Breast cancer and HPV infection: No evidence of a viral etiology of breast cancer in Indian women

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Abstract

**Introduction** Prevalence of HPV infection which causes cervical cancer is highest in India. The two clinically most relevant high risk human papillomavirus (HR-HPV) types 16 and 18 commonly associated with cervical cancer are also reported in many other epithelial malignancies at extra-genital organ sites including breast which is the second most common cancer and is showing a fast rising trend in urban India. The two transforming gene products E6 and E7 of HPV type 16 have also been shown to immortalize breast epithelial cells in vitro, but the role of HPV infection in breast carcinoma is highly controversial. Present study has therefore been undertaken to analyze the prevalence of HPV infection in both breast cancer tissues and blood samples from a large number of Indian women with breast cancer.

**Methods** The presence of all mucosal HPVs and the most common high risk HPV types 16 and 18 DNA sequences was examined by three different PCR methods - (i) L1 consensus PGMY 09/11, (ii) GP5+ / GP6+ primers and (iii) highly sensitive Real-Time PCR using GP5+/GP6+ primers where WHO international standard of HPV 16 was used as reference for determination of copy number.

**Results** A total of 262 patients comprising 228 biopsies and corresponding 142 blood samples collected from four different regions of India with significant socio-cultural, ethnic and demographic varieties were tested but, none of the biopsy or blood samples from any region showed positivity for HPV DNA sequences in conventional PCRs either using PGMY 09/11 or by GP5+/GP6+ primers. Further testing of these samples by Real time PCR also demonstrated absence of HPV DNA sequences both in the tumor and blood samples of breast cancer patients, the majority of whom were in premenopausal stage and diagnosed to have infiltrating ductal carcinoma.
Conclusions Observation of complete absence of HPV DNA sequences either in the blood or in the tumor DNA of the same breast cancer patients even after employing the highly sensitive real time PCR disproves the possibility of any role of oncogenic genital HPV infection in the pathogenesis of breast cancer in Indian women.

Key words: Human papillomavirus (HPV), Breast carcinoma, Polymerase chain reaction (PCR), Real Time PCR.
Introduction

Breast cancer is the second most common cancer in the world, after the cancer of the lung, affecting one in eight women during their lifetime, but it is the leading cancer among women worldwide [1]. In India breast cancer is the second most dominant cancer in women, but it is showing a fast rising trend in major metropolitan cities in India [2]. Though various clinico-epidemiological and demographic risk factors both exogenous as well as endogenous including mutations in breast cancer susceptibility genes \textit{BRCA1} and \textit{BRCA2} [3, 4], sex-steroid hormones and family history have been strongly implicated with the development of breast cancer, the mechanism(s) of breast carcinogenesis is still not clearly understood.

Specific types of high risk human papillomaviruses (HR-HPVs) are considered potent human carcinogens which primarily cause cervical cancer but are also linked with other anogenital cancers and cancers of other organ sites [5] such as oral [6-8], esophagus [9-11], nasopharyngeal carcinoma [12-14] and retinoblastoma [15-17]. In India HPV is found to be present in almost 100% of cervical cancer patients and prevalence of high risk HPV type 16 is exceptionally high (~90%). HPV being an epitheliotropic DNA virus it has been detected in many carcinomas at extra-genital sites of epithelial origin [18-28]. The two transforming proteins E6 and E7 of high-risk HPVs have been shown to interact and inactivate tumor suppressor proteins p53 and Rb respectively [29-31]. Furthermore, these transforming proteins of high-risk HPV types 16 and 18 have also been shown to immortalize human mammary epithelial cells in-vitro [32, 33]. Even introduction of low risk HPV 6 E6 into the normal mammary cells leads to immortalization and modulation of p53 [34], Bak [35] and Gps 2 protein [36] functions. These observations strengthened a possible role of not only high risk but also low risk HPVs in the development of breast cancer. Several viruses such as Epstein-Barr Virus (EBV) [37-39], human equivalent of murine mammary tumour virus
and cytomegalovirus and HHV-8 including human papillomavirus (HPV) have been implicated in the etiology of human breast cancer. This has however, not confirmed by other authors [43-44]. Reports on the infection of HPV in breast cancer are not only limited but also highly controversial. While several authors [45-49] including our previous report on fine needle aspirated breast cancer cells [50] did not find any HPV infection in breast cancer, a moderate frequency of 20-45% HPV infection was reported by many authors [51-60]. Recently, a very high frequency of HPV infection ranging from 60 to 85% occurrence of HPV in breast cancer has been reported [61-64]. This prompted us to re-look into the role of HPV in a large number of breast cancer cases that were previously analyzed for mutations in BRCA1, BRCA2 and p53 tumor suppressor genes [65] and collected from all four regions of India. We used both conventional consensus L-1 PCR, GP5+ / GP6+ as well as highly sensitive real time PCR to detect HPV DNA sequences in tumour as well as corresponding blood samples of same breast cancer patients. The results reconfirm complete absence of HPV infection in breast cancer patients in India.

