Human papillomavirus and skin tags: Is there any association?

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ABSTRACT

Background: Low-risk human papillomavirus (HPV) infections are related to the genesis of various benign lesions. In an isolated report available, HPVs have been implicated in the causation of skin tags too. **Aims:** The present study was designed to detect the existence of low-risk HPV types 6 and 11 in cutaneous soft fibromas (skin tag) in north Indians. **Methods:** A total of 37 cases of skin tags from various sites were analyzed. Highly sensitive and comprehensive polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays were done for the detection of low-risk HPV types 6 and 11. **Results:** The results revealed the presence of HPV DNA 6/11 in 48.6% of the skin tags examined by PCR-RFLP. **Conclusion:** This result corroborates the hypothesis that HPV plays a part in the etiology of benign lesions like cutaneous soft fibromas. The identification of HPV 6/11 in these lesions, which are benign proliferations of the skin, further expands the spectrum of HPV-linked lesions.

Key Words: Human papilloma virus, Skin tags

INTRODUCTION

Human papillomavirus (HPV) is known to be the most ubiquitous of the human viruses. Over 100 HPV types have been identified till date. In healthy population, most of these HPV types appear to establish a latent infection of the skin, mostly as normal flora residing in hair follicles. The numerous HPV types differ in their biological properties and oncogenic potential. Types with high oncogenic potential (which always express the early proteins E6 and E7) are able to transform keratinocytes on their own.[1] The HPV genome usually remains episomal; but in transformed cells, the viral DNA is frequently integrated into the host DNA.[1]

Skin tag, or soft fibroma, is a common benign condition, which consists of a bit of skin which projects from the surrounding skin.[2] Histologically, skin tag is a polypoid lesion with overlying mildly acanthotic epidermis. There is a loose, edematous fibrovascular core with mild chronic inflammation. Fibroepithelial polyps, or acrochordons, often develop in areas of skin friction. Certain HPV types are found to be associated with the pathogenesis of benign lesions like papillomas of larynx, conjunctiva; respiratory papillomatosis[3]; etc. We undertook the present study with the aim of establishing the presence of low-risk human papillomavirus types 6 and 11 using polymerase chain reaction (PCR) systems in a benign cutaneous lesion like skin tag in a tertiary care hospital setting in India.

METHODS

Those subjects who presented to the dermatology outpatient clinic with skin tags on multiple sites and were otherwise healthy and willing to participate in the study were enrolled. After obtaining informed consent, biopsy specimens from skin tags were obtained from neck, dorsum of hand and axilla from 37 patients. Ten biopsy specimens from the normal skin surrounding skin tags were also analyzed. In a few cases, biopsies from two different skin tags of the same patient were analyzed.

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DNA extraction

Briefly, the tissue specimen was teased and suspended in 500 μL of lysis buffer containing 1% sodium dodecyl sulphate (SDS) and 0.01% proteinase K in Tris-EDTA (TE) buffer (pH 8.0) and incubated at 55°C overnight. After phenol-chloroform extraction, the DNA was precipitated with chilled isopropanol and resuspended in TE buffer. The prepared DNA was quantitated spectrophotometrically, and PCR for β-actin gene was done for each sample as control reaction to check the quality of material.

E1-PCR analysis

All the samples were subjected to PCR using primers specific for E1 open-reading frame of the HPV genome. The sequence of the forward primer was 5′-TATGGCTATTCTGAAGTGGAA-3′ and that of the reverse primer was 5′-GATATACCTGTTCTAAACCA-3′.[4] The reaction was carried out in a volume of 20 μL containing 2 μL 10X Taq buffer, 10 pmol of each of the sense and antisense primers 250 μmol dNTP mix, 1.5 units of Taq polymerase (Invitrogen, USA), and distilled water. Three microliters of template DNA was added for each reaction. The plasmid DNA containing HPV genome types 6 and 11 was used as a positive control in the reaction. Reaction was performed in a DNA thermal cycler (Eppendorf, Germany) as per the understated protocol.

Ten minutes of denaturation at 94°C for the first cycle, followed by 1 min each of denaturation at 94°C, annealing at 48°C, and extension at 72°C for 33 cycles was done. The last cycle was extended for 10 min at 72°C. The electrophoresis of amplified products was done, and the gel was stained with 0.5 μg/mL ethidium bromide to visualize the amplified PCR product. A 526-594-base pair (bp) band was visualized in the samples positive for HPV on a UV transilluminator. The picture was captured on a gel documentation system (Imagemaster, Pharmacia Biotech, Sweden).

