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Detection of human papilloma viral particles in some acral vascular tumors

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Abstract

While many HPVs can be considered commensal and part of the microbiota of healthy tissue, long-term persistent infection with HR-HPVs increases the risk to invasive cancer. Vascular proliferation is a wide and complex spectrum. Many appear as hamartomas in infancy; others are acquired neoplasms. Twenty-four patients with vascular acral tumors who attended Dermatology Outpatient Clinic of Minia university hospital in a period of 6 months duration were enrolled in this study. Detection of HPV was carried out by polymerase chain reaction (PCR) amplification using consensus primers (My09, My11). The integrity of the DNA amplification was done by the amplification of housekeeping gene at 268 bp. Only one skin biopsy out of 20 of the pyogenic granuloma patients was positive for HPV particles by PCR while two skin biopsies with angiokeratoma were positive for HPV PCR test. Two patients with Kaposi sarcoma were positive for HPV PCR test. Further studies need to be conducted on wide scale of patients with vascular tumors to confirm that Human papilloma virus could be accused as a causative agent, and we recommend the trial for HPV vaccination as a way of prevention of these tumors.

Keywords: Detection, HPV, pyogenic granuloma, kaposi sarcoma, angiokeratoma

Full length article *Corresponding Author, e-mail: Doaaa.hamdy7@gmail.com

1. Introduction

Infection with HPV types, results in a spectrum of subclinical and clinical manifestations, ranging anywhere from silent infection to benign warts or papillomas on the skin and genitalia. While many HPVs can be considered commensal and part of the microbiota of healthy tissue, long-term persistent infection with HR-HPVs increases the risk to invasive cancer [1]. Vascular proliferation is a wide and complex spectrum. Many appear as hamartomas in infancy; others are acquired neoplasms. In this study we searched for HPV particles in three vascular tumors (pyogenic granuloma, solitary angiokeratoma and Kaposi sarcoma). Pyogenic granuloma (PG), refers to a common, acquired, benign vascular tumor that arises in tissues such as the skin and mucous membranes [3]. Clinically it presents as a solitary, red, pedunculated papule that is very friable, but it may present as a sessile plaque. It shows rapid exophytic growth, with a surface that often undergoes ulceration [4]. Histologically, a lobular capillary hemangioma consists of lobular aggregates of capillary-sized vessels, separated by fibromyxoid stroma with each lobule containing a central feeder vessel. Angiokeratoma presents as single or multiple reddish or blackish hyperkeratotic papules, about 10-mm in diameter. Solitary angiokeratoma occurs most frequently on the lower extremities, penis, vulva and clitoris and rarely in the oral cavity [10, 11]. Histopathological examination shows hyperkeratosis, dilated capillaries, large cavernous, endothelial-lined, and blood-filled spaces extending deep into the reticular dermis and subcutaneous fat [11]. Kaposi sarcoma is a confusing soft tissue tumor with a diversity of presentations and courses. Kaposi sarcoma occurs commonly in patients with immunosuppression [13]. Clinically, it presents as a violaceous pink to purple plaque on the skin or mucocutaneous surfaces. Lesions may be painful with associated lymphedema and secondary infection[15]. Histologically, Kaposi sarcoma progresses through 3 distinct clinical stages: patch, plaque, and nodular. The patch stage of Kaposi sarcoma is characterized by a spindle cell proliferation of irregular, complex vascular channels dissecting through the dermis with the promontory sign. Extravasated red blood cells, hemosiderin-laden macrophages, rare hyaline globules, and perivascular lymphocytes and plasma cells are also frequently identified. The plaque stage of Kaposi sarcoma has an increasing prominence of the features seen in the patch stage with extension into the subcutis, and more prominence of the

hyaline globules intra- and extra-cellularly [16]. Cellular pleomorphism is minimal, and there are few mitotic figures [16]. The nodular form, the pleomorphism increases and mitotic figures become more prominent [17]. The slit-like lumens are enhanced as well [16].

