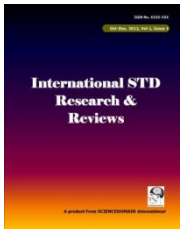

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Oncogenic Human Papillomavirus Detection in Penile Lichen Sclerosus: An Update

Maria Rita Nasca[1*], Francesco Lacarrubba[1], Gianluca Paravizzini[2] and Giuseppe Micali[1]

[1]Dermatology Clinic, University of Catania, Italy.
[2]Clinical Laboratories "Girlando and Paravizzini", Catania, Italy.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MRN and GM designed the study, authors MRN and FL retrieved samples for PCR examination, author MRN managed the literature searches, wrote the protocol, and the first draft of the manuscript, and author GP provided PCR assistance. All authors read and approved the final manuscript.

Short Research Article

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ABSTRACT

Aims: Aim of this study was to better address a possible association of human papillomavirus (HPV) infection with penile lichen sclerosus (LS).
Study Design: Paraffin-embedded penile biopsies obtained from adult patients with genital LS retrieved from institutional pathology files were evaluated.
Place and Duration of Study: The study has been performed in the Dermatology Clinic of the University of Catania, Italy, spanning a 19-year period.
Methodology: We previously demonstrated a high (17.4%) HPV detection rate in a study on 46 patients with genital LS. In this retrospective analysis we extended the analysis to a larger number of patients in order to strengthen these former data. HPV infection was assessed by polymerase chain reaction (PCR) in paraffin-embedded penile biopsies obtained from the glans or inner foreskin of 92 adult patients with penile LS and in brush cytology smears of penile healthy mucosa from an equal number of randomly selected control males matched for age. Statistical evaluation was performed using conditional logistic regression analysis.
Results: PCR disclosed the presence of high risk HPV infection in 22.83% of LS patients (HPV 16:16 cases; HPV 18:1 case; HPV 31:1 case; HPV 45:2 cases; HPV 68:1 case) vs

**Corresponding author: E-mail: nasca@policlinico.unict.it; mnasca@fastwebnet.it;*

15.21% of controls, (HPV 16:4 cases; HPV 31:1 case; HPV 53:1 case; HPV 56:1 case; HPV 68:1 case; HPV 70:1 case; HPV 81:5 cases). Statistical regression analysis confirmed that the rate of oncogenic HPV infection was higher among patients with genital LS than among healthy controls (χ^2=8.26; P<.01; OR=3.59).
Conclusion: These data suggest a possible pathogenetic interplay between LS and oncogenic HPV infection in the development of LS-associated penile cancer.

Keywords: Lichen sclerosus; human papillomavirus infection; penile carcinoma.

1. INTRODUCTION

Lichen sclerosus (LS) is a chronic inflammatory dermatosis typically affecting the genitals in both sexes. Its etiology and pathogenesis are still unclear. Penile LS is a disease less commonly observed compared to vulvar LS. Clinically, it is usually characterized by multiple, coalescing whitish and atrophic papular lesions involving the glans and prepuce, sometimes leading in the late stage to acquired phimosis, preputial adhesions, and meatal stricture. Its real incidence is difficult to determine and it is probably underestimated, since many patients may not seek medical attention until complications occur. Treatment is not standardized, and several medical (topical, intralesional or systemic), physical and surgical therapeutic approaches have been suggested [1].

In the past, some clinical studies have remarked on the possible link between genital LS and penile squamous cell carcinoma (SCC) [1-5]. Others have highlighted for penile SCC a likely causative role of infection from oncogenic “high risk” Human Papillomavirus (HPV) types [6-7]. On the other hand, other findings, including that of oncogenic HPV infections in some cases of penile carcinoma arising on LS [8-9], have suggested a more complex interplay between these two factors in at least a subset of patients developing penile carcinoma.

In a previous study we reported the association of penile LS and oncogenic HPV infection [10]. In our opinion, assessing the presence of oncogenic HPV infections in LS patients represents a crucial step to achieve a full knowledge of the events that lead to penile SCC development. Therefore, based on these considerations, we decided to extend our former investigational study to a larger series of patients in order to obtain more significant results.