Restriction fragment length polymorphism (RFLP)

PCR products obtained from E1-PCR were purified by using the Invisorb® Spin PCRapid Kit (Invitek, Berlin) as per instructions of the manufacturer. Subsequently, purified PCR products were digested with 10 units of restriction enzyme *Alu1* at 37°C for 2 h and electrophoresed on a 3% agarose gel. *Alu1* digestion of amplified fragment from 'low-risk' HPV types 6 and 11 formed the same pattern (a large fragment of 555 bp).[4]

Statistical analysis

Statistical analysis was performed with the Fisher exact test; a *P* value less than or equal to 0.05 was considered significant.

RESULTS

Thirty-seven cases of skin tags from different sites including neck, dorsum of hand, and axilla were recruited in the study. Male:female ratio was approximately 3:1. Majority of the patients (57%) were in the age group of 26 to 55 years. Mean age was 41 years (range, 15-65).

PCR using consensus primers spanning the E1 open reading frame showed presence of mucosotropic HPV types in 48.6% (18/37) of the samples [Figure 1]. The biopsy specimens from the normal skin surrounding skin tags were negative for HPV DNA [Figure 1]. The E1-PCR products were then subjected to RFLP, which specifically identifies HPV types 6 and 11. All samples (18/18, 100%) positive by E1-PCR showed HPV 6/11 sequence patterns (a single band of 555 bp) (Figure 2, lane 6 to lane 9). A few cases, where biopsies from two different sites of the same patient were analyzed, gave the same results. Table 1 shows the distribution of HPV DNA types 6 or 11 in skin tags from different patients with respect to their sex, age, and localization of the lesion. There was no significant correlation with respect to sex, site of lesion, or age of the patient.

DISCUSSION

The subtropical and tropical areas of the globe harbor large number of infections. The reason can be attributed

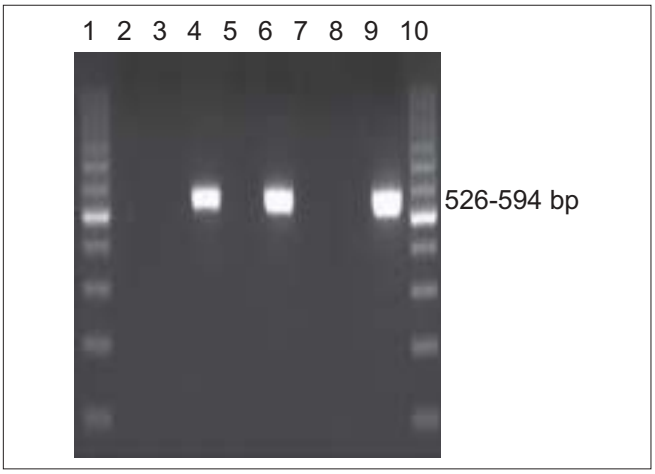

Figure 1: Representative picture of 1.5% agarose gel electrophoresis showing post-PCR products in normal skin tissue and skin tags from different patients [positive band is of amplified HPV DNA of 526-594 base pairs (bp)]. Lane 1: DNA marker (100-bp ladder); lane 2: negative control; lane 3: normal skin from patient 1; lane 4: skin tag from patient 1; lane 5: normal skin from patient 2; lane 6: skin tag from patient 2; lane 7: normal skin from patient 3; lane 8: skin tag from patient 3; lane 9: plasmids DNA containing the HPV genome types 6 and 11 (positive control); lane 10: DNA marker (100-bp ladder)

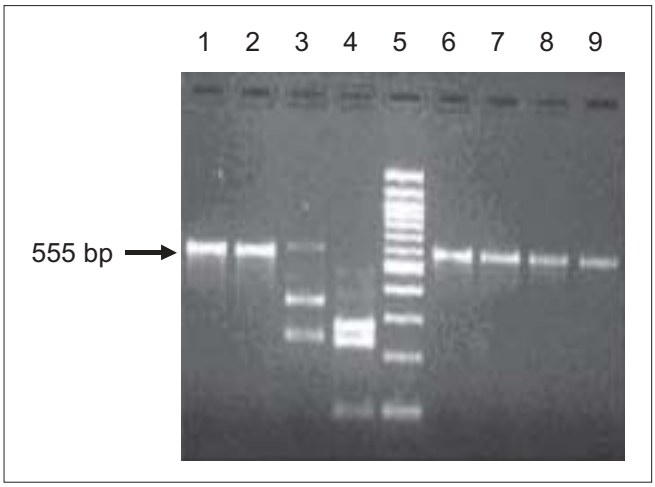

Figure 2: Electrophoresis on 3% agarose gel showing the *Alu1*-digested PCR products of the E1-PCR from different skin tag samples and from positive controls. Lane 1: plasmids DNA containing the HPV genome types 6 (555 bp); lane 2: plasmids DNA containing the HPV genome types 11 (555 bp); lane 3: plasmids DNA containing the HPV genome types 16 (343 bp and 240 bp); lane 4: plasmids DNA containing the HPV genome types 18 (267 bp, 238 bp, and 90 bp); lane 5: DNA marker (100-bp ladder); lane 6 to lane 9: test samples from different patients

