Screening of human papillomavirus infection in women with systemic sclerosis

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ABSTRACT

Objective. *High risk human papillomaviruses (HR HPV) are associated with risk of cervical dysplasia and carcinoma. The risk is increased in patients with immune deficiency or auto-immune disease as systemic lupus erythematosus. Currently, no data are available about the human papillomavirus status in women with systemic sclerosis (SSc).*

Methods. *Thirty-one women with SSc were evaluated for cervical HPV infection and dysplasia, and compared to fifty age-matched control. Cervical swabs were tested by the INNO-LiPA assay®. Serum antibodies against HPV 16 and 18 were assessed using enzyme-linked immunosorbent assay in the SSc group.*

Results. *The overall HPV frequency was comparable between SSc and controls (32% vs. 38%), as well as the HR HPV frequency (28% vs. 34%), but infection by ≥2 HPV was two times more frequent in the SSc group (50% vs. 26% of the HPV positive samples). The most prevalent genotype was 52 in the SSc group (12%), and 52/53 in the control group (8% for both). Pap smears were within the normal range. Seropositivity for HPV 16 and 18 was 13% and 6.5%, respectively. A diffuse systemic sclerosis and a younger age at first intercourse were more frequent in cases of overall HPV positivity. Current smoking and a higher number of sexual partners were only observed in cases of seropositivity.*

Conclusion. *This is the first study to evaluate HPV status in women with SSc. HR HPV52 was the most common genotype with a greater multi-HPV infection rate. This result needs to be confirmed in a larger study.*

Introduction

Persistent infection by high-risk oncogenic HPV (HPV HR) is a necessary cause for the development of preneoplastic and malignant cervical lesions (1). Together with DNA detection, HPV serology is a good marker of cumulative HPV infection in epidemiologic studies (2).

Risk factors for cervical HPV infection are smoking, young age at first intercourse, high number of sexual partners, past of sexual transmitted infections (*herpes, chlamydia trachomatis, HPV),* and hormonal contraception (3). In systemic lupus erythematosus (SLE), HR HPV infection and high grade squamous intra-epithelial lesions (HGSIL) rates are increased compared to the general population (4).

Systemic sclerosis (SSc) is an uncommon connective tissue disease, of still unclear pathogenesis, resulting in dermal and internal organ fibrosis. SSc is associated with an increased overall risk of malignancies, especially lung, liver and haematologic (5).

A Canadian study has shown an increased prevalence of abnormal Pap smears in women with SSc, compared to the general population (25.4% *vs* 13.8%). The major risk factors were a diffuse form and smoking (6). However, in this study and in the literature, Pap smear abnormalities and HPV status have not been evaluated.

We report the genotype distribution of HPV and HPV-related lesions in the cervix of women with SSc, the humoral response anti-HPV16 and HPV18, and the SSc-related clinical factors for HPV infection.

Materials and methods

The procedures followed were in accordance with the standards of the responsible local committee.

Sample collection and patient data

Cervical swabs were collected between November 2010 and April 2011, from women with diffuse or limited SSc (7), followed in the department of internal medicine or dermatology, in the University Hospital of Besançon (France).

Exclusion criteria were: pregnancy, morphea, and total hysterectomy. Written consent to participate was signed. Patient data recorded were:
i) SSc-related: type, time of duration since diagnosis, treatments;
ii) general: age at first intercourse, parity, number of sexual partners, current smoking, history of sexually transmitted infections (STI).
The HPV genotypes and HPV-related lesions in the cervix of women with SSc were compared with control samples from age-matched women followed in the department of gynecology in the University Hospital of Besançon (France) (ratio: 2 controls for 1 case). Control women with specific follow-up for dysplasia or cervical cancer were excluded.

Cytopathologic study
Abnormal Pap smears were classified using the Bethesda system in atypical squamous cells of undetermined significance (ASCUS), low-grade or high-grade squamous intra-epithelial lesions (LGSIL, HGSIL), or adenocarcinoma/squamous cell carcinoma.

DNA isolation and HPV typing
Cervical swabs were placed in 1 mL of Digene specimen transport medium (STM). DNA was isolated from 200 µL of each STM sample using the QIAmp DNA blood Mini Kit (Qiagen, Courtaboeuf, France).
HPV genotyping was performed, as previously described (8), with the INNO-LiPA HPV Genotyping Extra test (Innogenetics, Gent, Belgium), which allowed the identification of 28 HPV genotypes, classified in HR HPV or probably HR (pHR) (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82), low risk (LR) HPV (6, 11, 40, 43, 44, 54, and 70) or HPV of unknown risk (69, 71, and 74).