**Materials and methods**

**Breast cancer patient population and sample collection**

A total of two hundred sixty two-breast cancer patients comprising 100 biopsies from New Delhi, 43 biopsies and corresponding blood samples from Jammu & Kashmir, 65 biopsy and blood samples from North-East region (Meghalaya, Manipur, Dibrugarh and Silchar), and 24 blood samples from familial breast cancer patients from Goa and 20 biopsies and 10 blood samples from southern region (Bangalore). The majority of them were suffering from infiltrating ductal carcinoma and biopsies and blood samples were collected directly from the surgical OT of Lok Nayak Hospital, New Delhi, Regional Institute of Medical Sciences,
Imphal, Assam Medical College, Dibrugarh, Silchar Medical College, Silchar, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Jammu and Kashmir, Goa Medical college, Goa and Kidwai Institute of Oncology, Bangalore in chilled phosphate buffered saline and stored at -70º C till further processing. Written informed consent was obtained from all the subjects included in the study and was carried out in accordance with the principles of the Helsinki Declaration and clinico-epidemiological details were taken from their clinical records. The study was approved by the Institutional Ethics Committee. Both sporadic as well as familial breast cancer and none of the whom had family history of any other cancer were included and all cases were inhabitants of India. The age of the breast cancer patients recruited varied from 25-80 years.

**DNA extraction and detection of HPV DNA sequences by Polymerase Chain Reaction (PCR)**

Genomic DNA from breast tumor biopsies and blood were isolated using standard Proteinase K digestion and phenol chloroform extraction method routinely being employed in our laboratory [66, 67]. The DNA concentration was measured either on an ethidium bromide-stained 1% agarose gel using Hind III-digested lambda marker or by spectrophotometric methods.

For the detection of HPV DNA, both conventional as well as real-time PCR methodology were employed using most common L1 consensus primers MY 09/11 or GP5+/GP6+ derived from HPV genome. HPV 16 plasmid DNA served as positive control whereas human placental DNA served as negative control. Amplification of β-globin gene as well as
exon 5 of p53 tumor suppressor gene served as internal control to examine quality and integrity of breast tumor DNA.

**Conventional PCR using L1 consensus MY 09/11 and GP5+/GP6+ primers**

Approximately, 100-200 ng genomic DNA was utilized for conventional PCR according to the routinely followed protocol of our laboratory [66] on a DNA Engine Tetrad (MJ Research, USA). Detection of HPV was carried out using consensus primers (MY09/MY11) located within the conserved L1 region of HPV genome (forward primer, 5'-GCM CAG GGW CAT AAY AAT GG-3', reverse primer 5'-CGT CCM ARR GGA WAC TGA TC-3' where M= A+C, W= A+T, Y= C+T, R= A+G). Consensus primers GP5+ (5'-TTT GTT ACT GTG GTA GAT ACT AC – 3’ and GP6+ (5'-CTT ATA CTA AAT GTC AAA TAA AAA – 3’) [68]. β-globin gene sequences (forward primer, 5’-GAA GAG CCA AGG ACA GGT AC-3’, reverse primer, 5’-CAA CTT CAT CCA CGT TAC ACC -3’) and p53 of exon 5 forward primer (5’-TAC TCC CCT GCC CTC AAC AA – 3’), reverse primer (5’- CAT CGC TAT CTG AGC AGC GC -3’) were used as internal controls.