2. Patients and methods

Twenty-four patients with vascular acral tumors who attended Dermatology Outpatient Clinic of Minia university hospital in a period of 6 months duration were enrolled in this study. Informed consents were taken from all patients or parents of patients under the age of 18 years of age before enrollment into the study. The study was approved by the local ethical committee of scientific research at Faculty of medicine, Minia university. (Approval No: 679-9/2020). Skin biopsies were taken from the patients After histopathological analysis with H&E stain, patients were divided into 3 groups:

- Group I: Included 20 patients with pyogenic granuloma
- Group II: Included 2 patients with solitary angiokeratoma
- Group III: Included 2 patient with Kaposi sarcoma

DNA was extracted from tissues after deparaffinization using DNA extraction kit and stored at -80°C in aliquots until required. This was done using Qia-amplification extraction kit (Qiagene, USA. Detection of HPV was carried out by polymerase chain reaction (PCR) amplification using consensus primers (My09, My11). The integrity of the DNA amplification was done by the amplification of housekeeping gene at 268 bp.

Denaturation at 95°C for 5 min. PCR reaction was carried out for 40 cycles under the following conditions:

- Denaturation at 95°C for 1 min
- Annealing at 55°C for 1 min
- Extension at 72°C for 2 min

Then final extension cycle of 72°C for 7 min. was done.

Detection of PCR Amplification Products Using Gel Electrophoresis and Ultra-Violet Light Transillumination

Samples were prepared for loading by adding 2 µl loading buffer to10 µl of the PCR reaction mixture. The PCR marker was also loaded into one of the wells. The amplified product of HPV by consensus primers gives 450 bp.

2.1. Performing the Electrophoresis

The power supply was programmed to give 60 volts for about 20 minutes. The gel was taken for viewing on ultra-violet transilluminator.

3. Results

The current study was conducted on 24 patients with acral vascular soft tissue tumors attending the Dermatology out-patient clinic, Minia University Hospital, Minia, Egypt. Of these patients, 14 patients were females (58.33%) and 10 patients were males (41.66%) and their age ranged from 11 to 68 years. In group I which included 20 patients with pyogenic granuloma of which 12 patients were females (60%) and 8 patients were males (40%). Their age ranged from 11 to 48 years and the duration of the lesions ranged from 6 months to one year. Only one (male patient aged 48 years with a dome shaped vascular papule on the sole of the foot) skin biopsy out of 20 of the pyogenic granuloma patients was positive for HPV particles by PCR. Histologically the type of pyogenic granuloma was the cellular one and showed stromal cellularity comprising diffuse endothelial cells throughout the stroma with little evidence of lumen formation (figure 2). In group II, it included 2 patients with angiokeratoma one of them was a male (50%) and the other one was a female (50%). The male aged 15 years and had solitary angiokeratoma since birth while the female aged 67years and had a solitary angiokeratoma of 15 years duration. The 2 skin biopsies were positive for HPV PCR test. Histologically both showed hyperkeratosis, dilated capillaries, large cavernous, endothelial-lined, and blood-filled spaces extending deep into the reticular dermis (figure 3). In group III that included 2 patients with Kaposi sarcoma, they were 2 males aged 65 and 68 years with disease duration of 7 and 10 years respectively. Their skin biopsies were positive too for HPV PCR testing. Histologically both showed a spindle cell proliferation of irregular, complex vascular channels dissecting through the dermis, extravasated red blood cells and perivascular lymphocytes (figure 4).

4. Discussion

The role of HPV in the pathogenesis of non-melanoma skin cancers was initially described in epidermodysplasia verruciformis and transplant recipients. High-risk types, mainly HPV 16, are strongly linked with anogenital carcinomas and among cutaneous lesions, implicated in Bowen's disease and squamous cell carcinoma (SCC) [19]. HPV particles were detected by PCR in patients with pyogenic granuloma in a study published in 2015 by Vázquez-Martínez OT, et. al., [20] and eight out of 18 patients were positive for the test but in this study one case only out of 20 tested positive for HPV PCR and was of the proliferating type with no history of proceeding trauma or coincident HPV infection. HPV infection associated with angiokeratoma has been described in a case presenting on vulva with coexisting positivity for HPV-6 [21], and have been described too in one case on the elbow of a child and HPV type 58 and 59 was accused as a causative agent [22]. Here in this study both patients with angiokeratoma was tested positive for HPV PCR in general but the specific type wasn't defined because of lack of facilities in our lab, and this goes with this case report by Khullar G. et. al., [22]. Human papilloma virus was detected by PCR technique in 2 patients with Kaposi sarcoma in this study and this up to our knowledge is the first report of this association.

