

Thirty one Squamous Cell Carcinoma (SCC), and 9 Conjunctival Intraepithelial Neoplasia (CIN) specimens were included in the study. DNA extracted from all of the samples was amplified using the internal control β globin gene primers, which showed a product of 248 bp (Figure 1). Thus we confirmed the quality of DNA extracted from fixed tissues was suitable for PCR. Amplification of the DNA with HPV-specific primers showed that all OSSN cases were negative for conserved HPV sequences (Figure 2), whereas the positive controls for were amplified.

## DISCUSSION

HPV DNA was not detected “and in conjunctival intraepithelial neoplasias (OSSN)” carcinoma samples by nested PCR using primers sets of the HPV consensus L1 region. The relative amounts of HPV DNA detected in Positive samples yielded strong signal that negative tissue specimen is specific for HPV L1 orf. These results suggest that there maynot be any role of HPV in the development of cancer however type specific PCR and a large number of PCR should perform to check the presence of HPV genome in conjunctival neoplasia. We have also reported absence of HPV in esophageal squamous cell carcinoma from Northeastern region.<sup>[6]</sup>

A Study from Hyderabad, 48 patients with OSSN of specimens included 36 squamous cell carcinomas and 21 conjunctival intraepithelial neoplasias. All of the cases were found to be negative for high-risk HPV using polymerase chain reaction and ISH-CARD assay.<sup>[4]</sup> Another study from AIIMS, Delhi analysed 65 cases Immunohistochemically where, 35 were papillomas and 30 were CSCC. 17.1% conjunctival papillomas stained positive for HPV antigen, all cases of CSCC were negative for HPV. There was no correlation between koilocytosis or actinic keratosis and the detection of HPV antigen.<sup>8</sup> The association between HPV and CSCC is variable in different geographical areas and also depends on the methods of detection used. However, Sensitive, polymerase chain reaction assay for consensus HPV sequences, did not show the presence of high-risk HPV in CSCC. Thus, HPV appears to play no significant role in the TO Etiology of OSSN in Northeastern part of India.

Even though the clinical and histologic spectrum of squamous cell carcinoma is expected to be the same everywhere, the notable difference in the age and the absence of HPV in our population could point toward

some other undetermined etiologic factors that could explain the geographic differences. We therefore conclude that HPV unlikely to play a significant role in the to Role In The Oncogenesis of OSSN in patients of Northeastern Indian origin.

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