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BRIEF REPORT

No evidence for an association of human papillomavirus and breast carcinoma

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Abstract Breast cancer represents a serious public health problem worldwide, for its high incidence, morbidity, mortality, and its high cost treatment. It is known that breast cancer is a multifactorial disease, possessing various risk factors, among which include hormonal factors, genetic and environmental. The role of viruses in breast carcinogenesis is controversial. This study aims to evaluate the expression of HPV 6, 11, 16, and 18 by polymerase chain reaction (PCR) in invasive ductal breast carcinoma. We analyzed 90 women diagnosed with invasive ductal breast carcinoma, of which the extracted DNA was amplified, quantified and tested for DNA subtypes 6, 11, 16, and 18 by PCR. The research carried out in 79 samples of HPV DNA, proved negative. Our study demonstrates no association between the most prevalent types of HPV and breast cancer.

Keywords Human papillomavirus · Breast · Carcinoma · PCR

Introduction

Breast cancer is the most frequent malignancy among women in western countries. In Brazil it is the important cause of cancer-related death with approximately 49.240 new cases seen each year [1]. The etiology of breast cancer remains unknown. Many risk factors have seen associated, including hormones, family history, diet, and alcohol consumption [2]. Viral oncogenesis as an etiological factor for breast cancer has been addressed in several studies but remains controversial [3].

Human papillomaviruses (HPVs) are a family of small DNA viruses that infect epithelial cells of the skin and mucosa. The role of theses virus infections has been established by the regular presence of HPV DNA in retrospective tumor biopsy specimens, detailed molecular virology, and epidemiologic studies pointing to these HPV infections as a major risk factor for cervical cancer [4]. The presence of HPV DNA sequences in breast cancer has been reported by some authors in invasive breast carcinoma [5–7]. However, others authors have reports negative results in invasive breast cancer [8–10]. These controversies led us to conduct this study.

Methods

Patients

Ninety paraffin-embedded tissue samples from women with histological confirmed primary ductal breast carcinoma were obtained from the Pathology Department's archives between January 2006 and September 2009. The inclusion criteria were previous diagnostic of ductal invasive carcinoma and no prior treatment for breast cancer.

Study design

In a total of 90 patients diagnosed with invasive ductal carcinoma were studied by PCR the presence of subtypes of HPV 6, 11, 16, and 18.

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DNA extraction

The DNA extraction method used was with enzymatic digestion with proteinase K [11]. We performed manual cut outside the area, removing the excess paraffin. It was inserted into 1.5 ml tube containing 50 µl of TET buffer (10 mM TRIS–Cl, 10 mM EDTA, and Tween 20—0.5%) and 10 µl of proteinase K at 20 mg ml^{-1} and incubated on dry bath for 12 h. Then proteinase K was inactive for 15 min at 94°C. After inactivation, was added 1 ml of saturated phenol (Life Technologies, Gaithersburg, MD, USA) and buffered phenol (pH 8.0), and the tubes centrifuged at 4,200×*g* for 20 min. The supernatant was transferred to another tube and then added 1 ml of phenol, chloroform, and isoamyl alcohol (25:24:1), homogenized, and centrifuged at 4,200×*g* for 20 min. The supernatant was again transferred to another tube of 1.5 ml. For precipitation of genomic DNA were added to 2–3 volumes of absolute ethanol and one-tenth volume of 7 M ammonium acetate (sigmaE, St. Louis, USA) overnight. Tube was then centrifuged at 19,600×*g* for 20 min and the precipitated DNA washed with 70% ethanol and after evaporation of ethanol at room temperature, the precipitate was dissolved in 30–50 ml of TE buffer (10 mM Tris–HCl, pH 7.4, and 1 mM EDTA, pH 8.0) and kept at 4°C until quantification.

Quantification and amplification of DNA

The quantity and purity of genomic DNA obtained were determined by optical density spectrophotometer (DU-640, Beckman, Palo Alto, CA, USA) to identify the amount of DNA in mg/µl and subjected to PCR to control the size of the extracted material, since PCR-based molecular assays depend on the integrity of DNA. Amplified segments of 100, 200, 300, 400, and 600 bp of human genes trombaxano synthase (TBXAS1), recombinant human activator gene (RAG1), gene for zinc-finger of human promyelocytic leukemia (PLZF), and two exons AF4 gene, respectively, were used for control of viability according to the protocol described by Van Dongen et al. [12].

