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To cite this version:

Maria Rita Nasca, Maria Concetta Potenza, Laura Alessi, Gianluca Paravizzini, Giuseppe Micali. ABSENCE OF POLYMERASE CHAIN REACTION-DETECTABLE HUMAN PAPILLOMA VIRUS IN ERYTHROPLASIA OF QUEYRAT USING A COMPARATIVE CONTROL GROUP. Sexually Transmitted Infections, 2010, 86 (3), pp.199. 10.1136/sti.2009.039230 . hal-00557459

HAL Id: hal-00557459

https://hal.science/hal-00557459v1

Submitted on 19 Jan 2011

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ABSENCE OF POLYMERASE CHAIN REACTION-DETECTABLE HUMAN PAPILLOMA VIRUS IN ERYTHROPLASIA OF QUEYRAT USING A COMPARATIVE CONTROL GROUP

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Word count: 1300

Key words: Human Papillomavirus – Penile carcinoma –Virology – Skin disease – Anogenital cancer - Polymerase chain reaction – Erythroplasia of Queyrat - In situ carcinoma

Competing Interest: None declared

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ABSTRACT

Objectives

High risk human papillomavirus (HPV) types have been linked with genital carcinomas. However, the exact relation between HPV and erythroplasia of Queyrat (EQ), e.g. in situ carcinoma of the glans and prepuce, is still unclear. Aim of this study was to detect the presence of lesional HPV DNA in patients affected by EQ.

Methods

Paraffin-embedded biopsies were obtained from the glans or inner foreskin of 11 adult uncircumcised patients (mean age, 67.7 years; range, 57-79 years) with EQ. An equal number of randomly selected uncircumcised healthy control patients underwent a single brush cytology smear of the penile mucosa. Biopsy specimens and brushings were then assayed by a highly sensitive two-step nested polymerase chain reaction (PCR) technique based on MY11/MY09 consensus primers and general GP5+/GP6+ PCR primers, followed by cycle sequencing. Statistical evaluation was performed using conditional logistic regression analysis.

Results

None of the EQ or control samples proved to be positive for the presence of HPV DNA.

Conclusions

Our findings do not support the hypothesis that there is a considerable risk of EQ development in patients with HPV infection. Prevalence of HPV infection in patients with EQ has been seldom investigated and available data are relatively scant and

controversial. Therefore, the relationship between HPV infection and the risk of progression of EQ into SCC remains a matter of debate, and further investigations are needed in order to confirm the role of HPV in delineating this risk.

Introduction

Erythroplasia of Queyrat (EQ) is a carcinoma *in situ* that mainly occurs on the glans penis, the prepuce, or the urethral meatus of elderly males. Causes of EQ are unknown.[1] Clinically, it appears as a sharply demarcated, velvety bright reddish plaque or plaques on the glans or on the foreskin. Patients may show erythema, scaling, crusting, and difficulty in foreskin retraction. Many have pruritus or pain locally, while a few report bleeding. EQ usually progresses slowly. Transformation into invasive squamous cell carcinoma (SCC) has been reported in up to 33% of cases. Ulceration suggests that EQ may have transformed to invasive SCC, with a risk of penile submucosal involvement, regional lymph node metastases, or distant metastatic disease.[2]

Histologic examination of EQ shows findings similar to those of Bowen disease (BD), an *in situ* carcinoma characterized by lack of maturation of the surface epithelium and partial or total replacement of mature keratinocytes with disorderly, dysplastic, and sometimes dyskeratotic cells that display nuclear atypia. Marked epidermal acanthosis and parakeratosis and an inflammatory lymphohistiocytic infiltrate in the dermis are common findings. Compared with BD, EQ more often shows epithelial hypoplasia, with fewer multinucleated cells and fewer dyskeratotic cells but an increase in the number of plasma cells in the chronic inflammatory infiltrate.[2]

A 1% annual incidence of genital human papillomavirus (HPV) infection has been estimated among sexually active people.[3] In the last few decades there has

been an increasing trend of new diagnoses both in the United States and in Europe, reflecting not only an increasing incidence resulting from changes in sexual behaviour, but also greater ascertainment of cases through more testing and improved diagnostic methods [4]. In men, depending on the population studied, sampling method and anatomic site, the prevalence of HPV-DNA positivity ranges widely, often reaching highest prevalence rates among selected high risk people [5].

In the present study, we investigated the presence of lesional HPV DNA in a series of patients affected by EQ.

Methods

Paraffin-embedded biopsies were obtained from the glans or inner foreskin of 11 adult uncircumcised patients (mean age, 67.7 years; range, 57-79 years) that were histologically consistent for EQ and were selected for HPV evaluation by polymerase chain reaction (PCR).

A control group consisting of an equal number of randomly selected uncircumcised males matched for age and attending our dermatology clinic for nongenital complaints was also enrolled in the study. Patients with a positive history of anogenital warts or any other HPV-related disorder were excluded from both the control group and the EQ group. Also excluded were those patients whose sexual partners had a past history of anogenital HPV infection and/or cervical intraepithelial neoplasia (CIN) or invasive neoplasia. Control patients agreed to undergo a careful

clinical inspection (no instrumentation) to confirm no evidence of excludable disorders.