5. Conclusion

Further studies need to be conducted on wide scale of patients with vascular tumors to confirm that Human papilloma virus could be accused as a causative agent and we recommend the trial for HPV vaccination as a way of prevention of these tumors.

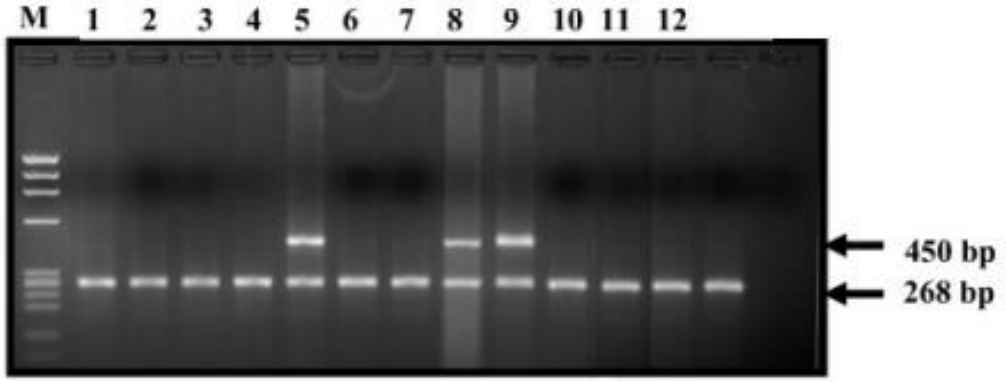

Figure 1: Agarose gel electrophoresis of HPV using consensus primers showing PCR amplification at 450 bp and the house keeping gene at 268 bp.

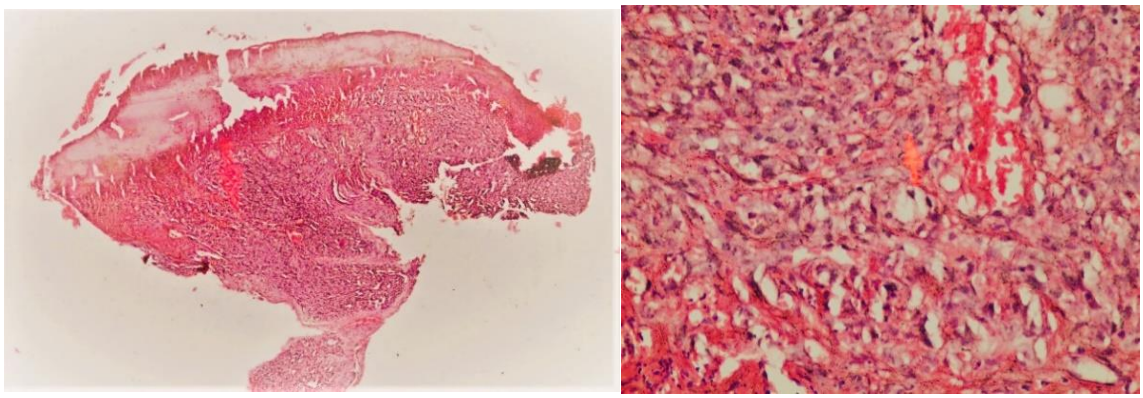

Figure 2: low-power and high-power magnification of cellular type of pyogenic granuloma

