

Skin mediated human papillomavirus infection in breast: A report of four cases

Saimul Islam¹, Hemantika Dasgupta¹, Mukta Basu¹, Anup Roy², Neyaz Alam³, Gautam Kumar Mandal⁴, Susanta Roychoudhury⁵, Chinmay Kumar Panda¹

From ¹Departments of Oncogene Regulation, ³Surgical Oncology and ⁴Pathology, Chittaranjan National Cancer Institute, ²Department of Pathology, Nil Ratan Sircar Medical College and Hospital, ⁵Saroj Gupta Cancer Centre and Research Institute, Thakurpukur, Kolkata, West Bengal, India

Correspondence to: Chinmay Kumar Panda, Department of Oncogene Regulation, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata-700026, West Bengal, India. Tel: +91-33-24743922, Fax: +91-33-2475-7606. E-mail: ckpanda.cnci@gmail.com
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ABSTRACT

To address the ambiguity of different modes of human papillomavirus (HPV) transmission in breast, the immunohistochemical expression of two oncoproteins E6/E7 of HPV16 was analyzed in primary breast cancer (BC) and adjacent normal skin of 4 samples. The patients were of 35–55 years old having no previous history of cancer. The E6/E7 expressions were evident in both skin and BC. In skin, high/moderate cytoplasmic expressions of E6/E7 proteins were seen in all samples, whereas in BC, high/moderate cytoplasmic expressions of the proteins were observed in 2–3 samples. Thus, it seems that HPV infection in the breast may occur through the skin.

Key words: Breast cancer, breast skin, E6/E7 oncoproteins, human papillomavirus, human papillomavirus transmission

Breast cancer (BC) is a frequent disease (25%) among women worldwide [1]. In India, its occurrence is about 18.5% [1]. Different etiological factors such as hormonal status, tobacco habits, alcohol consumption, and family history have been suggested to be associated with the disease [2]. In addition, human papillomavirus (HPV) infection has also been suggested to be an etiological factor in the development of the disease [3]. Globally, the HPV infection varies differentially (23.3–86%) in BC [4]. However, in India, its frequency varies between 0 and 26% [4,5]. Recently, our group also showed that HPV16 was prevalent subtype in Indian BC population [6]. However, there is still doubt in its possible mode of infection of HPV in breast tissue: (1) Transmission through blood, lymphatic systems or any other body fluid in patients with previous history of HPV-positive uterine cervical lesions, (2) transmission through oral sexual activity, and (3) transmission through nipple or microlesion of breast skin [7,8]. To address this ambiguity, in the present study, we have detected HPV in four primary BC samples and adjacent skin of the corresponding patients having no previous history of cervical lesions.

CASE REPORT

The four breast tumor specimens were collected during the year 2011–2014 from Chittaranjan National Cancer Institute (CNCI), Kolkata, India, after appropriate approval of the Institutional Ethical Committee. Written informed consent was obtained from either of the patients or next kin of the respective patients. The age of the patients ranged from 35 to 55, with no previous history

of cancer and tobacco habits (Table 1). One of the four cases has undergone chemotherapy before surgery. Among four cases, two tumors showed poorly differentiated form, whereas other two cases showed moderately differentiation. Depending on receptor status, two of four cases were belonging to luminal B, whereas one case falls in HER2-positive BC subtypes. After starting of therapy, three patients died within 5 years, but one patient is surviving even after 24 months. The detailed descriptions of four cases are given in Table 1.

Detection of HPV16 E6 and E7 Proteins Expression

The expression of HPV16 E6 and E7 proteins was analyzed in the tumors and adjacent skin samples by IHC using paraffin-embedded tissue sections [9]. About 5 μm thick sections were dewaxed, rehydrated, and incubated in 10 mM citrate buffer (pH-6.0) at 85°C for 40 min to retrieve the antigens followed by incubation with primary antibodies at a dilution of 1:200 at 4°C for overnight. Goat polyclonal IgG for E6 (sc-1584) and mouse monoclonal IgG for E7 (sc-6981) were purchased from M/s Santa Cruz Biotechnology, CA, USA. HRP-conjugated rabbit anti-goat IgG (sc-2768) and HRP-conjugated goat anti-mouse IgG (sc-2005) as secondary antibodies (M/s Santa Cruz Biotechnology, CA, USA) for E6 and E7, respectively, were added at 1:500 dilutions. The slides were developed using 3-3' diaminobenzidine as the chromogen and counterstained with hematoxylin. The staining intensity (1=weak, 2=moderate, and 3=strong) and the percentage of positive cells (<1=0, 1–20 =1, 20–50=2, 50–80=3, and >80=4) were detected by two observers