2. METHODOLOGY

2.1 Characteristics of the Sample

In the present study we investigated the presence of HPV DNA in paraffin-embedded penile biopsies obtained from the glans or inner foreskin of 92 randomly selected adult patients (mean age 61 years; range 25-89 years) with penile LS retrieved from our pathology files spanning over a 19-year period. Histopathologically, all patients labelled as LS showed hyperkeratosis, atrophy of the stratum malpighii with hydropic degeneration of basal cells, pronounced edema and homogenization of the collagen in the upper dermis, and inflammatory infiltrate in the dermis. No light microscopic signs of koilocytosis or intraepithelial neoplasia were detected in any patient. All patients denied previous sexually transmitted diseases (STDs), including anogenital warts, whereas information on their female partners on this regard were lacking. No reliable data were available about disease

duration. In all patients there was no positive history of prolonged treatment with topical or systemic corticosteroids or other immunosuppressive agents.

Data obtained were compared to a control group unrelated to the consultation date of the cases consisting of an equal number of consecutively randomly selected males matched for age, successively attending our Clinic for non-genital complaints. Patients with positive history of LS, phimosis, chronic balanitis, and past or present history of anogenital warts, or any other HPV-related disorder were excluded. We also excluded those patients whose wife or sexual partners had a past history of anogenital HPV infection and/or cervical intraepithelial (CIN) or invasive neoplasia. Control patients agreed to undergo a careful naked-eye clinical inspection that did not show evidence of disease, followed by a single brush cytology smear of penile mucosa for HPV testing.

2.2 DNA Extraction

Three 10µm thick sections were cut with a microtome from each paraffin embedded LS sample. DNA extraction was performed using a Qiagen kit (QIAamp DNA micro kit) and following the manufacturer's instructions. Sample adequacy, DNA quantity and integrity were established by spectrophotometric analysis. Each sample was monitored through amplification of the β-globin housekeeping gene.

2.3 HPV Detection and Genotyping

Ten ul of DNA solution from Biopsy specimens and brushings were assayed by a highly sensitive two-steps nested polymerase chain reaction (PCR) technique based on general MY11/MY09 PCR primers and GP5+/GP6+ consensus primers, followed by cycle sequencing. In the first step, 30 cycles of DNA amplification were performed in a reaction mixture of 50 µl containing 10mM Tris-Cl pH 8.3, 50mM KCl, 2.5mM $MgCl_2$, 200µM of each dNTP, 2.5units 1U of Taq DNA polymerase and 0.05mM of MY11/MY09, with denaturation at 94° for 1min, annealing at 55° for 1.5min, and 72° for 1min followed by extension at 72°C for 5min. In the second reaction, 2ul of PCR product were amplified in a reaction mixture of 50µl containing 10mM Tris-Cl pH 8.3, 50mM KCl, 2.5mM $MgCl_2$, 200µM of each dNTP, 2.5 units 1U of Taq DNA polymerase and 0.05mM of GP5+/GP6+, with denaturation at 94° for 30sec annealing at 47° for 30sec, and 72° for 30sec followed by extension at 72°C for 5min. Agarose gel of 10ul of nested PCR product revealed visible bands in the HPV. The reactions were purified, and cycle sequencing was performed as previously described to determine the type of HPV using an automated genetic analyzer [11-12]. The homology of the consensus sequence was then compared with those of known HPV types available through the Gen Bank database.

Statistical evaluation was performed using conditional logistic regression analysis.

3. RESULT

Among LS cases, PCR disclosed the presence of HPV DNA in 21 cases (22.83%) (Table 1). Infections with five different HPV types were detected, of which HPV 16 (76.2%), was the most prevalent type, followed by HPV 45 (9.5%) and HPV 18 (4.8%), HPV 31 (4.8%) and HPV 68 (4.8%). Surprisingly, only oncogenic HPV types were detected in our LS series, and no infection from low-risk HPV strains was found.

Among controls, the total rate of HPV infection, including non oncogenic types, was 14 out of 92(15.21%) (Table 2). Of these, 28.6% harbored HPV 16, and the remaining ones respectively HPV 81 (35.7%), HPV 31(7.1%), HPV 53(7.1%), HPV 56(7.1%), HPV 68 (7.1%), and HPV 70(7.1%). High risk HPV infection occurred in 7 out of 92 patients (7.61%). Comparing 92 patients with penile LS and 92 healthy controls, statistical regression analysis confirmed that the rate of oncogenic HPV infection was higher among patients with genital LS than among healthy controls (χ^2=8.26;*P*<.01;OR=3.59).