Briefly, the method involved a 25µl reaction mix containing 100-200ng DNA, 10mM Tris-Cl (pH 8.4), 50mM KCl, 1.5mM MgCl2, 12.5µM of each dNTP (dATP, dCTP, dGTP and dTTP), 5 pmoles of each oligonucleotide primer and 0.5U Taq DNA polymerase (Perkin-Elmer Biosystems, Foster City, CA, USA). The temperature profile used for amplification constituted an initial denaturation at 95°C for 5min followed by 30 cycles with denaturation at 95°C for 30sec, annealing at 55°C for 30sec and extension at 72°C for 30sec which was extended for 4-7min in the final cycle. The oligonucleotide primers were either synthesized in an automated Applied Biosystems DNA Synthesizer (Model 381A, Applied Biosystems Inc.,
Foster City, CA, USA), and HPLC purified or commercially got synthesized from Microsynth GmbH, Balgach, Switzerland.

**Quantitative Real-time PCR**

Highly sensitive real time PCR was performed for detection of low copy HPV infection in breast cancer tissues and plasma of sporadic patients using GP5+/GP6+ consensus primers with Biorad SYBR Green Supermix kit and iCycler real time PCR machine (Biorad, Hercules, CA). Quantitative PCR was calibrated using 10 fold serial dilutions of HPV16 International Standards (IS; ranging from $10^4$-10$^1$) supplied under WHO Global HPV LabNet program and was capable of detecting absolute copy number of viral DNA. Background genomic DNA from HPV-negative cell line C33a was used as negative control and for dilution of standards was done as prescribed by WHO. Briefly, the amplification was performed in 25µl reaction mix containing 5 pmol each of GP5+/GP6+ (amplicon ~140bp) primers as per the protocol provided by the manufacturer. The amplification ramp included first step for 3 min at 95°C for initial denaturation, followed by denaturation cycle of 20 sec at 95°C, an annealing cycle of 15 sec at 40°C and an elongation and readout cycle of 30 sec at 72°C for 45 cycles. The specificity was verified by a dissociation curve analysis. Linear plots of the log copy number vs the number of the threshold cycles was consistently obtained for HPV16 L1 consensus amplification and the correlation coefficient was between 0.995 to 1.000 in each run.

**Results**

We screened 228 breast cancer biopsies along with 142 blood samples collected from 262 breast cancer patients belonging to four different regions of the country comprising New
Delhi, Jammu & Kashmir, North-east states, Goa and Bangalore. All cases diagnosed with infiltrating ductal carcinoma and others were employed for detection of human papillomavirus infection in biopsy and blood DNA using both conventional as well as highly sensitive real-time PCR methods. The patients belonged to age group of 25-80 years with a mean age of 51.47±SD16.69 years. Sixty two percent of the patients were in pre-menopausal stages while rest formed post-menopausal group. Clinical staging revealed 14, 30, 55, 56, 62 and 11 percent of the patients belonging to stage I, IIa, IIb, IIIa, IIIb and IV respectively, whereas histopathologic grading revealed 45, 119 and 64 percent of the tumors in grade I, II and III respectively. All the patients were of Indian origin and inhabitants of either New Delhi, North-East region, south, Jammu & Kashmir or Goa region. None of the patients showed family history of any other cancer.

No HPV infection was detected using consensus primers located within the conserved L1 region of HPV genome by performing conventional PCR in any of the 228 breast tumor and 142 blood DNA specimens, while β-globin showed amplification of 268bp product in all cases. All these tumor samples were again processed for highly sensitive Real-Time PCR but not a single sample was found positive for any of the HPV infection. In both the PCR methods HPV 16 plasmid and human placental DNA were used as positive and negative control respectively. We also checked these samples with PGMY primers, but again all the samples were negative for any of HPV infections. Studies world-over showing positive as well as negative results for the presence of HPV infection in breast cancer and benign breast tissues are listed in Tables 1 and 2. Table 3 lists of the reports that show different results from the same country.
Discussion

