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Human Papilloma Virus Type and Recurrence Rate After Surgical Clearance of Anal Condylomata Acuminata

THESE

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Corrélation entre type de Human Papilloma Virus et récidive après traitement chirurgical de condylomes acuminés anaux

Le condylome acuminé anal (CAA), transmis par contact sexuel, résulte d'une infection par *Human Papilloma Virus* (HPV). Son traitement chirurgical est grevé d'un taux de récidive de 4-29%. Le but de cette étude était d'identifier une éventuelle corrélation entre type d'HPV présent dans les CAA excisés chirurgicalement et taux de récidive de la maladie.

Cette étude rétrospective porte sur 140 patients opérés au Centre Hospitalier Universitaire Vaudois de CAA, entre 1990 et 2005. Le diagnostic lésionnel a été confirmé par un examen histomorphologique. Le(s) type(s) d'HPV présent(s) dans ces lésions a été déterminé par *Polymerase Chain Reaction* (PCR). Les patients ont donné leur accord à cette analyse et complété un questionnaire. Une éventuelle corrélation entre récidive de CAA, type d'HPV et status HIV a été recherchée.

HPV 6 et 11 sont les virus les plus fréquemment découverts (51% et 28%, respectivement) chez les 140 patients (123H/17F). Trente-cinq (25%) d'entre eux ont présenté une récidive. HPV 11 était présent chez 19 (41%) sujets. Ceci est statistiquement significatif ($P<0.05$), en comparaison aux autres HPVs. Il n'y a par contre pas de différence significative entre la fréquence de récidive des 33 (24%) patients HIV-positifs et le reste du collectif.

HPV 11 est donc associé à un taux de récidive de CAA significativement élevé. Un suivi strict des patients atteints est nécessaire pour identifier une récidive et la traiter sans délai, notamment lorsque HPV 11 est présent. Ces résultats innovateurs soulèvent la question de la nécessité de pratiquer une typisation virale systématique sur les lésions excisées. La justification d'une telle attitude demande toutefois encore d'être confirmée.

of the lesion(s) and patient preference. It consisted of a comprehensive perianal, anal, and rectoscopic examination followed by excision of all visible warts starting with the perianal region. An en bloc excision was achieved with scissors with a free margin of normal tissue. The site of excision was then systematically cauterized with diathermy. Resorptive sutures were only exceptionally used on perianal skin or mucosa, to improve the scarring process. If disease was extensive or consequent anal stenosis feared, excision was carried out by sequential procedures with an intermittent maximum delay of 6 weeks.

Histologic Analysis

All specimens were submitted to standard histologic examination, to confirm diagnosis of condylomata acuminata (S.Y., H.B.). The most representative lesions were used for HPV genotyping.

HPV Genotyping

DNA was purified from fresh biopsy samples or paraffin-embedded material using the Magna Pure DNA isolation kit (N°3003990, Roche, Rotkreuz, Switzerland) on the Magna LC robot (Roche). Negative controls accompanied each set of DNA extractions assess contamination. Typing was performed at the Institute of Microbiology as part of its routine diagnostic testing using accredited procedures (EN17025) that will be published elsewhere (Estrade et al., In press). Briefly, HPV DNA was detected by polymerase chain reaction (PCR) and genotyped by reverse line blot hybridization (RLBH). PCR was performed with 5 μL DNA in triplicate 50 μL reactions using the PGMY primers, according to Gravitt et al.,[24] with slight modifications. The PGMY11 primers and one of the HLA primers were biotinylated to detect the amplicon in the genotyping by RLBH. PGMY primers were used at 80 nM, HLA at 40 nM in 1 × PCR buffer II (Applied Biosystems, Rotkreuz, Switzerland), 0.2 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, and 1.25 units of AmpliTaq Gold (Applied Biosystems). HLA-DQA primers[25] were used as an internal standard to assess the quality of the DNA and absence of PCR inhibitors. Positive controls (HPV16 DNA from Caski cells) and negative controls (PCR-grade water) were systematically analyzed to ensure appropriate sensitivity of the reaction (at least 100 copies of HPV DNA) and absence of contamination, respectively. Following PCR, 5 μL of each reaction were analyzed by gel electrophoresis and staining with ethidium bromide. Samples were considered informative if the HLA or the HPV DNA fragment could be detected. RLBH was performed on positive samples by RLBH with a miniblotter (MN 45, Immunetics, Boston) essentially as described by Kaufhold et al.[26] using a panel of 31 HPV type-specific probes (high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, MM4 [Type 82], and MM9 [Type 73]; low-risk: 6, 11, 34, 40, 42, 44, 53, 54, 57, 70, and MM8 [Type 84]; undetermined-risk: 26, 55, 66, and MM7 [Type 83]) arrayed on a reusable, negatively-charged nylon membrane. This procedure has been validated in the course of the first World Health Organization international collaborative study on detection of human papillomavirus DNA.[27] To identify types not represented in the panel of probes, sequencing was performed on PCR-positive/hybridization-negative samples with sequencers from Applied Biosystems using the Big Dye Terminator chemistry (BDT v.1.1, Applied Biosystems) and PGMY11 primers.