Table 1. PCR findings of 21 patients with concurrent LS and HPV infection

Patient	Age	HPV type
1	70	HPV 16*
2	72	HPV 16*
3	43	HPV 16*
4	65	HPV 16*
5	78	HPV 16*
6	65	HPV 16*
7	60	HPV 16*
8	60	HPV 16*
9	60	HPV 16*
10	67	HPV 16*
11	71	HPV 16*
12	79	HPV 16*
13	80	HPV 16*
14	46	HPV 16*
15	59	HPV 16*
16	82	HPV 16*
17	70	HPV 18*
18	89	HPV 31*
19	77	HPV 45*
20	71	HPV 45*
21	66	HPV 68*

** High risk HPVs*

Table 2. PCR findings of 14 healthy patients with subclinical HPV infection

Patient	Age	HPV type
1	64	HPV 16*
2	70	HPV 16*
3	58	HPV 16*
4	58	HPV 16*
5	46	HPV 53
6	68	HPV 70
7	56	HPV 81
8	59	HPV 81
9	58	HPV 81
10	70	HPV 81
11	32	HPV 81
12	57	HPV 31*
13	59	HPV 68*
14	41	HPV 56*

** High risk HPVs*

4. DISCUSSION

The relationship between LS and risk of genital cancer development has been clearly defined in female patients. Previous studies reported LS as a precancerous condition, as 3% to 7% of vulvar SCCs occur on genital LS [13-14]. Two different pathways of vulvar carcinogenesis have been proposed: one associated with HPV infection prevalent in young patients, and another arising on LS [15-16].

More recently, a similar association in men has also been proposed. This linkage has been delineated on the basis of evidence that includes the histopathologic finding of LS adjacent to tumor tissue in excision specimens from patients with penile SCC [4,8,17-19], as well as the observation of malignant changes in 9.3% of cases of penile LS in a series of 86 patients [2,3,5] suggesting a statistically significant risk of developing penile carcinoma in males with genital LS. In addition, our research group demonstrated a striking association between specific histopathologic LS features and an enhanced risk of SCC development in affected male patients. Seven out of 9(78%) cases of penile cancer were associated with a histological pattern characterized by epidermal thickening, loss of normal keratinocyte cytoarchitectural differentiation, presence of mitoses, and apoptosis. This pattern could be interpreted as areas of reactivation within a chronic atrophic stage [9], that, as suggested also by other recent investigations on vulvar LS [20], may considerably shorten the lagtime to tumor progression [14].

The role of oncogenic HPV in promoting penile SCC is another debatable point. HPV detection in penile SCC may range widely, but in Europe it is reported in about 45-48% of cases [21-22]. It has been proposed that only peculiar histopathological features are strictly associated with oncogenic HPV infections. Using PCR testing, it has been demonstrated that HPV DNA positivity strongly correlates histopathologically with either basaloid or warty changes (47%) or purely basaloid changes (75%), and is only weakly associated (11%) with typical keratinizing SCC [23]. Accordingly, two main morphologically distinct subtypes of in situ carcinoma have been identified that are considered likely precursors of their invasive counterparts: the squamous keratinized, characterized by keratinizing epithelium that shows variable degrees of cellular atypia, and the basaloid warty, which shows small basaloid cells with poorly defined borders or koilocytosis as prominent microscopic features and, unlike the simplex type, is frequently related to HPV infection [24]. On the basis of these observations, the existence of two different independent pathways in penile carcinogenesis has been suggested: one with possible sexual transmission after infection by oncogenic HPVs in young adults, and another of unknown cause, unrelated to HPV infection and cervical carcinoma in the sexual partners, mostly affecting older people, and possibly associated to preexisting longstanding LS [17,19,25].

On the other hand, it has been observed that oncogenic HPV infections and LS may coexist and that they may also occur together in penile carcinoma arising on LS [2,8].

In the past years, many studies have been carried out with conflicting results to better define the oncogenic role of HPVs in patients with LS by assessing their presence in LS tissue specimens [7-8,10,26].

HPV detection rates as high as 16% and 21% before and after topical corticosteroid therapy, respectively, were demonstrated in a study on 19 men, 6 of whom reported a positive history of penile warts but no clinical or histopathological evidence of HPV infection at enrollment [27]. A HPV detection rate of 65.5% was demonstrated in 23 children with

penile LS aged 4 to 14 years using both in situ hybridization and PCR techniques [28]. Another PCR study, performed in 17 patients with penile SCC, showed HPV positivity in 1 out of 8 SCCs associated with LS, compared to 5 out of 9 patients with non-LS-associated SCC [29]. A recent study has also shown the presence of LS in about 18% of HPV positive SCC penile samples, although no difference in the prevalence of LS between HPV positive and HPV negative samples was recorded [30]. Finally, a study whose aim was to investigate the role of HPV infection and expression of the tumor suppressor protein p16INK4J and Ki67 in the pathogenesis of penile cancer demonstrated a strong immunostaining for this protein correlated with HPV16/18 infection in both penile LS and penile SCC [8].

