No Evidence of Human Papilloma Virus Infection in Basal Cell Carcinoma

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Abstract

Background: Basal cell carcinoma (BCC) is the most common skin cancer among whites, and several risk factors have been discussed in its development and progress. Detection of human papilloma virus (HPV) deoxyribonucleic acid (DNA) BCCs in some studies suggests that the virus may play a role in the pathogenesis of this disease. Several molecular studies showed conflicting reports. Aims: The purpose of this study was to investigate the association between HPV and BCC using polymerase chain reaction (PCR).

Materials and Methods: HPV DNA detection was done for 42 paraffin-embedded tissue specimens of BCC and 42 normal skin samples around the lesions by PCR using GP5+/GP6+ primers. Results: HPV DNA was not found in any of the 42 samples of BCC, and only one normal skin sample around the lesions was positive for HPV DNA by PCR.

Conclusion: In this study, no statistically significant difference was seen between the presence of HPV DNA in BCC and normal skin around the lesion, and HPV is not likely to have an important role in the pathogenesis of BCC.

Key Words: Basal cell carcinoma, human papillomavirus, polymerase chain reaction

What was known?
Detection of human papilloma virus deoxyribonucleic acid in basal cell carcinomas (BCCs) in some studies suggests that the virus may play a role in the pathogenesis of this disease. Several molecular studies showed conflicting reports. There was no previous study regarding this point in Iranian population with BCC.

Introduction

Basal cell carcinoma (BCC) is the most common skin cancer among white population and comprises about 75% of all skin cancers. The mortality rate of this cancer is low, but it can rarely grow invasively and cause extensive tissue destruction. The metastasis rate of BCC is very low. The risk of BCC is related to environmental and individual factors.1]

In addition to ultraviolet radiation, older age and male gender are considered risk factors for BCC. Recently, the association between smoking and BCC in young women has been demonstrated. Although human papilloma virus (HPV) infection is an essential risk factor for a number of epithelial cancers, the etiologic relationship between HPV and keratinocytic malignancies such as squamous cell carcinoma and BCC has not been elucidated.1] The role of HPV in development of skin cancer was first demonstrated in the rare hereditary disease epidermodysplasia verruciformis, but no etiologic relationship between HPV and BCC in general population has been proved, and the results of different studies are contradictory.2-13] Therefore, we set out to assess the association between HPV and BCC in Asian population by using polymerase chain reaction (PCR) method for the first time.

Materials and Methods

In this case-control study, 52 paraffin-embedded specimens diagnosed with BCC and 52 paraffin-embedded samples of normal marginal skin of the same lesions were evaluated for the presence of HPV deoxyribonucleic acid (DNA) using PCR. (The minimum sample size in accordance to the reference number 10 with consideration of the prevalence of HPV positivity in BCC and healthy skin 52% and 16% respectively and with consideration of confidence coefficient of 95% and by using the formula \( n = \frac{1.96^2 \times 0.52(1-0.52) + 0.16(1-0.16)}{0.52-0.16} \times 2 = 24 \) was calculated as 24, but we have used the sample size of 52 for more reliable conclusions).

A total of 52 excisional biopsy samples of BCC from 2002 to 2011 were selected from archives of pathology department of Imam Reza Hospital. Paraffin-embedded samples and corresponding microscopic slides were extracted from the archive. Then, normal marginal skin samples of the lesions were selected and were sampled using 5mm punch by correspondence with paraffin-embedded blocks. Paraffin-embedded blocks of them were prepared and were subject to 4 µm sections to confirm the absence of BCC in the sample. Microscopic slides were then reviewed, and microscopic sub type of BCC was determined. Clinical data including age, sex,
and location of the lesion in each patient were collected using their records.

Five 20 µm sections of paraffin embedded blocks were prepared in sterile conditions, and the sections were put in sterile Eppendorf tubes for deparaffinization procedure, DNA extraction and PCR. Blocks with incomplete archive files and the samples in which β-globin gene was negative in PCR and was not qualified for PCR were excluded from the study.

