MALE BREAST CANCER: NO EVIDENCE OF HUMAN PAPILLOMAVIRUS ETIOLOGY

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ABSTRACT

Background and Aims: Once hypothetical, the association between virus and cancer is a reality in many diseases. A possible role of Human papillomavirus (HPV) in breast carcinogenesis is conflicting. The purpose of this study was to investigate the existence of HPV in archival breast cancer specimens of male patients.

Material and Methods: The Pathology Department’s archives between 1998-2008 were scanned. Tissue blocks of 27 male breast cancers and 27 gynecomastia tissue specimens were included to the study. DNA was extracted according to the manufacturer’s protocol.

Results: This study was not able to demonstrate HPV infection nor in male breast cancer or gynecomastia tissue specimens.

Conclusions: This is the first study investigating the presence of HPV in male breast cancer samples. All investigated tumoral, and non-tumoral samples were negative for HPV DNA. According to our findings, the possibility of HPV function in breast oncogenesis decreases.

KEYWORDS Human papillomavirus; HPV; male; breast cancer; virus

Introduction

Aetiology of the breast cancer, despite being the most prevalent cancer in women, remains unresolved [1]. Among the possible risk factors involved in this disease, possible viral aetiology has gained significant interest.

Viruses are involved in nearly 20% of all human cancers [2,3]. The presence of Human papillomavirus (HPV) [4-6], Mouse mammary tumor virus (MMTV) [7, 6], Epstein-Barr virus [8,6], Simian virus 40 [9], MMTV-like env sequences [10], and bovine leukemia virus [11, 12] has been demonstrated in some studies. Some hypotheses were proposed to explain the possible viral oncogenesis in breast cancer. HPVs are generally related to genital (cervical, anal, vulvar, and penile) cancers. However, its evidence in breast cancer is controversial [13]. It has been proposed that high-risk HPV infection can induce cell invasion and metastasis in breast cancer [14-16].

To the best of our knowledge, there has been no study investigating the presence of HPV DNA in male breast cancer specimens. We hypothesised that, if HPV played a role in breast cancer tumorigenesis, then it would be detected not only in female but also in male breast cancer tissues.

This study aimed to investigate the presence of HPV in archival specimens of breast carcinoma from male patients.

Methods

All formalin-fixed paraffin-embedded blocks (FFPE) of male breast cancer specimens between 1998-2008 were retrieved from the archives at the Pathology Department of Istanbul University, Cerrahpasa School of Medicine, Istanbul, Turkey. As a comparative group, unselected FFPE tissue blocks of gynecomastia patients were selected.
DNA was extracted by the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Manheim, Germany). FFPE specimens about 25-50 mg were manually cut outside the area. For every specimen we used different gloves, blades and collecting plates. Sample lysis, washing and DNA elution steps were performed according to the manufacturer’s Isolation of Nucleic Acids from Formalin-Fixed Paraffin-Embedded Tissue 13 www.roche-applied-science.com 2.8 Protocol.

**HPV DNA investigation**

To detect HPV DNA in the eluted DNA samples, two-step nested PCR analysis utilising consensus primers MY09/11 and GP5+/6+ was performed. Primers MY09 (5’-gctcmmarrggwactgac-3’) and MY11 (5’-gcmaggwctaayaatgg-3’) amplify 450 base pair (bp) long fragment in the L1 region of the HPV genome. Consensus primers GP5+ (5’-ttggtcttggttagatc-3’) and GP6+ (5’-gaaaataacgttaatca-3’) produce 150 and 140 bp fragments of the L1 region of the viral genome consecutively [17]. Besides, to exclude the PCR inhibitors leading to false negative results and to verify the integrity of the DNA obtained, a second PCR amplification by subjecting the sample with primers targeted to the human β-globin gene was performed. PCR procedure of the isolated DNA for HPV detection was described previously [17]. During PCR analysis positive and negative controls were included. PCRs were run on the PTC-200 thermal cycler (MJ Research, Inc., MA, USA). Amplicons were then electrophoretically (Mighty Bright, Hoefer Scientific Instruments, San Francisco) analysed on a 1.5% agarose gel stained with ethidium bromide.

**Results**

A total of 27 specimens of male breast cancer and 27 specimens of gynecomastia were retrieved.

The median age was 41 (range, 17-97) and 59 (range, 22-81) years in the gynecomastia and breast cancer groups, respectively. Among patients with breast cancer, some data of the two patients were missing. Of the remaining 25 patients, 13 (52%) had left sided, 10 (40%) had right sided, and two (8%) had bilateral breast cancers.

