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ORIGINAL ARTICLE

Human Papilloma Virus in Skin Tags

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ABSTRACT

Background: *There are two clinical classes of Human papillomaviruses (HPV); the high-risk HPV and low-risk HPV. HPV is associated with a variety of cutaneous and mucosal manifestations. Among the cutaneous manifestations are flesh colored papilloma known as skin tags. Researchers reported possible relation to some metabolic illnesses and syndromes and controversial results concerning the role of low-risk HPV types in the pathogenesis of skin tags.* ***Aim of the work:*** *To investigate the presence of HPV low risk types 6, 11 and high risk types 16 & 18 DNA in skin tags.* ***Methodology:*** *Thirty patients with skin tags (25 females and 5 males) at different body sites were included in this study. Tissue specimen were collected from the lesions and within an area 5cm away from the lesion (served as control). DNA was extracted and polymerase chain reaction (PCR) was used to detect HPV DNA of low-risk types 6&11 and high-risk types 16& 18.* ***Results:*** *HPV DNA 16& 18 was not detected in any tested specimen. HPV DNA 6& 11 or both was detected in 25 specimens (71.4%) out of the 35 samples collected from 30 patients with skin tags. Only four non-lesional specimens (13.3%) were positive for HPV DNA (2 for HPV6 and 2 others for HPV11). This might support the association of HPV and skin tags, however, a study with larger population sample is recommended. There was no significant relation between the site of skin tag and HPV DNA positivity neither between this positivity and any previously reported risk factors.*

INTRODUCTION

Skin tags are flesh colored, pedunculated benign masses with a smooth surface [1]. Skin tags usually involve flexural areas such as the neck being the most commonly involved site, axilla, and groin and in rare cases genital and anal areas [2]. Skin tags are more common in elderly people, especially in the post-menopause women. Histologically, a skin tag has a fibrovascular core with mild chronic inflammation [3].

Human Papilloma Virus (HPV) is a small DNA Papovavirus. Currently over 200 different types of HPV have been described [4]. HPV is associated with a variety of cutaneous manifestations including plantar warts, common wart, plane warts, anogenital warts and epidermodysplasia verruciformis. Mucosal manifestations include oral warts and condylomata, nasal and conjunctival papilloma, focal epithelial hyperplasia, laryngeal papillomatosis as well as cervical lesions [5]. Transmission of HPV requires the inoculation of the virus into cells of the basal epithelial layer, which is thought to occur in sites prone to micro injuries [6].

Human Papilloma Viruses are classified into high risk HPVs that induce malignant transformation as types 16& 18 and low risk HPVs such as 6&11 that are never reported in invasive squamous carcinoma [7]. To distinguish between present and past HPV infection, serology is an unreliable method. Consequently, demonstration of HPV in the tissues depends on the identification of viral nucleic acid. DNA amplification by polymerase chain reaction (PCR) can be used to detect HPV in a given tissue [8].

Dianzani and coworkers in [9] reported HPV types (6, 11) DNA in 88% of biopsies of skin tags and a statistical association of skin tags with diabetes mellitus and acromegaly has also been suggested [1]. The relation between HPV and skin tags is not yet clear and previous studies on HPV in skin tags provided contradictory results [1, 10, 11].

Aim of the Work

The aim of this study was to evaluate the relationship between HPV low risk types (6, 11) and high risk types (16, 18) of Human Papilloma Virus and skin tags pathogenesis.

METHODOLOGY

Subjects:

This study was carried out on 30 patients with multiple skin tags (25 females and 5 males) with exclusion of patients with verrucae anywhere on the body. Patients were recruited from the outpatient clinic

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Patients and Methods

1. An informed consent was obtained from each participant included in the study. The diagnosis of skin tags was clinically based and confirmed by histopathological examination when needed. All patients included in this study were subjected to a detailed history taking, full clinical general and dermatological examination and a set of laboratory investigations.