Serologic testing for antibodies against HPV16/18
The detection of serum IgG antibodies against HPV16/18 was assessed in the SSc group, in the Unit INRA UMR 1282, François Rabelais University, Tours (France), using enzyme-linked immunosorbent assay. Briefly, HPV16 and HPV18 virus-like particles (VLP) were produced in sf21 insect cells using recombinant baculoviruses encoding the L1 gene of HPV16 and HPV18, and purified. Microtiter plates were coated overnight at 4°C with 100 µL of VLP preparation and one hour at 37°C with 200 ng of bovine serum albumin (BSA) in 200 µL of PBS (pH: 7.4). Plasma samples were diluted at 1:20, and tested (100 µL) in duplicate. Background reactivity of the BSA-coated wells was subtracted. The cut-off level for HPV seropositivity was an optical density equal or superior to 1.

Data analysis
Continuous variables were expressed in means and standard deviations, and categorised variables in percentages and numbers. Because of the small sizes, comparisons were only descriptive without statistical analysis.

Table I. Characteristics of SSc women at baseline.

	n (%)
Age (year), mean (SD)	59.8 (11)
Diffuse cutaneous SSc	6 (19)
Limited cutaneous SSc	25 (81)
Duration since SSc diagnosis (year), mean (SD)	9.4 (8,2)
Past or current SSc-treatment	5 (16.1)
Hydroxychloroquin	3 (9.7)
Corticosteroids	2 (6.4)
Methotrexate, others IS	2 (6.4)
Number of sexual partners, mean (SD)	3.2 (2,6)
Age at first intercourse (year), mean (SD)	18.7 (2.7)
Parity, mean (SD)	2.6 (2)
Smoking	5 (16.7)
Past of STI	0

SSc: systemic sclerosis; IS: immunosuppressive agents; STI: sexually transmitted infection.

Results
Thirty-one women with SSc were included, with a mean (SD) age of 59.8 (11) years old. Limited SSc represented 81% of cases (n=25). Characteristics of SSc women are summarised in Table I. Fifty cervical control samples were collected from women with a mean age of 60.2 (8.3) years old.
Of the 31 SSc women, 25 had interpretable HPV detection. Eight (32%) were positive for overall HPV DNA, among which 7 (87.5%) for HR or pHR HPV DNA. Among the 50 cervical control samples, 19 (38%) were positive for overall HPV DNA, among which 17 (89.5%) for HR or pHR HPV DNA (Table II).
The most common genotypes were HPV52 among the SSc women, and HPV52 and HPV53 in the control group. Multiple infections (≥2 HPV) represented respectively 50% and 26.3% of the positive samples in the SSc group and the control group (Table II).
All the Pap smears were within the normal limits in the two groups, except one HGSIL in the control group, but with a normal colposcopy.
All the 31 SSc women had a HPV serology. Antibodies against HPV16 and HPV18 were detected respectively in 4 and 2 cases (13% and 6.5%). Only one patient (3.2%) was positive for both antibodies. Women with anti-HPV16 antibodies were negative for HPV16 DNA: one was HPV52 positive, one was co-infected by HPV52/66, and the two other were HPV DNA negative. Women with anti-HPV18 antibodies were HPV18 DNA negative, but one was infected by HPV52.
A diffuse SSc, a shorter duration, and a younger age at first intercourse were most frequently observed among women HPV DNA positives, compared to HPV DNA negatives (Table III). Current smoking was 3 to 4 times more frequently in women seropositive for anti-HPV 16/18, compared to seronegative. A greater number of sexual partners, a diffuse SSc with a shorter duration were more frequent in seropositive women.

Table II. Frequency of HPV and genotypes in SSc and control groups.

HPV result	SSc (n=25)	Control samples (n=50)
Overall HPV	8 (32%)	19 (38%)
HR or pHR HPV	7 (28%)	17 (34%)
LR HPV	-	1 (2%)
HPV of unknown risk	1 (4%)	2 (4%)
≥2 HPV	4 (16%)	5 (10%)
HPV6	-	1 (2%)
HPV16	2 (8%)	3 (6%)
HPV31	1 (4%)	3 (6%)
HPV39	1 (4%)	-
HVP45	-	1 (2%)
HPV51	2 (8%)	-
HPV52	3 (12%)	4 (8%)
HPV53	-	4 (8%)
HPV66	2 (8%)	1 (2%)
HPV68	-	1 (2%)
HPV74	1 (4%)	2 (4%)
HPV82	-	1 (2%)
HPVX	-	3 (6%)

SSc: systemic sclerosis; HR: high risk; pHR: probably high risk; LR: low-risk; HPVX: HPV DNA positivity witheout possible typing with the INNO-LiPA assay.