Sectioning of the paraffin block was done with single-use disposable blades to eliminate the risk of intersample contamination. Standard procedures to avoid PCR contamination were strictly followed: work was done in separate laboratories (pre- and post-PCR analysis).

Definition

Recurrence was defined as the advent of new lesion(s) within 12 months after curative surgery. This delay was chosen arbitrarily, as the literature is vague about this definition. Furthermore, in patients without visible warts after treatment, there is no way to verify whether the viral infection has been completely cleared or whether it is present and latent, at the site of previous resection or on adjacent mucosa.[2] As clinical warts depend upon the presence of HPV, it is difficult to determine whether ACA will recur in this context.

Follow-Up

Two groups of patients were defined: those without and those with recurrent disease. New lesions were surgically excised and diagnosis of ACA was confirmed by histology. Recurrence rates and time to recurrence after surgery were calculated.

Statistical Analyses

To compare patients' distribution, Pearson homogeneity test (χ^2) was used. When theoretical numbers were <5, the Monte-Carlo simulation test was used with 2000 simulations.[28] Wilcoxon test was performed for age. Statistical analysis was performed with R software (version 2.2.1).[29] To fulfill basic statistical rules for analysis and thus to obtain independent data, only the first lesion was considered. Significance was assumed to occur at $P < 0.05$.

RESULTS

One hundred forty consecutive patients were included in this study. Median age was 35.3 years (SD = 11.7). Of the 123 (88%) males, 40 (32%) declared themselves to be heterosexuals, 73 (60%) homosexuals, and 10 (8%) bisexuals. Of the 17 (12%) females, 16 (94%) were heterosexual and 1 (6%) was bisexual.

Recurrence occurred in 35 (25.0%, 1 female and 34 males) patients, 21 to 357 days after curative surgery (median 120 days). Median age of the nonrecurrent and recurrent group was 36.4 (SD = 12.4) and 31.9 (SD 1 = 8.7), respectively. Age and gender did not represent a risk factor for recurrence ($P > 0.05$).

Median follow-up was 32 months (SD = 35.4); this included both the clinical follow-up and the information provided in the returned questionnaires (of the questionnaires sent out, 71% were returned).

HPV Types

Initially, 144 patients had histologically confirmed primary ACA; 4 of them were excluded because conclusive typing of HPV was not possible (no DNA could be amplified). In the remaining 140 (123 M/17 F) patients, 17 different HPV types (2a, 6, 11, 16, 18, 27, 31, 40, 44, 45, 51, 53, 58, 59, 61, 66, and 68) were identified.

HPV 6 and 11 were the most frequently encountered viruses, representing 84 (51%) and 46 (28%) out of the 165 HPVs, respectively. Recurrence was detected in 16 cases with HPV 6 ($P > 0.05$) and in 19 cases with HPV 11 ($P < 0.05$). As a result of the high prevalence of HPV 6 and 11, data are stable and classic χ^2 test has been used.