2. Skin specimens' collection

Samples were obtained under aseptic condition and local anesthesia by injection of Mepivacaine hydrochloride. A new scalpel for each sample was used then specimens were collected in sterile containers with saline. A total of 65 biopsies were collected as follows;

- Thirty-five lesional biopsies (skin tags) were obtained from 30 patients (for five patients two skin tags were obtained from two different body sites of the same patient).
- Thirty non-lesional biopsies (Taken from free skin within 5cm^2 surrounding the skin tag).

3. Polymerase chain reaction (PCR) was used to detect HPV types 6, 11, 16 and 18 DNA.

3.1. Genomic DNA extraction

The DNA extraction kit used was supplied by Thermo-scientific (Sigma) and the manufacturer's recommendations were followed.

3.2. HPV DNA amplification by polymerase chain reaction (PCR)

PCR was carried out for HPV types 6,11,16 and 18 using the primers listed in table (1) (Sigma, Egypt). With each set of reactions, a positive control represented by the house-keeping β-actin gene and a negative control without a DNA template were included. PCR was carried out in a 25µl reaction volume containing 1µl (~50 ng) of extracted DNA, 1µl of each pair of primers 12.5µl of 2x *Taq* premix Mastermix (Sigma) and 9.5µl sterile double-distilled water. The PCR program involved an initial denaturation step at 95°C for 5 min followed by 35 cycles of a denaturation step at 95°C for 30 seconds, a primer annealing step at 54- 56°C for 30 seconds, an extension step at 72°C for 30 seconds, and a final extension step at 72°C for 10 min.

All PCR products were analyzed by running (10µl) of the product on 1% (w/v) agarose (Sigma) by gel electrophoresis performed at 4 V/cm in Tris borate EDTA buffer [TAE buffer (0.5M Tris acetate, 5.7% acetic acid, 10mM EDTA pH 8.0] containing 0.5µg/ml ethidium bromide (Innogenetics N.V.) stained with 0.5µg/ml ethidium bromide, and photographed under a UV transilluminator by using a Digital Kodak Science 120 system. A DNA ladder digest of 1 kb (GeneRulerTM 50bp DNA, Fermentas Life Sciences) was used as a molecular weight marker.

Statistical analysis:

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 17. - Qualitative data were described as numbers and percentages. Chi-square test, Fisher's exact test and Monte Carlo test were used for comparison between groups, as appropriate. - Quantitative data were described as means (SD) after testing for normality by Shapiro-Wilk test. - "*P* value ≤0.05" was considered to be statistically significant.

Table (1): HPV 6, 11, 16 and 18 and β-actin gene PCR primer sequences used in this study.

Primer's name	*5 `to 3 Primers' sequences ` sequence*	*Amplicon Size*	*Reference*
β-actin F	TCC TGT GGC CAT CCA CAA CT	364bp	GeneBank accession number
β-actin R	GAA GCA TTT GCG GTG GAC GAT		EF 095208.
HPV16F	TCA AAA GCC ACT GTG TCC TG	139bp	12
HPV16R	CGT GTT CTT GAT GAT CTG CA		
HPV18F	ACC TTA ATG AAA AAC CAC GA	450bp	13
HPV18R	CGT CGT TTAGAGTCGTTC CTG		
HPV6F	GTT ATC GCC TCC CCC AAA T	103bp	14
HPV6R	ATC TGG CTT TTC CTT TTC AGG		
HPV11F	TTG CGA AAG GAA CAA ATG TTT	159bp	14
HPV11R	GGA AGA CAC CAA TGA GCC ACT		

RESULTS

Patients included in this study were 30 (25 females and 5 males) with skin tags at different body sites including the neck, axilla, back and abdomen. Their ages ranged from 14 to 60 years with a mean of (40.2 ± 11.9) years and a mean Body Mass Index (Kg/m^2) of 33.9±8.4.