Table 1: Clinical and histopathological history of the four HPV16-infected breast cancer cases

Parameters	Case-I	Case-II	Case-III	Case-IV
Case description	A 54-year-old woman: Mammography and FNAC were used to diagnosed in right-sided breast tumor	A 35-year-old woman: FNAC detected breast cancer in left sided	A 55-year-old woman: FNAC diagnosed left-sided breast cancer	A 55-year-old woman: USG detected solid and heterogynous growth in left-sided breast and was confirmed cancer by FNACC
Symptom with duration	Lump in right breast for 1 year	Lump in left breast for 7 months	A fungating mass in left breast for 2.3 year	Breast lumps increasing in size for 8 months
Local examination finding	A 2×3 cm hard nodule was palpated in breast upper and outer quadrant	A 7×6 cm lump was felt in upper and outer quadrant with matted axillary nodes	A fungating bleeding mass in upper quadrant in left breast along with axillary nodes	A 2×1.5 cm mass felt in upper and outer quadrant
Family history	She has no previous family history of cancer	Previous family history of cancer was absent	Patients has no previous family history of cancer	Family history of cancer was absent
Previous history of cancer	Absent	Absent	Absent	Absent
Patients habits'	The patient has no any tobacco habits	The patient was not habited with any type of tobacco	Any type of tobacco habit of patients was absent	The patient was not habited with any type of tobacco
Treatment sequence	Surgery followed chemotherapy and radiotherapy	Neo-adjuvant chemotherapy followed by surgery then completion chemotherapy and radiotherapy	Surgery followed chemotherapy	Surgery followed chemotherapy and radiotherapy
Surgical description	MRM	MRM	Toilet mastectomy	MRM
Surgical specimen description	Mastectomy specimen of 17.5×16×6 cm mass, in which skin flap of 16×17.5 cm was present with an area of ulceration 1.5×1.5 cm near lateral margin of the specimen was observed. In addition, 3×4.5×1.7 cm mass was observed beneath the unaltered area. 11 lymph nodes identified, largest being 1.5×1 cm and smallest 0.3 cm	N/A	N/A	Mastectomy specimen of 18×16×6 cm mass in which skin flap was 14×6 cm. In this specimen, 2×1.5×4 cm area was ulcerated which was 1 cm away from cut margin
Histopathology of Breast tumor	Invasive Ductal Carcinoma with Grade-III	Invasive Ductal Carcinoma with Grade-III	Ductal carcinoma-Grade-II	Infiltrating Ductal Carcinoma with Grade-II
Auxiliary lymph node histopathology	4/11 lymph nodes show metastatic deposit	9/11 lymph nodes show metastatic deposit	Lymph nodes positive (detail not known)	All 11 lymph nodes were free of tumor
Clinical stage of tumor	N/A	T3N1M0	T4N1Mx	T2N0Mo
Tumor size in specimen	2×3 cm	7×6 cm	N/A	2×1.9 cm
Parity (No of child birth)	N/A	She has four children.	She has three children.	She has five children. Among them, only two are alive
Estrogen receptor status	Negative	N/A	Positive	Positive
Progesterone receptor status	Negative	Negative	Positive	Positive
Her2 receptor status	Positive	Negative	Positive	Positive
Chemotherapy	4 cycles of FEC - 4 cycles of taxanes FEC=5-Fluro-uracil, epirubicin, and cyclophosphamide Taxanes=Paclitaxel	4 cycles of FAC - 6 cycles of Paclitaxel and Cis-platin. FAC=5-Fluro-uracil, adriamycin, and cyclophosphamide	6 cycles of FAC FAC=5-Fluro-uracil, adriamycin, and cyclophosphamide	6 cycles of FEC FEC=5-Fluro-uracil, epirubicin, and cyclophosphamide

(Contd...)