HPV typing

For detection of the subtypes of HPV included the use of primers targeted at specific segments of the genome of subtypes 6/11 (SL6/11.S: TAC ACT GCT CAA GGA CAT CG and SL6/11.A: GTG CGC AGA TGG GAC ACA C), 16 (VdB16.S: TGC TAG TGC TTA TGC AGC CAA and VdB16.A: ATT TAC TGC AAC ATT GGT AC), and 18 (VdB18.S: AAGGAT CCH GCA GCT GTC TGA and VdB18.A: CAC GCA CAC GTC TGG CAG GT) [13], which amplify fragments of 302, 152, and 216 base pairs, respectively. The amplification reaction was performed in a final volume of 25 ml, consists of 1.4× PCR buffer (Tris–HCl 100 mM/500 mM KCl), 3.0 mM magnesium chloride, 0.2 mM dNTPs, 0.3 mM each primer (Sl6-11.S/Sl6-11A, VdB16.S/VdB16.A, VdB18.S/VdB18.A), and 1.25 U Taq DNA polymerase. For each, amplification reaction was performed using 2 ml (0.1 mg/ml) of solution containing the DNA extracted previously. In each, battery of PCR amplification was always used as a negative control consisting of the same concentrations and the same reagents for the amplification reaction except the DNA and positive control DNA for each HPV type studied DNA (6/11, 16, and 18). The reaction amplification of DNA was performed in 500 ml tubes, incubated in the thermocycler (MJ Research PTC 150) with the following cycling conditions: 94°C—5 min (1×), 94°C—1 min, 55°C—1 min, 72°C—1 min (40×), 72°C—7 min (1×). After amplification, we used 10 ml of the PCR product of each sample added with 2 ml of loading buffer (0.25% bromophenol blue, 15% Ficoll type 400). The final volume was applied to gel poliacrilaminda 7%, subjected to electrophoresis at 250 V, vertical tube for 50 min. The gel was stained with silver nitrate, and the distinction of HPV types was performed by comparing the brands generates in the gel with the positive control of each subtype.

Results

The age of patients ranged 23–87 years with an average of 50.33 years and standard deviation of 12.22. As for staging, 2.2% were stage I, 52.2%, stage II, 42.11%, stage III, and 4.4%, stage IV.

The DNA extracted from 90 cases of breast carcinoma was quantified and subjected to PCR. After amplification, 11 cases (12.2%) that had less than 300 bp were excluded from the study.

Thus, 79 samples were tested for HPV DNA subtypes 6, 11, 16 and 18. In none of these samples the presence of viral DNA was observed.

Discussion

Breast cancer is one of the most prevalent malignancies throughout the world and important cause of cancer-related death among women in Brazil. Although many epidemiological risk factors have been identified, the cause of almost 95% individual breast cancer is often unknown.

Several studies suggest viral oncogenesis as an etiological factor for breast cancer, but it remains controversial. Di Lonardo et al. [14] were the first to demonstrate the association of HPV in 29% of 17 patients with breast cancer, identifying HPV 16 DNA by means of PCR.

De Villiers et al. [7] found HPV DNA sequences in 25 (86%) out of 29 breast cancers and in 20 out of 29 samples of the corresponding nipple (69%). The most prevalent HPV type detected was HPV 11 followed by HPV 6. HPV 18 was not found in any case and only 12% of the samples showed HPV 16. Damin et al. [5] detected HPV in 25 of 101 (24.75%) patients with breast cancer, and did not identify HPV in any of the 41 benign lesions studied. They detected mostly HPV 16 (56%) and HPV 18 (40%). In this study the authors suggest to complement the use of dot-blot hybridization to decrease our cases of false-negative.

Several studies failed to detect HPV positivity in breast carcinoma. Wrede et al. [9] studied a group of 95 women with breast cancer with regard to HPV 6b, 11, 13, 16, 18, 30, 31, 32, 33, 45, and 51. They found no evidence of HPV infection. Bratthauer et al. [10] researched the subtypes 6, 11, 16, and 18 in 13 cases of invasive ductal carcinomas, 15 papillomas, and 15 papillary carcinomas, without detecting any of the subtypes. Czerwenka et al. [15] also showed no association of HPV in 20 cases of Paget's disease. In these studies we used primers MY09 and MY11, which comprises the subtypes of high risk. Lindel et al. [8] studied in 81 Swiss women with breast cancer and a variety of subtypes of HPV through six different primers, including a total of 40 types 16, 18, 31, 33, and 45 and did not find any of the subtypes. De Cremoux et al. [16] studied the prevalence of subtypes of high (16, 18, 33, 45) and low risk (6, 11) in 50 breast cancers in women in France by PCR with the prime general GP5+/GP6+ was not detected in presence of these subtypes in neither case. None of our 79 cases samples were positive for HPV 6, 11, 16, and 18.

There are currently over 100 known subtypes of HPV, 40 of which hit the anogenital region. Of these, 18 are considered oncogenic and the remaining low risk. The HPVs 6 and 11 are the most prevalent among low-risk and seen in genital warts, while subtypes 16 and 18 found in 70% of cervical cancer. The prevalence of female genital infection with HPV types of high risk show a great variation depending on the population studied. Studies have shown a higher prevalence in women under 25 years and a linear decrease with advancing age [17]. Hence the low prevalence of detection of HPV in women over 45 years and variation of subtypes detected according to age could be one of the reasons for not detecting HPV in our patient group, which had an average of 50.33 years.

It is felt that the wide variety of results regarding the prevalence of HPV could be due to the lack of standardization of technique on the material to be obtained, the type and number of primers used, the lower prevalence of HPV in this age group in certain regions, and because the great variability of the subtypes most frequently detected. Although our study has not shown an association between HPV and breast cancer, more studies are essential to confirm or to exclude the role of this virus in mammary carcinogenesis.

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