Identification of HPV types 6 and 11 in skin tags using PCR

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ABSTRACT: The Human Papilloma Virus (HPV) infection is suspected to be one of the causes of skin tag lesions. In order to identify HPV types 6 and 11 in skin tag lesions using the Polymerase Chain Reaction (PCR) technique, this study is a descriptive study with a cross-sectional design involving 30 skin tag lesions. PCR examination of skin tag lesions was performed to identify HPV types 6 and 11. The collected data was processed and presented descriptively. In this study we found that eight (26.7%) skin tag lesions identified with HPV type 6, and six (20%) skin tag lesions identified with HPV type 11. From a total of 30 skin tag lesions that were examined using the PCR technique, 14 (46.7%) of the lesions identified with HPV types 6 and 11.

Keywords: skin tag, HPV, polymerase chain reaction

1 INTRODUCTION

A skin tag is a benign lesion composed of loose fibrous tissue that occurs mainly on the neck and major flexures as a small, soft, pedunculated protrusion (Quinn & Perkins, 2010). Skin tags may present singly or as multiple lesions, they tend to grow progressively and they do not involute spontaneously. These lesions are commonly found in the adult population over 40 years of age, and increase in incidence in the elderly (Tamega et al., 2010). Skin tags can manifest clinically as three types: multiple small papules, single or multiple filiform, and bag-like pedunculated growths (Shashikala et al., 2014). Histological findings show epidermis slight acanthosis and papillomatosis with a fibrovascular connective tissue core (Heenan, 2005; Ko, 2012).

To date, the aetiology or pathogenesis of skin tags is still unclear. Obesity, ageing, dyslipidemia, diabetes mellitus, pregnancy and Human Papilloma Virus (HPV) have been proposed as a potential aetiological or associated factor of the skin tag (Tamega et al., 2010; Erkek et al., 2011). The HPV infection has been suggested to be one of the factors involved in the pathogenesis of skin tags, and the first study to correlate this theory was conducted by Dianzani et al. in 1998. Dianzani and his colleagues were able to detect HPV DNA 6 and 11 in 88% of the samples of skin tags by using the Polymerase Chain Reaction (PCR) technique (Dianzani et al., 1998).

HPV is a small non-enveloped virus (50–55 nm) that contains a double-stranded closed circular DNA genome associated with histone-like proteins and protected by a capsid formed by two late proteins, L1 and L2 (Fermandes, 2012). Over 100 HPV types have been identified to date. HPV is an epitheliotropic virus that can induce squamous epithelial tumours (benign cutaneous and mucosal lesions) in many different associated anatomical localisations, which produces cytopathic effects in infected keratinocytes (Dianzani et al., 1998; Morshed et al., 2014).

HPV DNA can be detected in biopsy specimens by various methods, such as hybridisation procedures and PCR. PCR is the most widely used method for amplification of nucleic acids, and has a high sensitivity and specificity (Moljin et al., 2005). In this study, PCR was used to detect HPV DNA types 6 and 11 genomes in skin tag lesions.
2 METHOD

This study aimed to detect HPV DNA in skin tags by using the PCR technique. This study was performed on skin tag patients with a clinical and anamnesis diagnosis. Thirty skin tag biopsy specimens were obtained under local anaesthesia from body sites (colli, axillaris, auricularis, and femoralis). The specimens were preserved at below –20°C, and DNA was then extracted from the skin tag samples using a commercial DNA isolation kit from tissue (GeneAid®) according to the manufacturer’s instructions.

All of the samples were subjected to PCR, using primers specific to the HPV genome. The presence of HPV type 6 was investigated by PCR amplification of 258–361 bp (base pairs) target from the locus control region (LCR) using the sequence forward primer 5’-TAGGGACGGTCTCTATTC-3’ and reverse primer 5’-GCAACAGCCTCTGA GTCACA-3’. The presence of HPV type 11 was investigated by PCR amplification of 356 bp target from the L1 region using the sequence forward primer 5’-GAATACATGCGCCATGTGGA-3’ and reverse primer 5’-AGCAGACGTCCGTCTGC GAT-3’. The reaction was carried out in a volume of 20 μl containing 12 μl PCR fast ready mix (KAPA 2G®), 2.5 μl forward primer, 2.5 μl reverse primer, and 5 μl DNA isolate. The amplification cycles comprised 1 min at 95°C for initial denaturation, 15 seconds at 95°C for denaturation, 15 seconds at 55–65°C for annealing, 5 seconds/kb at 72°C for extension, and 10 mins at 72°C for the final extension using a thermocycler (Applied Biosynthesis™ Veriti 384®).