Table 1: (Continued...)

Parameters	Case-I	Case-II	Case-III	Case-IV
Radiation	N/A	EBRT completed in Co60-50Gy-25 fraction from 11.3.13–16.4.13. Boost chest wall dose - 7.5 Gy in 3 fractions	Not given (patient died)	Radiation not required.
Survival	14 months after starting of therapy	5 months after starting of therapy	58 months after starting of therapy	Still now, she is alive during 24 months after starting of therapy

FNAC: Fine-needle aspiration cytology, N/A: Not available. MRM: Modified radical mastectomy, HPV: Human papillomavirus, USG: Ultrasonography

Table 2: Expression pattern of E6 and E7 proteins of HPV16 in normal breast skin and adjacent breast cancer

Cases	Normal breast skin				Breast cancer			
	E6		E7		E6		E7	
	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm
Case-I	Absent	M	L	H	L	L	Absent	H
Case-II	L	H	M	H	H	L	H	M
Case-III	L	H	L	H	M	H	H	L
Case-IV	L	M	L	H	Absent	M	L	M

L: Low expression; M: Moderate expression; H: High expression. HPV: Human papillomavirus

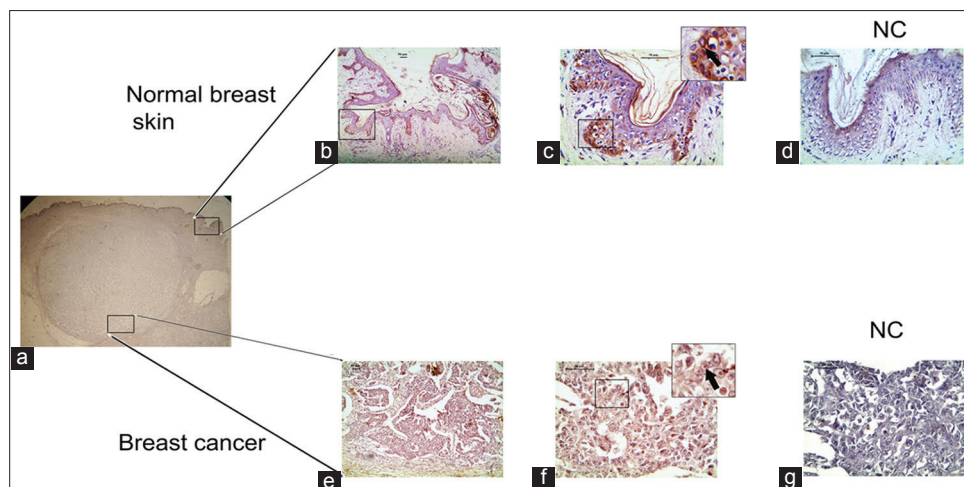


Figure 1: Immunohistochemical detection of E6 oncoprotein of human papillomavirus (HPV16)-positive breast carcinoma tissue and adjacent HPV16-positive normal skin: (a) IHC staining showed E6 protein expression in both normal skin and tumor tissue of the same BC sample. (b and c) IHC staining of E6 in HPV16-positive normal breast skin showed high cytoplasmic and low nuclear expression of E6 protein. (e and f) IHC of HPV16 E6 in BC showed high cytoplasmic and moderate nuclear expression of E6 protein. (d and g) IHC without primary antibody representing negative control [Magnification (a) is $\times 4$, (b and e) are $\times 10$, whereas (c and f) are $\times 40$, scale bars=50 μm]

independently and by combining the two scores. Then, the final evaluation of expression was done as follows: 0–2=low, 3–5=intermediate, and 6–7=high.

DISCUSSION

To understand the mode of HPV transmission in the breast, the expression of E6 and E7 oncoproteins of HPV16 was analyzed in primary BC and their respective adjacent normal skin tissues. Differential expression pattern of E6/E7 proteins was seen in BC and the respective skin (Table 2).