Despite breast cancer being one of the most frequently diagnosed cancers in women worldwide, the etiology and molecular pathobiology of breast carcinogenesis is still not clearly understood. In addition to various exogenous, endogenous and genetic risk factors, viral etiology of breast cancer implicating potential role of Epstein-Barr virus (EBV) [37-39], murine mammary tumor virus (MMTV) [40-41] and human papillomavirus (HPV) [69] has been demonstrated in several studies but remains highly controversial. Though the HPV infection, particularly the high risk-types 16 and 18, have been strongly associated with the development of cervical cancer [69] and recently two prophylactic vaccines, Gardasil and Cervarix against these two HR-HPVs are now available; such studies on breast cancer are not only limited but also controversial.

Our previous study on a limited number of patients [50] as well as several other reports worldwide [45-49] did not observe any infection of HPV in breast cancer, whereas a moderate (20-45%) to high frequency (60-85%) of HPV infection has been reported by many authors [51-60]. Recent reports of detection of very high rate of HPV infection in breast cancer up to 85% [61-64] prompted us to re-look into the prevailing condition of HPV infection in breast cancer which we refuted previously [50]. Since in earlier study we used mainly fine needle aspirated (FNAC) cells from breast cancer patients (n=26) including only four tumor biopsy specimens to detect HPV, we have now screened 228 sporadic breast cancer biopsy specimens and 142 blood samples from 262 cancer patients in the present study for the detection of HPV infection. The samples were collected from four different regions of the country and three different PCR techniques both conventional as well as the highly sensitive real-time PCR were employed. The L-1 consensus primers MY09/11 or PGMY 09/11 and GP5+/GP6+ which detect as may as 40 mucosal HPV genotypes, belonging to both
Of 21 previous studies done till date world over, fourteen reported occurrence of HPV infection in breast cancer and the authors have tried to put forward possible explanation for the presence of HPV infection in breast cancer. Though the spreading of genital HPV infection from one site to another is not known, studies have reported detection of HPV in peripheral blood lymphocytes in patients with urino-genital cancers [70-73] and head & neck cancers [74]. So, there is a possibility of systemic spread of oncogenic virus through organ perfusion [75] facilitating its entry into the breast. For this reason we have also tried to detect HPV in blood samples of 142 breast cancer patients from Bangalore, North-east region, J & K and Goa but none of them was found positive for HPV DNA sequences. However, detection of HPV in nipples and areolar region reported by de Villers et al. [62] may not be quite unlikely as there could be infection of cutaneous HPVs by mechanical path where the virus in scrubbed through the skin [62]. However, they apparently have no role to play in breast carcinogenesis.

It is also interesting to note that authors found both positive and negative results from the same region [45, 47, 61-62]. de Villiers et al [62] observed HPV DNA in 25 out of 29 samples (86%) in invasive breast carcinoma and in 20 out of 29 samples (69%) of the corresponding nipple samples collected from US. The most prevalent HPV genotypes detected in both benign and breast tumours were HPV 11 followed by HPV 6 which are low risk HPV-types that generally cause benign lesions and warts and are non-oncogenic. Only 12% cases were found to have infection of high risk HPV type 16 but no HPV 18 genotypes could be detected. Bratthauer et al [45] in contrast, analyzed 43 cases of breast cancer from
USA for the detection of HPV DNA but no HPV infection was observed in breast cancer cases.

Similarly, from Austria, Widschwendter et al [61] analyzed only 11 cases of breast cancer, of which 7 (63.7%) had HPV infection while Czerwenka et al [47] from the same country reported complete absence of HPV infection in breast cancer cases. The reason(s) for disparity of presence or absence of HPV infection in breast cancer cases from the same country is not understood. It may be primarily because of selection of primer types and the amplicon region in the HPV genome could be the possible explanation for the observed differences in the HPV detection. It may also in part, be attributed to cross-contamination from other organ sites, if infected with HPV.