Human papilloma virus DNA 16, 18 was not detected in any tested specimen. HPV DNA (6, 11 or both) was detected in 22 patients out of the 30 patients with skin tags as shown in **[Figure 1 and Table 2].** Regarding the lesional specimens, Twenty-five out of the 35 specimens (71.4%) were positive. In five patients, samples were taken from two different body sites of the same patient and positive samples (3/5)

carried the same HPV type. Only four non lesional specimens (13.3%) were positive for HPV DNA out of the 30 tested non lesional specimens. A statistical significance was detected for HPV DNA positivity in the lesional specimens when compared to the non lesional specimens (P <0.0001).

In the lesional specimens, twelve (34.3%) were positive for HPV 6 DNA, eight specimens (22.9%) were positive for HPV11 DNA and five specimens (14.3%) were positive for both HPV 6,11 DNA. In non- lesional samples, type 6 was present in 2 samples and type 11 in twoother samples. Twenty- five lesional samples (71.4%) were obtained from the neck, six samples (17.1%) from axilla, two samples (5.7%) from the abdomen and two samples (5.7%) from the back **[Figure 1 and Table 2]**. There was no significant relation between the site of skin tags and HPV positivity neither with the type of HPV (p>0.05).

The mean serum cholesterol of the study group was 178 ± 39 mg/dl. The fasting blood glucose (FBG) was 86.4±11 mg/dl and the mean post prandial blood glucose (PPBG) was 125.3±18.4 mg/dl. Only 3/30 individuals (10%) of the study group had a high serum cholesterol above the reference value (220 mg/dl). One patient (3.3%) had a high FBG above (110mg/dl) and five individuals (16.7%) had a high PPBG above (140mg/dl). Acanthosis nigricans was found in 12 individuals (40%) out of 30 individuals enrolled in this study, 6 individuals (20%) out of 30 patients had a high blood pressure and 19 were overweight/obese (BMI > 25).

Twelve patients (54.5%) out of 22 patients of the positive group were overweight. One patient (4.5%) had high serum cholesterol, four patients (18.2%) had a high blood pressure in the positive group and only two patients (9.1%) had high PPBG. There was no significant relation between the presence of these risk factors and HPV positivity in skin tags as shown in **Table (3).**

Table (2): PCR results for HPV 6 and 11 DNA in lesional and non lesional specimens

Biopsy sites	*Number of samples*		*HPV6 +ve No. (%)*	*HPV11 No. (%)*	*HPV6&11 No. (%)*	*Total +ve No. (%)*	*P value*
Back	L	2	1 (8.3)	1(12.5)	0 (0.0)	2/35 (5.8)	Monte Carlo test
	NL	2	0 (0.0)	0 (0.0)	0 (0.0)		
Abdomen	L	2	0 (0.0)	0 (0.0)	0 (0.0)	0/35 (0)	**P = 0.4**
	NL	2	0 (0.0)	0 (0.0)	0 (0.0)		
Axilla	L	6	3 (25)	2 (25)	0 (0.0)	5/35 (14.3)	
	NL	6	**1**	**1**	0 (0.0)	2/30 (6.66)	
Neck	L	25	8 (66.7)	5 (62.5)	5 (100)	18/35 (51.4)	
	NL	20	**1**	**1**	0 (0.0)	2/30 (6.66)	

L= lesional NL= Non lesional; Total PCR positive lesional specimens= 25/35= 71.4%
Total PCR positive non- lesional specimens= 4/30=13.3%