**Deparaffinization**

Xylool/ethanol method was used. A total of 1 mL xylol was added to 1.5 mL microtubes containing tissue sections. Next, the microtubes were incubated in laboratory temperature with constant rotation for 30 min, followed by centrifugation in 13000 rpm for 10 min, and the supernatant was decanted. These two steps were repeated once. After that, 500 mL 100% ethanol was added to these diment, and after several inversions the microtube was centrifuged at 13,000 rpm for 10 min, and the supernatant was decanted. This step was repeated once and the sediment was put in laboratory temperature for complete removal of ethanol.

**DNA extraction**

This procedure was performed manually. Extraction buffer consisted of 100 mM Tris-Cl with pH = 7.5 and Tween 20 at a concentration of 0.05%. A total of 400 mL extraction buffer and 20 mL protease K were added to each microtube. The microtubes were incubated for 3 h at 55°C. They were then placed in 100°C dry blocks to inactivate protease K. The microtubes were centrifuged in 5000 rpm for 10 min and the supernatant was used for PCR.

**PCR**

After DNA extraction from paraffin-embedded blocks, the quality of extracted DNA samples was determined using β-globin gene primers. For this purpose, GH20 and PC04 β-globin primers were used, which amplify a 260 bp fragment. The sequence of these primers was as follows:

GH20: 5’ GAA GAG CCA AGG ACA GGT AC 3’
PC04: 5’ CAA CTT CAT CCA CGT TCA CC 3’

PCR was done to amplify the β-globin gene segment using the above primers. The samples producing 260 bp segments using the above primers were considered favorable and were used to amplify HPV L1 gene. GP5+/GP6+ primers were used to evaluate the presence or absence of HPV sequences in extracted DNA samples. These primers amplified part of the highly preserved region of L1 genome in papillomavirus, and were able to detect several types including low- and high risk ones. The primer sequence was as follows:

GP5+: 5’ TTTGTTACTGTTGATATCATAC 3’
GP6+: 5’ GAAAAATAAACTGTTAATCATATTCC 3’

After amplification, 5 ml of each sample was electrophoresed on 2% agarose gel and stained using Green viewer dye for detecting the 142 bp HPV segment.

Data analysis was performed using SPSS 11.5 software, and comparison between case and control groups was done by Chi-square and Fisher’s exact tests.

**Results**

52 BCC samples and 52 normal marginal skin samples of the same lesions were evaluated for presence of β-globin gene using PCR. β-globin gene was negative in both BCC and normal skin around the lesions in 10 samples, which were excluded from the study. PCR was finally done in 84 samples including 42 cases of BCC and 42 cases of normal marginal skin to detect HPVs using GP5+/GP6+ primers.

The patients included 34 males and 8 females. Mean patient age was 61.07 years with a standard deviation of 12.19 years, with minimum and maximum ages of 26 and 81 years, respectively. Nodular BCC was the most common type with 36 cases (85.7%), and other types included keratotic (two cases) and one case of each of superficial, adenoid, and micronodular cases. The most common anatomic site was scalp with 13 cases (31.0%) and the least common was eyelids and neck, each with one case (2.4%). HPV DNA was not found in any of the BCC samples, and there was a single positive case of HPV DNA from normal marginal skin samples using PCR. According to Fisher’s exact test, the difference between case and control groups was not statistically significant ($P = 1.000$).

**Discussion**

Up to now, the etiologic relationship between HPV and BCC has not been established in general population, and the results of several studies are contradictory. In this study, 42 cases of BCC were assessed by PCR using GP5+/GP6+ primer for the L1 region of HPV genome, but HPV DNA was not found in any of them, and only one case of marginal normal skin samples was positive for HPV DNA by PCR, and the difference between the two groups was not statistically significant ($P = 1.000$).