All eligible control subjects underwent a single brush cytology smear of the penile mucosa for HPV testing. A (saline-wetted) cotton tip swab was rubbed on the glans, coronal sulcus and inner foreskin. Biopsy specimens and brushings were then assayed by a highly sensitive two-step nested PCR technique based on MY11/MY09 consensus primers and general GP5+/GP6+ PCR primers, followed by cycle sequencing.[6] This technique is able to detect 0.05-5 HPV DNA copies, has a sensivity of 10 fg to 1 pg, and covers all known HPV types.[7] SiHa and HeLa HPV-infected cell lines derived from human cervical carcinomas were used as positive controls for HPV analysis.[8-9] Statistical evaluation was performed using conditional logistic regression analysis.

Results

None of the EQ or control samples proved to be positive for the presence of HPV DNA, whereas SiHa and HeLa cell lines were all found to be positive (SiHa: HPV 16, 1 to 10 copies per cell; HeLa: HPV 18 10 to 50 copies per cell).[8-9]

Discussion

Main findings

Our retrospective analysis on a series of patients with EQ aimed to determine the frequency of associated HPV infection failed to detect the presence of HPV DNA

in all cases. Therefore, the findings of this study do not support the hypothesis that there is a considerable risk of EQ development in patients with HPV infection.[2, 10]

Relation to the existing literature

Prevalence of HPV infection in patients with EQ has been seldom investigated and available data are relatively scant and controversial.

EQ (which is usually diagnosed in men), BD (more frequently diagnosed in women), and BP are a group of disorder known also as penile intraepithelial neoplasia (PIN). These three clinical variants of PIN share identical histologic features of *in situ* SCC. The malignant potential, however, may very according to the disorder considered.[2] Various pathogenic factors have been implicated in the pathogenesis of PIN: chronic irritation, poor hygiene, traumas, and HPV infection.[11]

HPV infection may be latent, subclinical or clinically evident. The oncogenic potential of HPVs has now been unequivocally proven, and this finding has raised considerable concerns due to the worldwide distribution of HPV infection. High risk HPV types 16, 18, 31, 33, 39, 42, 51-54 have been linked with genital carcinomas. HPV 16 and 18 are more likely to be present in subclinical infections that may eventually become evident later in life. Although other routes of HPV transmission do exist, genital warts are predominantly transmitted by sexual contact.[5] HPV 16 is a high risk oncogenic virus associated with the development of genital SCC, although HPV-negative tumors have also been found. It is reported in approximately 70% of

the cases of genital BD (*in situ* SCC of genital skin).[10] Nevertheless, it is rarely found in EQ, e.g. *in situ* carcinoma of penile mucosa.

The exact relation between HPV and EQ is unknown.[10] It has been suggested that HPV infection may enhance the risk of SCC development.[2] However, a higher incidence of HPV infections in patients with EQ has not been demonstrated unequivocally. Previous studies analyzed only a limited number of patients.[1-2] In one study, 12 paraffin-embedded biopsies from 8 patients affected by EQ were tested for the presence of HPV DNA. Surprisingly, all patients were infected with the carcinogenic EV-associated cutaneous HPV type 8. Seven of 8 patients were coinfected with HPV16. Control biopsies from patients affected by inflammatory penile disorders were negative.[1] No positive controls were used. In another study, the relationship between HPV infection and external genital *in situ* SCC was investigated by PCR using consensus primers.[12] HPV DNA was positive in 22 (55%) out of 40 cases of bowenoid papulosis (BP), in all of 5 cases (100%) of BD and in 2 (33.3%) out of 6 cases of EQ. In all positive cases HPV 16 was detected.

Limitation of the study

EQ is a rare disorder, and its rarity hampers the collection of a wide case series able to provide reliable statistical data. In fact, the largest case series we found in the literature deals with a number of biopsy samples similar to ours that is a relatively limited number of cases (12 vs 11).[1] In addition, this study disclosed controversial findings, such as the presence of unusual HPV types, like HPV 8, whose significance is difficult to assess due to small sample size and that in our study we were unable to

confirm. In the absence of stringent confirmation of HPV presence in penile *in situ* carcinomas, a crucial role of HPV infection still remains debatable, and a causative role of other still unknown factors must be taken into account.

Meaning/future directions for research

The totally discordant data in our study are inconsistent with those by Wieland et al.[1] and may be related to private life and sexual behaviour of individual patients.[5] This issue is often difficult to thoroughly explore and should be better assessed in prospective research. The relationship between HPV infection and the risk of progression of EQ into SCC remains uncertain, and further investigations into the role of HPV and possibly other infectious agents are warranted.

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Word count: 1300

Contributions of each author to the project

Maria Rita Nasca MD PhD	participated in designing the study, and in generating, gathering, and analysing the data; she also participated in writing the paper;
Maria Concetta Potenza MD	participated in generating and gathering the data for the study;
Laura Alessi MD	participated in generating, gathering, and analysing the data; she also participated in writing the paper;
Gianluca Paravizzini MD	participated in generating the data for the study and provided technical assistance for PCR investigation;
Giuseppe Micali MD	participated in designing the study, in the analysis of the data, and in the revision of the paper.

Key messages

- This retrospective analysis failed to detect the presence of HPV DNA in a series of patients with EQ.
- These findings do not support the hypothesis that there is a considerable risk of EQ development in patients with HPV infection.
- Further investigations taking in consideration sexual habits are needed in order to confirm the role of HPV in delineating this risk.

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