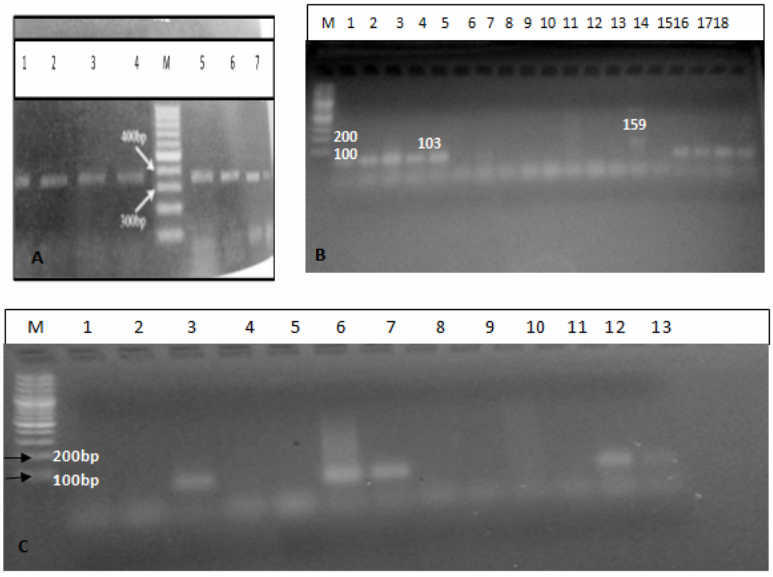

Fig. 1: PCR amplification of HPV DNA types 6, 11

(A): PCR amplification of human β-actin gene (House-keeping gene/positive control)
An ethidium bromide stained agarose gel electrophoresis showing PCR products of some lesional, non lesional and negative control specimens. Lane M: 1kb DNA ladder size marker, Lanes 1-4 and 5-7 show the 346bp band specific for human β- actin gene that was included as a positive control with all reactions and was positive in all.

(B): PCR amplification of samples for HPV types 6&11
An ethidium bromide stained agarose gel electrophoresis showing PCR products of some lesional specimens. Lane M: 1kb DNA ladder size marker, Lanes1-5 and 16-19 show the 103bp band specific for HPV6, Lane 14 shows the 159bp band specific for HPV11. Samples loaded in the rest of lanes were negative for both types.

(C): PCR amplification of samples for HPV types 6&11
Lane M: 1kb DNA ladder size marker, Lane 3,6 and 7 show the 103bp band specific for HPV6, Lanes 12&13 show the 159bp band specific for HPV11. Samples loaded in the rest of lanes were negative for both types.

Table (3): HPV positivity and risk factors in patients

Tested Parameters	*HPV +VE (N = 22) No. (%)*	*HPV -VE (N = 8) No. (%)*	*P value*
Serum cholesterol			Fisher exact' s test
High	1(4.5)	2 (25)	**P = 0.2**
Normal	21(95.5)	6 (75)	
Fasting Blood Glucose			Fisher exact' s test
High	0 (0.0)	1 (12.5)	**P = 0.3**
Normal	22 (100%)	7 (87.5)	
Post Prandial Blood Glucose			Fisher exact' s test
High	2 (9.1)	3 (37.5)	**P = 0.1**
Normal	20 (90.9)	5 (62.5)	
Obesity			Fisher exact' s test
Normal weight	12 (54.5)	7 (87.5)	**P = 0.2**
Overweight or obese	10 (45.5)	1 (12.5)	
Blood Pressure			Fisher exact' s test
Normal	18 (18.8)	6 (75)	**P = 0.6**
High	4 (18.2)	2 (25)	

DISCUSSION

The aim of the study was to investigate the presence of HPV low risk types (6, 11) and high risk types (16, 18) DNA in skin tags in order to evaluate the relationship between these types of HPV and skin tags. Therefore, we obtained 35 specimens of skin tags from 30 patients (for five patients, two skin tags were obtained from the same patient from two different sites), additionally 30 other biopsy specimens of normal skin within 5cm surrounding skin tags (non lesional specimens) were obtained as a control.

The age of the patients ranged from (14-60 years) with a mean age 40.2 ±11.9 years. Skin tags are more common in middle aged and older individuals with an equal prevalence in males and females [15]. However, most of the patients in our study were females (25 females and 5 males). The high female ratio in this study may be due to that females are seeking medical advice more than males.

We used PCR as a sensitive and specific method for detection of viral DNA in tissues [8]. HPV DNA (6 and/or 11) was detected in (71.4%) of skin tags biopsy specimens. In non-lesional skin the detection rate was only (13.3%). This suggests that HPV may be involved in the pathogenesis of these cutaneous lesions. Our results are in agreement with Dianzani et al., [9] who reported the presence of HPV DNA 6/11 in 88% of skin tags from 49 Caucasian patients using PCR. However, unlike our study, the ten specimens they obtained from non-lesional skin were all negative. In a study carried out by Al-Shaiji and AL-Buainian [1] thirty individuals with skin tags were included and close to our findings, they detected HPV6, 11 DNA in (77%) of skin tag biopsies however, they did not examine samples from the non lesional skin.

