Skin Tags in Relation with Human Papilloma Virus

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Skin tags are flesh colored papilloma found commonly on the sides of the neck, axilla, upper trunk and eyelids of the middle aged and elderly.[1] It has been suggested that there is statistical association of skin tags with diabetes mellitus[2] accromegaly[3] and as an aid to confirm Crohn's Disease through the biopsy of perianal skin[4] and adenomatous colonic polyp.[5] There are many synonymous names for skin tags according to variation in microscopic finding such as acrochordons, cutaneous tags, papilloma, fibroma molluscum, templetons, skin tags, eruptive lipo fibromata. If the tag shows the predominance of epidermis and little connective tissues the name fibroma would be an appropriate one. The term tag merely means (an appendage) and thus might fit for entire spectrum of these lesions.[6]

Clinically:
Skin tags present as small multiple furrowed papules specially on the neck and in the axillae.[1] It may present as apedunculated single bag like growth mostly on lower trunk.[7] Tags are usually harmless and asymptomatic but occasionally one may become inflamed and necrotic with severe tenderness on being twisted.[8] No significant sex preference was found (with male to female ratio of 1. 19:1.0).[5] The age incidences range from 10 to 50 years.[9]

Histopathologically:
The tag usually shows papillomatosis, hyperkeratosis and regular acanthosis and sometimes horn cysts within the acanthotic epidermis. The lesions may have loose or dense collagen; diffuse fatty tissue may be prominent with no evidence of inflammation.[8]

Etiology:
There is possible association between skin tags and colonic polyps.[10] It is also suggested that they may be skin markers of Gardener's Syndrome or Birt Hogg Dube Syndrome.[11 - 12] It was reported that multiple skin tags often appear in the second half of pregnancy and it has been suggested that they are probably due to hormonal factors.[13]
In 1998 Dianzany et. al., were able to detect human papilloma virus DNA in skin tags by PCR, suggesting a possible role in the etiology of skin tags and there is no documented or proven inter-human transmission. Human papilloma virus (HPV) is associated with benign cutaneous or mucosal lesions as well as with malignant ones and recently in several studies, HPV could be detected in normal skin samples. The presence of HPV is tested by DNA polymerase chain reaction (PCR) which was found to be positive in 35% of normal skin samples tested; suggesting that HPV DNA may be widely distributed in normal skin of the immunocompetent population in whom an intact immune system probably inhibits the development of clinical disease.[14]
HPV sequences have been detected in 10% of normal squamous epithelium of cervical swabs and molecular in situ hybridization was positive indicating latent papilloma virus infection[15- 16] while another study indentified HPV in 35 – 40% of women with abnormal papanicolau smear.
Venereal transmission of papilloma virus is generally accepted, and inapparent HPV infection is suspected in the male population. A study was done on 530 smears of clinically unaffected glans penis. Sulcus coronas of healthy men aged 16-79 years were hybridized with 32p- labeled HPV DNAs. None had any clinically visible lesions on the genital area.

Patient and method:
This study aimed to try to detect HPV DNA in skin tags by PCR. The study group consisted of 30 patients: 14 females and 16 males, attending dermatology department. Skin biopsy specimens were obtained under local anesthesia from body friction sites (neck, back, axilla) from patients with multiple skin tags with dimensions ranging from (3- 6x 2- 5). Five other biopsy specimens were taken from normal skin as a control.
The specimens were preserved under -70 ^{0}C then they were subjected to DNA extraction using Wizard genomic DNA purification. The DNA was extracted from the tissue biopsy and each sample was subjected to a consensus primer mediated PCR method which is able to amplify 450 base pair (bp) within the L1 open reading frame. The standard PCRs were carried out in 50ul. A two minutes initial denaturation step at 95 was followed by 30 cycle of amplification with a PCR processor. Each cycle included a denaturation, annealing and extension step. The reaction mixture was analyzed by electrophoresis on 2% agarose in triacetate EDTA buffer, stained with ethidium bromide to be visualized under ultra violet transilluminator.[19]
High pure PCR template was used to extract nucleic

acid from tissue samples taken. Cells are lysed during a short incubation with proteinase k in the presence of chaotropic salt (guanidine HCL), which immediately inactivates all nucleases. Cellular nucleic acids bind selectively to glass fiber in a special centrifuge tube; the nucleic acids remains bound while a series of rapid "wash-and-spin" steps remove contaminating small molecules. Finally, low salt elution removes the nucleic acids from the glass fiber. The process does not require precipitation, organic solvent extractions, or extensive handling of the nucleic acids.[18] DNA product obtained by PCR were digested with restriction enzyme Bam HCI (Concentration 1u/u1) incubated over night at 37°C and electrophoresed on 2% agrose gel then stained with ethidium bromide. Restriction with Bam HCI is positive with type 11 while human papilloma viruses types 6, 16, 31–33, 35, 36 and 5 give no restriction with Bam HCI.