Table III. Global and SSc-related factors of HPV infection.

	DNA HPV + (n=8)	DNA HPV - (n=17)	Ab HPV 16 + (n=4)	Ab HPV 16 - (n=27)	Ab HPV 18 + (n=2)	Ab HPV 18 - (n=29)
Number of sexual partners*	3.3 (2.5)	2.9 (2.9)	5.6 (1.5)	2.9 (2.5)	4.5 (4.9)	3.1 (2.5)
Age at first intercourse**	18.1 (0.9)	19.5 (3.4)	17.6 (2.1)	18.8 (9.8)	20.5 (6.4)	18.8 (2.7)
Parity	2.7 (0.9)	2.6 (2.5)	2.3 (2.1)	2.7 (2.1)	4 (1.4)	2.5 (2.1)
Smoking	12.5%	11.7%	50%	11.5%	50%	14.2%
STI	0%	0%	0%	0%	0%	0%
Diffuse SSc	25%	17.6%	25%	18.5%	50%	17.2%
Limited SSc	75%	82.3%	75%	81.5%	50%	82.8%
Duration of SSc**	7.1 (5,2)	11.7 (10)	5 (2.6)	10 (8.5)	2 (0)	10 (8.3)
SSc-related treatment	12.5%	11.7%	25%	14.8%	0%	17.2%

DNA: deoxyribonucleic acid; Ab: antibodies; STI: sexually transmitted infections; SSc: systemic scleroderma. *: mean (SD); **: (years), mean (SD).

A younger age at first intercourse and a history of SSc-related treatment were more frequent in women with anti-HPV16 antibodies. The number of pregnancies was higher in women with anti-HPV18 antibodies (Table III).

Discussion

The HR or pHR HPV frequency was comparable between SSc women and the control group (32% and 28% respectively). Barzon *et al*. reported also the presence of HR or pHR HPV DNA by INNO-LiPA® in 30 to 35% of the cervical swabs from 99 women aged of 55 years old or more (9).

HPV52 was the most common genotype in both groups. However, the small sample size can underestimate the prevalence of some HPV genotypes. Moreover, the hospital recruitment of the control group can overestimate the overall HPV prevalence, impending to highlight a difference between the two groups. Nevertheless, multi-HPV infections are near two times more frequent in the SSc group (50% *vs*. 26.3%).

Among women with SLE, HPV53, 58, 66, and 84 are the most prevalent genotypes (10), suggesting a facilitator effect of the auto-immunity in genital infection with less common HR or pHR HPV. However, in Gougerot-Sjögren syndrome or rheumatoid arthritis, Pap smears, colposcopy or HPV status are not different from the general population (11, 12).

Absence of concordance between HPV DNA positivity and corresponding humoral antibodies is linked to the fairly constant humoral response over the years, even after HPV DNA clearance (2). Cumulative seroreactivity anti-HPV16 and 18 is only of 5% in European women (2), suggesting that HPV seroreactivity in women with SSc could be superior since our cumulative frequency is about 20%.

A larger number of sexually partners, a younger age at first intercourse, and current smoking, are observed in SSc women with HPV infection. Among women of the same age from the general population, the number of sexually partners is the main risk factor for HPV infection, but smoking and age at first intercourse are not significant (13).

A diffuse SSc is more frequently observed in women HPV DNA positive and/or seropositive for HPV16/18. Currently, no such data are available in the literature. Diffuse SSc is associated with more important skin sclerosis and organ damage than limited SSc, and with a small overall survey at 10 years (14). We could think that, related to skin fibrosis, epithelium modifications in the cervix could occur in the transitional area, as a co-factor for penetration and/or HPV persistence. Conversely, viral infections are suggested to act as a co-factor for SSc development (15), but the role of HPV as not been evaluated to date.

Our study is the first to evaluate the HPV status in women with SSc. Overall HPV frequency and genotypes are not different from the hospital control group, but multi-HPV infections are two times more frequent in the SSc group. These results need to be confirmed in a larger and controlled study.

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