In normal breast skin, high/moderate cytoplasmic expression of E6 and E7 proteins was seen in basal cells with low/negative nuclear expression in the majority of cases (Figs. 1 and 2 and

Table 2). This indicates that breast skin may be one of target organs of the HPV infection. HPV DNA was detected in superficial layer of nipple epidermis, breast milk, ductal lavage fluid, and colostrum [10,11] and normal breast tissue [12]. HPV infection was also reported in normal perilesional skin of ear, wrist, forehead, leg, and temple [13]. The prolong infection of HPV in the skin has been shown to be associated with carcinogenesis [13,14]. Hence, the presence of E6 and E7 proteins in the basal layer of skin indicates the route of HPV transmission as suggested in uterine cervical epithelium [15].

Like skin, high/moderate cytoplasmic expressions of E6/E7 proteins were seen in 2–3 cases of BC (Figs. 1 and 2 and Table 2). However, high/moderate nuclear expressions of E6/E7 proteins were also evident in 2 cases. Similar, E6 and E7 protein expressions

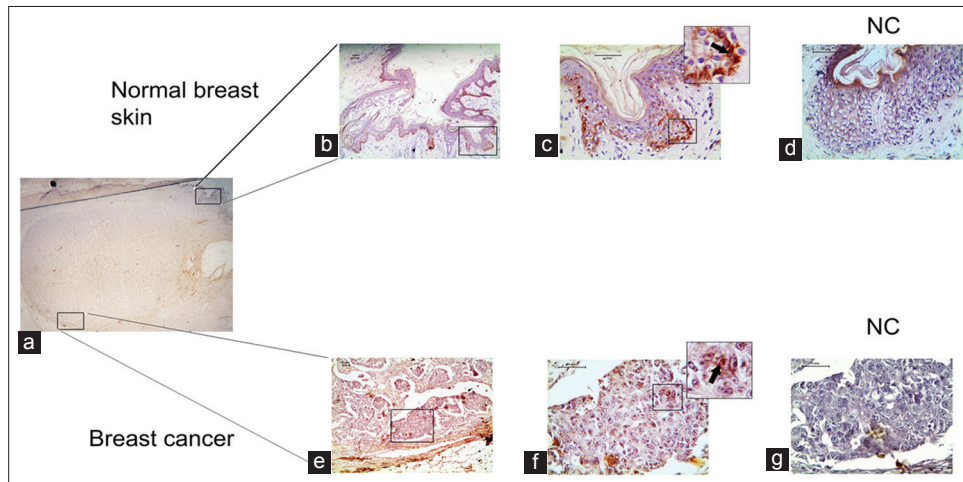


Figure 2: Immunohistochemical detection of E7 protein of human papillomavirus (HPV16) in breast cancer tissue and adjacent HPV16-positive normal breast skin: (a) IHC staining of E7 showed E6 protein expression in both normal breast skin and BC. (b and c) IHC staining of E7 in HPV16-positive normal breast skin showed high cytoplasmic and low nuclear expression of E7 protein. (e and f) IHC staining of HPV16 E7 in BC showed moderate cytoplasmic and high nuclear expression of E6 protein. (d),(g) IHC staining without primary antibody representing negative control [Magnification (a) is $\times 4$, (b and e) are $\times 10$, whereas (c and f) are $\times 40$, scale bars=50 μm]

were also detected in BC by Lawson *et al.* [16]. It was evident that the E6 and E7 oncoproteins of HPV16 could immortalize human mammary epithelial cells, indicating their importance in cellular transformation [17]. In addition, hrHPV DNA and its transcripts of E6 and E7 were also previously detected in BC though having a low copy number. Interestingly, reports pointed out that such low copy number of HPV16 is sufficient to bring about carcinogenesis as seen in HPV16-positive cervical squamous cell carcinoma cell line, SiHa [4,16,18].

CONCLUSION

The present report of the similar expression pattern of E6/E7 proteins in skin and adjacent BC indicates that HPV transmission in breast might have occurred through nipple or microlesion of breast skin in these cases. Although more samples need to be analyzed, it may perhaps be extrapolated to a model for plausible mode of transmission of HPV in breast tissue. This will be further useful to understand the HPV association in breast carcinogenesis along with its importance in prognosis and therapeutic implication in disease.

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