Interestingly, in Brazilian women, Damin et al [56] could not observe HPV DNA in benign breast disease, but HPV could be detected in 25% of patients with breast carcinoma. However, HPV infection was found to have no correlation with prognosis of the disease. Lindel and her colleagues [48] showed no evidence of HPV infection in 81 Swiss women with breast carcinomas using the SPF1/2 primers covering about 40 different low, intermediate and high risk HPV types. Similarly, de Cremoux et al [49] analyzed 50 invasive breast carcinoma tissues from French patients using consensus and type-specific primers but no HPV infection were observed in any of breast cancer cases. Wrede et al [46] analyzed a group of 95 British women with breast cancer for the infection of HPV 6b, 11, 13, 16, 18, 30, 31, 32, 33, 45 and 51 but they failed to detect any HPV infection. Kroupis et al [63] performed HPV test on 107 breast carcinoma in Greece and only 17 (15.9%) were found positive. Many of them were having multiple infections of 21 as many as high risk HPV types and majority (14/17; 67%) of them were positive for HPV16. Yu et al [53] analyzed
seventy-two patients from Shanghai, China and Tokushima in Japan and found 34.1% positivity for HPV33 alone but there was no evidence of HPV 16-positive breast cancers. Yet another study by Mendizabal-Ruiz et al [59] analyzed 67 breast cancer patients including 40 non-malignant tissues and a low 3/67 (4.4%) frequency of HPV infection was observed.

Several other authors from different parts of the world reported presence of HPV infection in breast cancer cases. It is, however, most important to note that not many studies have indicated or analyzed if the women with breast cancer recruited for HPV detection studies had also anogenital or oropharynheal infection of HPV. Though the route of HPV transmission from one organ to the other is not known, Hening et al [52] detected HPV 16 in 46% breast carcinoma patients who had a history of CIN III lesions. Most interestingly, all patients with HPV 16 positive for breast cancer correspond to same patients with cervical CIN III lesion with HPV 16 infection. Also, no high risk HPVs were detected in the primary breast lesions. So, it is essential to look for genital or oropharynheal infection of HPV in breast cancer patients to rule out the possibility of inter-organ transmission and/or cross contamination of viral infection. However, controversial published data suggest that various epidemiological factors and the history of HPV-associated diseases specific to the region may play a role in differential distribution of HPV in breast cancer.

In spite of exceedingly higher incidence of HPV infection in India as compared to that in Europe and in the USA including presence of other conducive risk factors such as early age marriage, multiple pregnancy, malnutrition, poor hygiene and healthcare, the complete absence of HPV infection in Indian women with breast cancer in the present study which is in confirmation of our earlier observation [50] does not suggest the role of HPV in breast carcinogenesis in Indian women. We however, do not preclude the possibility of yet
unidentified HPV playing important role in breast carcinogenesis. Furthermore, due to the changing social and psycho-sexual behavior particularly in urban Indian population, it may not be unlikely to observe in future, presence of HPV in breast tissue due to an exterior transfer of HPV form genitalia to breast.

**Conclusions:** Complete absence of HPV DNA sequences either in the blood or in the tumor DNA of the same breast cancer patients recruited from all four regions of the country even after employing the highly sensitive real time PCR disproves the possibility of any role of oncogenic genital HPV infection in the pathogenesis of breast cancer in Indian women.

**Abbreviations:** HPV = human papilloma virus; EBV = Epstein-Barr virus; MMTV = murine mammary tumour virus; PCR = polymerase chain reaction; Breast carcinoma

**Competing Interest:** There is no competing interest of authors of this paper.

**Authors’ contributions:** SH- carried out experiments, analyzed the data, and drafted the manuscript. S Hussain, DB, MMM - provided breast cancer biopsy samples, clinical diagnosis and information from J & K. UK, SP, AT, RK - to perform some of the experiments and critical reading of the manuscript. NJ, SS- has done all work related to real time PCR. TD- provided blood samples of breast cancer patients, diagnosis and clinical information from Goa. SC- provided breast cancer biopsy and blood samples, diagnosis and clinical information from Silchar. YMS- provided clinical breast cancer biopsy and blood samples, diagnosis and clinical information from Imphal. ACB – co-designed the study and revised the manuscript. KS- collection of blood and biopsy samples from Bangalore and critical reading of the manuscript. BCD- conceived and designed the study, interpret of data
and critically corrected and communication the manuscript. All authors have read and approved the final version of the manuscript.