Similar to our research, many studies have found no association between HPV and BCC. In the study of Wieland et al., approximately 60% of BCCs were not associated with HPV infection. No HPV DNA was detected in part of their study on 38 cases of BCC using single-round PCR with GP5+/GP6+. They concluded that their results did not support the role of HPV as an etiologic factor of BCC in immune competent individuals. In the study of Zhu et al., HPV DNA was not detected in PCR in any of the 13 cases of BCC. Berbert et al., Escutia et al., Forslund et al., and Ally
et al.,[3,6-8] in their studies have not indicated the role of HPV in BCC.

Zaravinos et al., Iftner et al., Meyer et al., and Paolini et al.,[1,3,9-11] have also noted the requirement for further studies to determine the etiologic relationship between HPV and BCC. Prevalence of HPV DNA in BCC in their studies has been 33% (5 out of 15), 27.8% (5 out of 18), 52%, and 70.3% (26 out of 37), respectively. In the study of Zakrzewska et al.,[12] 29 out of 50 BCC samples and 19 out of 50 marginal lesion biopsy samples were positive for HPV, which was a significant finding.

According to Andersson et al.,[13] where HPV 9 seropositivity has been recognized as a risk factor for BCC development in the future,[13] seropositivity for HPV 16, 18 is not a risk factor for BCC, and there was a single case of positivity for HPV in 221 BCC biopsy samples.[14]

The study of Wieland et al.,[4] in view of PCR method and the primers as well as prevalence of HPV DNA in BCC (none of 38 cases), was similar to the present study.

The difference in HPV DNA in normal skin and BCC in our study and the aforementioned studies could be due to the following:

a. Lack of association between HPV and BCC
b. Technical error: We have used highly sensitive and specific methods to detect HPV in BCC and marginal normal skin of the lesion; therefore, negative results of our study are not likely to be due to technical error

c. Sample type and method for PCR: Similar to our study, Paolini et al.,[11] used paraffin-embedded blocks but their results were different from ours perhaps due to different PCR methods. In the studies of Escutia et al., Zaravinos et al., Wieland et al., Iftner et al., and Berbert et al.,[2,4,6-9] in which HPV DNA incidence was higher than our study, snap frozen punch biopsy samples were used. The use of paraffin-embedded blocks may be a reason for low incidence of HPV DNA in our study. The incidence of HPV DNA in swab samples was higher than biopsy samples after removing superficial cellular layers.[6]

Application of disinfectant before skin biopsy and/or simple stripping of the skin using tape seem to be effective in positive HPV DNA results.[6-9]

d. PCR method, primer type, and number: The majority of successful methods to detect HPV DNA in skin tumors have used nested PCR, which suggest that the level of HPV DNA in the lesion is low.[6] In part of his study on 38 cases of BCC, Wieland et al.,[4] used single-round PCR and GP5+/GP6+ primers. HPV DNA was not found in any of the samples, which was similar to our study in terms of PCR, primer, and results. However, he used both single-round PCR and nested PCR using seven primer pairs to detect HPV DNA on 69 BCC samples in another part of his study, among which 30 cases (43.5%) were positive for HPV DNA, and 28 cases were positive in nested PCR using CP65/CP70 and CP66/CP69 primers.

According to our study results, HPV infection has not a significant role in BCC pathogenesis. This is the first study in Asian population using PCR method for detecting HPV in BCC. Further studies to compare HPV DNA in BCC using paraffin-embedded blocks, fresh tissue, and snap frozen punch biopsy as well as alternative PCR methods such as real-time PCR and nested PCR using more primers which are more sensitive than conventional PCR is recommended to detect HPV DNA in samples.

Acknowledgment

The authors express their profound gratitude for research deputy of Mashhad University of Medical Sciences for financial support and approval of the research proposal (No. 89928) related to thesis of Samaneh Jahanfakhr with code of 2593.

What is new?

Human papilloma virus infection has not a significant role in basal cell carcinoma pathogenesis.

References