Table 1: Distribution of HPV DNA type 6 or 11 in skin tags from different patients with respect to their sex, age, and site of lesion

Parameter	Total number of cases (*n* = 37)	Positive for HPV type 6 or 11 (*n* = 18, 48.6%)
Sex		
Male	27	13 (72.2)
Female	10	5 (27.7)
Age		
≤41	18	9 (50)
>41	19	9 (50)
Localization		
Neck	24	14 (77.7)
Dorsum of hand	8	3 (16.6)
Axilla	5	1 (5.5)

Figures in parenthesis indicate percentages

to the intense exposure of skin to sun. The ultraviolet (UV) irradiation has intense effects on skin immunology, including reduction in density and antigen-presenting ability of Langerhans cells in the epidermis, increased keratinocyte secretion of IL-10 and prostaglandin E2 with increased serum levels of IL-4.[5] In addition, there is induction of suppressive IL-12p40 homodimers by dendritic cells and macrophages. Thus UV irradiation seems to induce a resultant immunosuppressive effect with decreased TH1 cell activation.[6] This type of microenvironment is ideal for the survival of infection in healthy subjects living in tropical parts of the globe.

HPVs are epitheliotropic and host specific, with infection across the species being exceedingly uncommon. It has been postulated that HPV infection begins with the inoculation of virus into the interrupted epithelium and the interaction with a putative specific cellular receptor.[7] It is recognized that HPV following trauma of epithelium establishes a nonproductive infection of basal cells in the skin and mucosa, but it is only in the differentiated epithelia that HPV replicates.

The skin tags or fibroepithelial polyps are known to develop in areas of skin friction, leading to disruption of skin, which might serve as a route of entry for the virus. The presence of HPV DNA and mechanical friction seem to be significant cofactors in the pathogenesis of skin tags.[8] The immune status and genetic profile of the host, as well as the type of virus, may play a role in determining the clinical outcome of HPV infection. So the clinical behavior of soft fibromas may be reminiscent of that of recurrent laryngeal papillomas with respect to the fact that they spread locally in the same subject but rarely to other individuals.

In the index study, HPV 6/11 DNA was found to be present in 48.6% of biopsies from skin tags. The entire samples were subjected to PCR-RFLP, and the results of both recognized the presence of HPV 6/11 in 48.6% of the entire sample. The samples were negative for high-risk HPV types (data not shown) when subjected to polymerase chain reaction using consensus primers for high-risk types. The only available literature is a study by Dianzani *et al.*,[8] who have reported the presence of HPV 6/11 in 88% of the skin tags in Caucasian patients using PCR-RFLP technique. These observations strongly suggest that HPV, along with other cofactors, may be involved in the pathogenesis of these cutaneous lesions. Dianzani *et al.*[8] observed low quantity of HPV DNA in soft fibromas and explained their association with the clinical evolution of these lesions.

The consistent presence of certain 'low-risk' human papillomavirus types in the skin tag specimens from different sites of the same patient supports the viral etiology. Though the tropical geographical conditions favor infectious etiology in our patients, the lower frequency of the presence of HPV DNA in soft fibroma cells in the present study as compared to the frequencies reported in the literature could be due to difference in the sensitivity of the test, as well as the loss of HPV genome. The presence of HPV sequences in skin tags could aid their recurrence as well. The expression of early viral genes may contribute to stimulated cell growth, which leads to limited epithelial proliferation and formation

of acanthotic epidermis overlying edematous fibrovascular tissue.

The association of HPV type 6 with benign lesions is very old. It was in 1982 that HPV type 6 was observed in laryngeal papillomas with the help of DNA hybridization technique. A year later, HPV type 11 was also found to be associated with laryngeal papillomas.[9] However, the literature on the intracellular mechanisms through which HPV type 6 or 11 may immortalize cells is still fractional in comparison with data on the high-risk HPV types. Additional *in vitro* and epidemiological studies investigating the influence of genetic and environmental factors on the interaction of the HPV proteins with cellular proteins should provide valuable information on a possible role for HPV types 6 and 11 in the pathogenesis of these cutaneous lesions.

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