Detailed information on the clinical and pathological characteristics of the breast cancer patients is listed in Table 1. Between breast cancer patients, the FFPE blocks of five (Patient No: 8, 9, 10, 13, 18) tumoral tissues could not be retrieved. Instead, the FFPE blocks of the non-tumoral tissues (tumour bed and axillary lymph nodes) of these cases were used. These patients underwent excisional biopsies at other centres, and after the diagnosis of cancer, they were referred to our hospital for advanced surgery. At our hospital, we performed modified radical mastectomy (MRM) to these patients. In the pathological evaluation of the mastectomy specimens, no residual tumoral tissue was detected. In the pathological assessment of the axillary curettage specimens, two patients (No: 9, 13) had axillary metastases, and three patients (No: 8, 10, 18) did not have any metastases. All lymph nodes were included in the study. In one case (No: 2), only the FFPE blocks of the tru-cut biopsy specimens for receptor study were retrieved. All samples were positive for the β-globin gene and negative for PCR inhibitors. No HPV DNA sequences were found neither in 27 male breast cancer nor 27 comparative gynecomastia samples (Fig. 1).

**Discussion**

To our knowledge, this is the first study investigating the presence of HPV DNA in male breast cancer. In our study, all investigated tumoral and non-tumoral samples were negative for HPV DNA.

According to our theory, if HPV infection had a role in breast carcinogenesis, then it could be able to be demonstrated not only in female breast cancer samples but also in male breast cancer samples.

From the observation that HPV can immortalise breast epithelial cells and the presence of HPV in some adenocarcinomas, it was postulated that HPV could play a role in the pathogenesis of breast cancer [18].

The presence of HPV in breast cancer was first demonstrated by Di Lonardo et al. in 1992 [19]. The HPV DNA incidence among breast cancer patients was reported between 0-86%. According to a meta-analysis, when studies with zero prevalence of HPV were excluded, the overall prevalence of HPV in breast cancer was calculated as 30.30% [20]. In another meta-analysis, the risk of breast cancer was estimated to be 4.02-fold higher for HPV DNA-positive individuals [21].

Until the present study, all studies were performed on female breast cancer samples. Whether HPV is associated with breast cancer as a primary etiologic factor or as a cofactor was questioned. Studies that looked for a relationship between HPV infection and breast cancer were conflicting and were focused primarily on high-risk HPV types [22, 23].

An important argument against the vital role in the breast cancer was the fact that the risk of breast cancer was not increased in the chronically immunosuppressed heart or kidney transplant patients [24, 25]. Similarly, in human immunodeficiency infections, the rates of all virus-related cancers were increased, but prostate and breast cancer rates remained unchanged [24, 26, 27, 25]. The most likely explanation postulated by Lawson et al. [24] was that oncogenic mechanisms of HPV in breast cancer might be different and might not be affected by immunosuppression. Liang et al. [28] hypothesised that breast epithelial cells that partially lose control could become more susceptible to persistent HPV infection. Some studies proposed that HPV might play a role in the early steps of breast oncogenesis [24] including enhancement of APOBEC3B genes [29] that lead to increased risk of breast cancer and ‘hit and run’ phenomenon [30, 31] that could explain the lower detection rates in breast
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Carcinoma type</th>
<th>Size of the tumor (cm)</th>
<th>Lymph node status (positive/total)</th>
<th>Analysed sample</th>
</tr>
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<tbody>
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<td>ILC</td>
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<td>0/11</td>
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</tr>
<tr>
<td>2</td>
<td>40</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>Tru-cut biopsy</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>IDC</td>
<td>2</td>
<td>1/14</td>
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</tr>
<tr>
<td>4</td>
<td>22</td>
<td>Dermatofibrosarcoma</td>
<td>2.5</td>
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</tr>
<tr>
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<td>61</td>
<td>IDC</td>
<td>*</td>
<td>2/12</td>
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<tr>
<td>6</td>
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<td>2</td>
<td>20/22</td>
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<td>7/36</td>
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<td>12/12</td>
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<td>0/6</td>
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<td>4</td>
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<tr>
<td>27</td>
<td>45</td>
<td>IDC</td>
<td>1.8</td>
<td>10/11</td>
<td>Tumor tissue</td>
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</tbody>
</table>

cancer cells. Further, multiple viral DNAs were demonstrated in the same breast cancer samples suggesting a co-existence of HPV with other viruses as a possible mechanism in breast cancer tumorigenesis [32-34, 6]. The positivity of HPV infection in female breast cancer in previous studies could be attributed to some contamination artefacts. It was shown that women with positive urogenital infections had a 52% rate of HPV DNA in their peripheral blood mononuclear cells [35]. Ruiz et al. [36] showed a 4.5% HPV infection rate in breast cancer and attributed this to the HPV infection in the blood rather than the tumoral tissue. A critical study supporting the contamination artefact idea was performed in the European Oncology Institute, Milan, Italy [37]. In this study, β-catenate HPV rate was 15% in the ductal lavage fluid of high-risk women. However, band-stripping of the nipples before the ductal lavage decreased the HPV positivity to 0%.