DNA amplification:

Was performed by including the reaction mix for 30 cycles in a thermocycler. Each cycle consisted of denaturation at 95 C for 30 sec followed by annealing at 55 C for 30 sec and extension at 72 C for 30 sec with initial delay for 2 min at 95 C at the beginning of the first cycle and 5 min delay at 72 C at the end of the last cycle. [19] To detect amplification product agarose gel electrophoresis is used to separate molecules. It is based upon charge, size and shape. [20]

Results:

HPV DNA was detected in 23 patients out of 30 (77%) patient with skin tags , only one sample out of 5 in the control group was positive (20%) . There is highly significant correlation between patient and control group regarding positive cases with P<O. 01.

16 samples showed HPV type 6 with restriction band level at 450bp and 7 samples showed type 11 with no restrictions, with band at 450bp.

We had 12 female and 11 male in the positive group, 2 female and 5 male in the negative group there was no significant correlation with sex and presence of HPV DNA with P>O.05.

The mean age in the positive group was 40 + 14.2, while in the negative group 41 + 9.8, when both group were matched in age there was no significant different with p>O.05 .

We had 4% obese patient in the negative group and 13% in the positive group with no significant correlation between obesity and HPV detection, with P>O.05.

There was no significant correlation between the localization of skin tag and the presence of HPV except for the back where 30% of the patient of the positive group had skin tags on their back while no one in the negative group had tags on the back with P<O-.05.

Discussion:

In the present study, we have detected human papilloma virus (HPV) type 6 and 11 DNA in a high percentage 77% of biopsies from skin tags which suggested that this virus may be involved in the pathogenesis of skin tags, no significant correlation was found between the presence of (HPV) DNA in skin tags and their localization except for the back, this may be because the back is more subjected to trauma of friction. There is no significant correlation with sex, age or obesity.

Dianzani and his colleagues (1998) were able to detect HPV DNA 6, 11 in skin tags by molecular hybridization and PCR, the percentage of PCR positive sample is higher in hybridization which is consistent with higher sensitivity of PCR compared to molecular hybridization and it also indicates that HPV DNA is present in low amount in soft fibroma.

Suzuki and his co-workers (1995) demonstrated the high association between the precense of HPV DNA types and other benign tumours, like recurrent laryngeal papilloma which seems to behave like skin tags, in being able to spread locally in the same subject but rarely to other individuals. Those mentioned studies are consistent with our study showing the possible role of HPV in benign tumours with behaviors similar to skin tags.

HPV has been detected in vocal cords of patients without papillomatosis, Rihkanen and his colleagues (1994) suggested that additional events is necessary to produce clinical infection, with HPV, such as the effects of trauma, steroid hormones, sepsis, malnutrition immuno suppression or the presence of virus coinfection. [21 - 22]

HPV sequences have also been detected in clinically and histologically normal squamous epithelium indicating latent papilloma virus infection, by molecular in situ hybridization on cervical swabs 10% of samples that are normal on papanicolaou staining are positive for HPV DNA, [15] [24] also HPV DNA was detected in clinically normal newborn tissues taken from the foreskin these study results point out that HPV DNA can be found in the absence of the overt disease. [25] This result is inconsistent with our study where HPV DNA was detected in 20% of the controlled cases.

Concerning the HPV status of the PCR negative samples in our study several possibilities exist. These samples could have carried on or only very few HPV sequences, HPV could have been damaged during fixation process, or HPV sequences might have been present that were not amplified by the primer used. In the future, wider use of novel HPV isolates in the probe panels will improve our knowledge about the association of HPV and skin tags, and we could possibly detect the co-factors that together with HPV DNA, the degree of cellular differentiation and mechanical friction seem to be significant co factors in the pathogenesis of skin tags and the almost constant association between HPV DNA and skin tags indeed suggests a role for HPV in the pathogenesis of these cutaneous lesions.

Summary and Conclusion:

Soft fibroma or skin tags are flesh colored pedunculated tumors with a smooth surface. Histologically they are polypoid tumors with a flattened acanthotic and hyperpigmented epithelium. The dermis has loose connective tissue with dilated blood vessels. Soft fibromas are benign lesions that arise mainly at sites of friction (neck, back, axilla). No interhuman transmission has been documented, nor viral etiology has been demonstrated, but some recent studies suggest that human papilloma virus (HPV) may have a role in pathogenesis of skin tags. [26] There is suggested statistical association of skin tags and diabetes mellitus, [2] Acromegaly, [3] and crohns disease. [4]

In our study, we explore the relationship between human papilloma virus and skin tags. Skin biopsy specimens were obtained from 30 patients with multiple skin tags. Biopsy specimens from normal skin were analyzed from five healthy volunteers. DNA was extracted from all the obtained specimens by polymerase chain reaction PCR. 77% of the samples were positive for the presence of HPV DNA and 20% only in normal skin, seven samples showed type 11 and 16 samples showed type 6 HPV. Statistical analysis showed highly significant difference between the patients and the control in HPV DNA detection. No significant correlation was found between the presence of HPV DNA in skin tags and sex, age or obesity of the patient. From those results we concluded that human papilloma virus is related to skin tags and it could be an etiological agent for development of skin tags.

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