For analysis of the PCR product, 5–7 μl amplification product were electrophoresed on 2.5% agarose gel (Bioron®) in Tris Acetate EDTA (TAE) buffer with 1 ul ethidium bromide (Invitrogen®). Gel was visualised on an ultraviolet transilluminator; visualising the 258–361 bp fragment was interpreted as a positive result for HPV type 6 and visualising the 356 bp fragment was interpreted as a positive result for HPV type 11. Precautions to avoid cross-contamination and false-positive results were taken in every assay.

3 RESULT

In this study, 30 samples of skin tag lesions were obtained from 16 skin tag patients (6 men and 10 women), who were in the age group 20 to 60 years. Samples from patients were taken from the following areas: 20 (66.7%) samples from the colli region, 7 (23.3%) samples from the axilla region, 2 samples (6.6%) from the auricularis region, and 1 (3.3%) sample from the femoralis region.

Analysis of the presence of HPV DNA by PCR showed that 14 (46.7%) of the 30 positive samples contained HPV DNA, 8 (26.7%) of the positive samples contained HPV type 6 with restriction band level at 258 bp (Figure 1), and 6 (20%) of the positive samples contained HPV type 11 with restriction band level at 356 bp (Figure 2).

Figure 1. Gel electrophoresis PCR with HPV type 6. Line 1 weight marker 50 bp ladder, line 2 is positive control, line 3 is negative control. Sample nos. 1, 5 and 8 showed a positive band (258 bp).
DISCUSSION

HPV is epitheliotropic and host-specific, with infection across the species being uncommon (Androphy & Kirnbauer, 2012). HPV infection occurs through inoculation of the virus into a viable epidermis through breaks in the epithelial barrier. Maceration of the skin is probably an important predisposing factor. Animal models using HPV virions demonstrate that attachment to heparan sulfate proteoglycans on the basement membrane is a required initial step in natural infection. A furin protease then cleaves L2, inducing a conformation change that allows binding to an unidentified basal cell receptor. This experimental model explains how PV reserve infection for and specifically target epithelial basal cells (Androphy & Kirnbauer, 2012).

Suzuki and his co-workers demonstrated the high association between the presence of HPV DNA types and other benign tumours, such as laryngeal papillomas (Al-Shaiji & Al-Buainian, 2005). The clinical condition of skin tags is closely similar to that of mucocutaneous papillomatosis and their clinical behaviour may be reminiscent of that of laryngeal papillomas, which might raise the suggestion of a common aetiology. Since HPV has been detected in many papillomas, they may consequently be responsible for the development of skin tags (Sallam et al., 2003). The result of this study was in accordance with a study by Gupta et al. (2008), who previously reported the presence of HPV 6/11 DNA in 48.6% of biopsies; however, the results of this study were lower in comparison with the study conducted by Dianzani et al. (1998), who reported the presence of HPV type 6/11 in 88% of skin tag samples, and the study by Sallam et al. (2003), who reported that 23 (76.6%) out of 30 positive samples contained HPV DNA type 6/11.

The finding of HPV DNA types 6 and 11 in biopsies of the skin tag lesions suggests that this virus may be involved in the pathogenesis of these cutaneous lesions. It has been known that skin tags or fibroepithelial polyps are developed in areas of skin that are prone to rubbing or friction (Pezeshkpoor et al., 2012) and can lead to disruption of the skin, which might serve as a route of entry for the virus. The presence of HPV DNA and mechanical friction seem to be significant co-factors in the pathogenesis of skin tags (Shashikala et al., 2014). It has been postulated that HPV infection begins with the inoculation of the virus into the interrupted epithelium and the interaction with a putative specific cellular receptor (Gupta et al., 2008). HPV infection involves squamous epithelium and can cause growth stimulation, cell proliferation and the formation of pathologic cells (Morshed et al., 2014) through the role of E5 oncoprotein, which can activate growth factor receptors, epithelial hyperplasia, papillomatosis, hyperkeratosis and koilocytic change that are regarded as cytopathic effects of HPV (Shashikala et al., 2014).
5 CONCLUSION

In this study the presence of HPV types 6 and 11 genomes in the skin tag lesions from different sites could support the viral aetiology theory, but cannot be considered as proof of the aetiological role, because the results of this study are not of a high percentage. The presence of HPV DNA that can affect cellular differentiation and mechanical friction seem to be significant co-factors in the pathogenesis of skin tags.

REFERENCES