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### Table-1: List of studies identified HPV DNA sequences in breast cancer tissues.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Study report</th>
<th>Sample size</th>
<th>Total HPV positivity n(%)</th>
<th>HPV 16 n(%)</th>
<th>HPV 18 n(%)</th>
<th>HPV 16/18 n(%)</th>
<th>Other HPV types n (%)</th>
<th>Country</th>
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<tr>
<td>1</td>
<td>Di Lonardo et al., 1992</td>
<td>70</td>
<td>7 (10)</td>
<td>7 (100)</td>
<td>0</td>
<td>0</td>
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<td>19 (46.3)</td>
<td>19 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>3</td>
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<td>72</td>
<td>19 (26.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>19 (HPV 33)</td>
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<td>4</td>
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<td>32</td>
<td>14 (43.8)</td>
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<td>0</td>
<td>0</td>
<td>14 (HPV 33)</td>
<td>China</td>
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<tr>
<td>5</td>
<td>Liu et al., 2004</td>
<td>17</td>
<td>6 (35)</td>
<td>3 (50)</td>
<td>1 (17)</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>6</td>
<td>Damin et al., 2004</td>
<td>101</td>
<td>25 (24.7)</td>
<td>14 (56)</td>
<td>10 (40)</td>
<td>1 (4)</td>
<td>0</td>
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<tr>
<td>7</td>
<td>Widschwendter et al., 2004</td>
<td>11</td>
<td>7 (63.7)</td>
<td>7 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>8</td>
<td>de Villiers et al., 2005</td>
<td>29</td>
<td>25 (86.2)</td>
<td>3 (12)</td>
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<td>0</td>
<td>22 (88)</td>
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<tr>
<td>9</td>
<td>Kroupis et al., 2006</td>
<td>107</td>
<td>17 (15.9)</td>
<td>14 (82.3)</td>
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<td>0</td>
<td>3 (17.6)</td>
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<td>10</td>
<td>Kan et al., 2005</td>
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<td>24 (48)</td>
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<td>24 (100)</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>50</td>
<td>37 (74)</td>
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<td>20 (54)</td>
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### Table-2: List of studies that did not detect HPV DNA sequences in breast cancer tissues.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Study report</th>
<th>Sample size</th>
<th>Total HPV positivity n(%)</th>
<th>HPV 16 n(%)</th>
<th>HPV 18 n(%)</th>
<th>HPV 16/18 n(%)</th>
<th>Other HPV types n (%)</th>
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<td>4</td>
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<td>0</td>
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<td>0</td>
<td>Switzerland</td>
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<tr>
<td>6</td>
<td>de Cremoux et al., 2008</td>
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<td>France</td>
</tr>
<tr>
<td>7</td>
<td>Hedau et al. (Present study)</td>
<td>262*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>India</td>
</tr>
</tbody>
</table>

* 228 biopsies plus 142 blood samples from 262 breast cancer patients.

### Table-3: Country with both positive and negative reports of HPV DNA sequences in breast cancer tissue.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Study report</th>
<th>Sample size</th>
<th>HPV positivity n(%)</th>
<th>HPV 16 n(%)</th>
<th>HPV 18 n(%)</th>
<th>HPV 16/18 n(%)</th>
<th>Other HPV types n (%)</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bratthauer et al., 1992</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td>Authors</td>
<td>Year(s)</td>
<td>HPV Frequency</td>
<td>Other Year(s)</td>
<td>Country</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>de Villiers et al., 2005</td>
<td>29</td>
<td>25(86.2)</td>
<td>3(12)</td>
<td>USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Czerwenka et al., 1996</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>Austria</td>
<td></td>
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<td></td>
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<tr>
<td>4</td>
<td>Widschwendter et al., 2004</td>
<td>11</td>
<td>7(63.7)</td>
<td>7(100)</td>
<td>Austria</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:

2. National Cancer Registry Programme Report (Indian Council of Medical Research), 2005


42. Tsai JH, Tsai CH, Cheng MH, Lin SJ, Xu FL, Yang CC. Association of viral factors with non-familial breast cancer in Taiwan by comparison with non-