Some authors proposed a causative role of HPVs on genital LS development, following a hypothetical immune response triggered by viral antigens [28]. Others suggested that patients with penile LS may be at higher risk for HPV infection as a result of a genetically determined inability to clear the virus [4] or, alternatively, as a consequence of prolonged treatments with potent topical corticosteroids or calcineurin inhibitors [27,31]. Moreover, it has also been hypothesized that patients with LS may be more susceptible than normal subjects to cancer development following infection by oncogenic HPV types [4]. However, laboratory data supporting these interesting hypotheses are lacking.

Our group had previously demonstrated a high HPV detection rate in patients with genital LS. Oncogenic HPV infection was found in 8(17.4%) out of 46 patients with genital LS, compared to 4(8.7%) out of an equal number of randomly selected control healthy subjects matched for age [10]. On the basis of this observation, in this present research study we performed a retrospective analysis on a larger series of 92 male patients with genital LS and on an equal number of healthy males without genital disease aimed to confirm the findings of the previous study by extending the investigation to a broader case series. Our findings strengthen the former data, detecting oncogenic HPV infection in 22.83% of LS patients. A potential limitation was failure to detect by DNA sequencing co-infection with more than one viral type. Investigations using different methods of genotyping would thus be advisable in order to rule out this possibility.

Noteworthy, the present study identified asymptomatic HPV penile carriage at a lesser degree in 15.21% of healthy subjects with no history of anogenital warts. Other studies have reported a latent genital infection in 7% to 10% of healthy men with no evident clinical or peniscopic abnormalities [13,22,32].

It is interesting to remark that in our series HPV detection was higher among cases than controls in both reports, but the figures differed: 8/46(17.4%) and 4/46(8.7%), respectively, in the subgroup of formerly described patients [10], and 15/46(32.6%) and 10/46(21.74%), respectively, in the newly recruited subgroup. Since no variations in sampling techniques, genital sites sampled, and HPV DNA detection methods used, that might explain such different outcomes, have been made during the course of this research study spanning through a 19-year period, it is reasonable to assume that the difference recorded among the two subgroups may reflect a tendency towards an increase of HPV prevalence with time in the population studied. Whereas this finding is to be related to a general increasing trend of HPV infections, possibly due to changes in sexual habits, among the global population of sexually active non vaccinated individuals, cannot be established based on the data available from our study.

Although a potential bias is represented by the fact that the control samples were not collected at the same time of the LS samples retrieved from our past pathology files, it is

also of note that in our series the mean age in HPV-positive LS cases (68.1 yrs) was higher compared to that of HPV-positive controls (56.9 yrs). Previous studies have shown that there does not appear to be an association between age and HPV prevalence in men, in which the majority of HPV infections is reported to clear in less than 12 months (median time to clearance 6 to 7.5 months) [33]. Our finding of a different mean age between HPV-positive cases and HPV-positive controls may reflect a longer duration of HPV infection in LS patients as a result of a defective clearing capability.

Finally, logistic regression analysis of data from the 92 case-control pairs matched for age confirmed a strong association between penile LS and high-risk HPV infection. These data may support the assumption of a link between LS and increased risk of cancer development. On the other hand, further data would be necessary to strengthen this hypothesis. These include more detailed information on history of STDs, both in the patients suffering from LS of our series and in their female partners, that were unfortunately unavailable.

5. CONCLUSION

The relationship between HPV infection and the risk of progression of male LS into SCC is matter of debate. In our group of LS patients we demonstrated a significantly increased detection of oncogenic HPV infection compared to controls. This finding might be more than coincidental and reflect a lower, potentially iatrogenically induced ability of LS patients to clear the virus, that may pose them at higher risk of malignant degeneration and penile SCC development [4,25,29]. In order to confirm an effective role of HPV infections in enhancing this risk, further prospective studies considering the role of other important cofactors, such as sexual habits and STDs history of the patients and their partners, are warranted.

CONSENT

All authors declare that written informed consent was obtained from the patients and is available.

ETHICAL APPROVAL

All authors hereby declare that the study has been examined and approved by the appropriate ethical committee (IRB).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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