Proving the presence of HPV DNA in breast cancer specimens does not mean that HPV has an activity in the pathogenesis of breast cancer [2]. Transcription of HPV DNA demonstrated the biological activity of the HPVVs to RNA and expression of HPV E7 proteins [24]. In his study, Aguayo et al. [2] performed a second test on HPV16 positive FFPE tissues of breast cancer and could not demonstrate E6 and E7 transcripts in any of the HPV16 positive specimens.

In this study, absence of HPV DNA in the study samples could be due to: (a) oldness of our tissue blocks, (b) damage of cellular DNA by formalin and paraffin fixation [38-41, 21], and (c) type of primer sets directed against HPV [38, 42].

Investigation of HPV presence was recommended to be performed in fresh breast cancer specimens. However, Zhou and colleagues [20] could not demonstrate any statistically significant difference regarding HPV presence between fresh tissues and paraffin-embedded tissues.

Due to the rareness of male breast cancer, it is difficult to perform a prospective study with fresh tissue specimens within a limited time in a single centre.

We utilised consensus primers (MY09/11 and GP5+/6 primer set) directed against the L1 region of the HPV genome. It is put forward that, primers specific to the E6/E7 region of the HPV genome are more reliable compared to L1 consensus primers because while L1/E1 regions are lost during the viral DNA integration, E6/E7 regions are preserved and never lost [35, 38, 42]. Ong et al. [43] investigated the presence of HPV DNA in 92 breast cancer samples for both mucosal and cutaneous HPV types. When they performed the analysis for only the mucosal types (GP5+/6 primers) their results were all negative. However, when they performed second PCR with primers (FAB 59/64) designed to detect cutaneous HPV types, the results were 35% positive. The authors suggested a combination of primers designed to cover a broader spectrum of cutaneous and mucosal HPV for detection of HPV in breast carcinoma. Since in the present study we utilised only primers directed against mucosal HPV types, this could be one of the factors for our negative results.

Damin and colleagues [44] postulated that higher rates of HPV infection could be the reason for high rates of breast cancer in Brazil. However, a study from Brazil performed by Silva et al. [45] did not demonstrate HPV infection in any of the breast cancer patients. Likewise, despite higher HPV infection rate in Korea, its rate in breast cancer was found to be 6.5% [38].

In a study performed in Japan, out of 124 breast cancer patients included to the study, 21% were positive for HPV DNA, but since the viral load was very low it was concluded that HPV could not be attributed as a risk factor in breast cancer aetiology [46]. Association between HPV infection and molecular subgroups of the breast cancer was first studied by Piana et al. in 2014 [47]. The authors investigated the HPV prevalence in triple negative breast cancer (TNBC) and non-TNBC series. According to their results, HPV positivity was 15% in the TNBC series. The non-TNBC series were HPV negative, and the difference was statistically significant. They hypothesized that TNBC could be the most likely type of breast cancer related to viral aetiology due to its poor differentiation. Similarly, in a study performed by one of the authors of this paper (FA), the prevalence of TNBC among male breast cancer was calculated as 2.3%, and when subdivided, among non-Hispanic white race it was found to account for 1.8% of the patients (unpublished data). On the contrary, the TNBC phenotype accounts for 10-24% of invasive breast cancers in women [47]. According to the results of this study, it could be hypothesised that, if HPV was mostly associated with TNBC subtypes, then the absence of HPV in our study patients could be due to the low prevalence of this subtype in male breast cancers.

Conclusion

Consistent with the results of this study, HPV is less likely to play a role in breast oncogenesis. Other improved methods like Real-Time Quantitative Reverse Transcription (RT-qRT) PCR combined with laser capture microdissection and Next Generation Sequencing for investigation of HPV presence in the archival breast cancer paraffin blocks may yield different results [14].

If the aetiology of breast cancer was a virus, to prove this, further studies with some methodological and technological improvements are needed.

Competing Interests

There were no financial supports or relationships between authors and any organisation or professional bodies that could pose any conflict of interest.

Research ethics

All procedures performed in studies involving human participants were by the ethical standards of the World Medical Association Declaration of Helsinki. The manuscript conforms to the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals.

Patient consent

At the time frame of this study, informed consent was not a requirement for retrospective studies at our institution.

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Note

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References