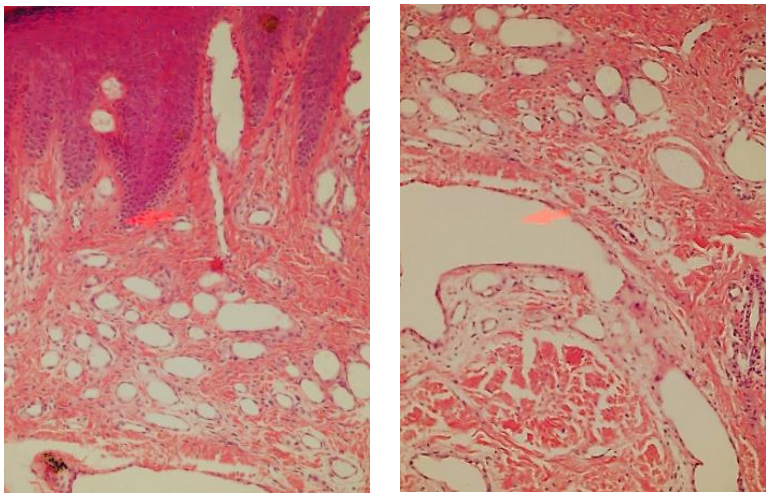

Figure 3: Low and high power and angiokeratoma showing hyperkeratosis and dilated blood vessels in the papillary and extending to the reticular dermis.

Table 1: Summary of the study results

Group number	Age	Sex	Disease duration	Number of Positive cases for HPV PCR test	Number of negative cases for HPV PCR test
Group I (pyogenic granuloma) (20 patients)	11-48 years	12 females 8 males	From one moth to one year	1	19
Group II (angiokeratoma) (2 patients)	15 years 67 years	Male female	Since birth 15 years duration	2	0
Group III (Kaposi sarcoma) (2 patients)	65 years 68 years	Male Male	7 years 10 years	2	0

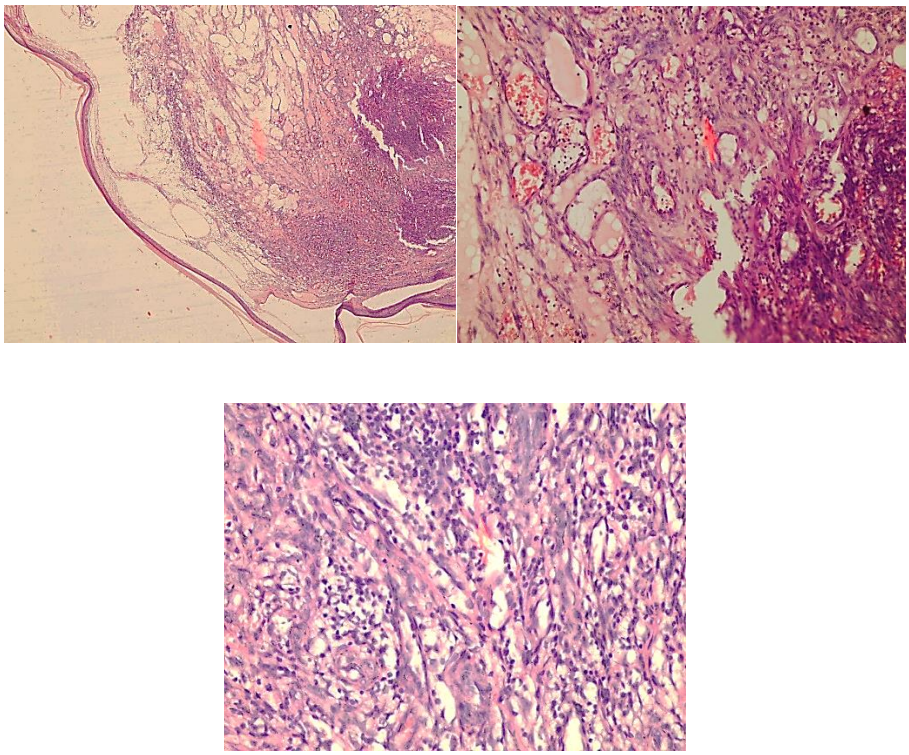

Figure 4: low, medium and high power of Kaposi sarcoma showing spindle cell proliferation of irregular, complex vascular channels dissecting through the dermis, extravasated red blood cells and perivascular lymphocytes.

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