A few years later, Gupta et al. [10] reported that 48.6% of skin tags specimens (taken from 37 Indian patients) were positive for both HPV6/11 DNA while, specimens surrounding skin tags were negative for HPV DNA. The positivity rate in their study is lower than that reported by us and that of Dianzani et al. [9] and AlShaiji and AL-Buainian [1]. This variation may be attributed to differences in the sensitivity of the test or the loss of HPV genome as the authors explained.

On the contrary, a study carried out in Iran reported no relationship between low risk/high risk HPV and skin tags as all skin tag biopsies (taken from 50 patients with skin tags) were negative for HPV DNA [11]. There are several practical issues that may explain the discrepancy between studies including: enrollment size, source of materials and difference in the sensitivity of the detection technique. Geographic difference in distribution of HPV may be a source of such discrepant results. Frequent exposure to sun rays in tropical and subtropical area reduces the dermal immunity by decreasing the number of Langerhans cells and facilitating skin infection with HPVs [10] and this may be an important factor in our population.

The presence of HPV DNA in skin tags cannot be considered as a proof for an etiologic role. Cutaneous HPV is a part of the microbiological flora of the healthy human skin. Asymptomatic carriage of HPV on the skin was described and may reflect viral shedding from cutaneous reservoir that may be the hair follicle [16]. However, in the current study, the detection of HPV 6/11 DNA in a statistically significant high percentage of skin tags (71.4%) and its absence in the majority of non lesional skin samples (only 4/30 were positive, i.e.13.33%) suggests a possible true role for HPV in the development of skin tags. This is further supported by the consistent results obtained when we examined

samples from two different sites of the same patient. The expression of early viral genes may disturb cell cycle regulation stimulating cell growth. This leads to limited epithelial proliferation and the formation of acanthotic epidermis overlying edematous fibro-vascular tissue as explained by Gupta et al. [10].

Although the current study demonstrated the presence of HPV DNA in more than two thirds of skin tags, 28.9% of skin tag samples were still negative for the four tested virus types. This means that HPV may only be a contributing rather than a sole factor in skin tags' development. Friction is thought to be an important factor in skin tag pathogenesis causing skin disruption hence opening a route for HPV entry. The immune status of the host may also contribute [17].

Common sites for skin tags include the neck, axillae and eyelids [2] and less common sites include groin, abdomen and back. In our research, most of skin tags were located on the neck similar to the finding of Gupta et al. [10] and Shah et al. [18]. We found no significant relation between HPV positivity rate or virus type and the site of skin tag and this was in agreement with Gupta et al. [10]. On the other hand, Al-Shaiji and AL-Buainian [1] found a significant relation between the localization of skin tags on the back and presence of the virus.

Regarding the possible risk factors for skin tags development -in our study- hypertension was detected in 20% of the patients, diabetes in 16.7%, high serum cholesterol in 10%, obesity in 63.3% and acanthosis nigricans in 40% of patients. We found no relation between the positivity of HPV DNA in skin tags and the presence or absence of obesity, hypertension, diabetes or hypercholesterolemia. Dianzani et al. [9] reported a similar result and also did Shaiji and AL-Buainian [1]. However, Shah et al. [18] found that the mean levels of Body Mass Index, blood pressure, Fasting Blood Glucose, and total cholesterol were significantly higher in patients of the study group than those in control group suggesting that skin tags represent a cutaneous sign for metabolic syndrome.

In conclusion, human papilloma virus may be a contributing factor in the development of skin tags. The detection of HPV DNA in skin tags may expand the spectrum of HPV-related skin conditions. This association may have a practical therapeutic importance in the future. However, further studies with larger sample size are needed to support these results.

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