Other viruses were rarely present, respectively identified once (HPV 2a, 27, 31, 40, 58, and 61), twice (HPV 44, 45, 59, and 68), thrice (HPV 51, 53, and 66), 5 times (HPV 18), and 8

TABLE 1. Association Between HPV Type and Recurrent Disease

HPV Type	Nonrecurrent Disease n (%)	Recurrent Disease n (%)	P
2	1 (1.0)	0 (0.0)	1.000*
6	68 (64.8)	16 (45.7)	0.073†
11	27 (25.7)	19 (54.3)	0.004†
16	8 (7.6)	0 (0.0)	0.203*
18	4 (3.8)	1 (2.9)	1.000*
27	1 (1.0)	0 (0.0)	1.000*
31	1 (1.0)	0 (0.0)	1.000*
39	1 (1.0)	0 (0.0)	1.000*
44	1 (1.0)	1 (2.9)	1.000*
45	0 (0.0)	2 (5.7)	0.051*
51	2 (1.9)	1 (2.9)	1.000*
53	3 (2.9)	0 (0.0)	0.570*
58	0 (0.0)	1 (2.9)	0.253*
59	0 (0.0)	2 (5.7)	0.065*
61	1 (1.0)	0 (0.0)	1.000*
66	3 (2.9)	0 (0.0)	0.582*
68	2 (1.9)	0 (0.0)	1.000*
Negative	6 (5.7)	1 (2.95)	0.672*

*χ^2 with simulation.
†Classical χ^2.
HPV indicates Human Papilloma Virus.

times (HPV 16). For these rarely identified viruses, the Monte-Carlo simulation test[28] was used with 2000 simulations.

Seven among 140 (5%) patients had no HPV type identified in the surgically excised specimen. One of them presented with recurrence. Details about association between recurrence and HPV types are reported in Table 1.

Mixed Infection (More Than 1 Virus Identified in 1 Single ACA)

One hundred five among 140 (75%) patients were infected by a single HPV, and 24 of them recurred. Twenty-eight among 140 (20%) patients presented a mixed infection, and 10 of them recurred. When compared, neither none, single nor mixed infections were associated with statistically higher recurrence rates $P > 0.05$ (Table 2).

Among the 10 patients with mixed infections which recurred, 9 contained HPV 11, and 1 did not. There was a significant correlation between mixed infection associating HPV 11 and recurrence compared to mixed infection without HPV 11 ($P < 0.05$).

HIV Status

Thirty-three (24%) patients were HIV-positive, 97 (69%) HIV-negative, whereas in 10 (7%) patients HIV status was not known. These 10 patients were not included in the association test between HIV and recurrent disease. Five of the 33 HIV-positive patients, whereas 30 of the 97 HIV-negative, presented with recurrence. Showing that HIV-positive status was not a risk factor for recurrence ($P > 0.05$). However, the CD4 counts of HIV-positive patients were not known at the point of surgery.

TABLE 2. Distribution of Recurrence Between None, Single, and Mixed Infections

Virus	Nonrecurrent Disease n (%)	Recurrent Disease n (%)	P
None	6 (5.7)	1 (2.8)	0.351*
Single	81 (77.1)	24 (68.6)	
Mixed	18 (17.2)	10 (28.6)	
Total	105	35	

*χ^2 with simulation.

DISCUSSION

Possible correlation between HPV type and recurrence of ACA has been little studied and remains debatable. Nevertheless, ACA are very frequent and, apart from HIV seropositivity, no other specific risk factor for recurrent ACA has been described in the literature.

For the purposes of this article, to obtain independent data and to fulfil basic statistical rules for analysis, only the first lesion was considered. According to the definition of recurrence (mentioned in Definition under Materials and Methods), only recurrences within 1 year were considered. Interestingly, the earliest clinical wart described after the period of 12 months was at 20 months. This "8-month-gap" suggests that the definition currently used can be considered as adequate. Unfortunately, no other data were available to support this statement. However, it is noted that Nathan et al.[30] also considered their patients as cured after a 12-months disease-free period after laser ablative therapy for HPV-associated anal canal disease.

Among the 140 patients, 7 (5%) had no HPV identified. These patients should arguably be excluded. However, 1 of these 7 patients presented with recurrence. All 7 have therefore been included in the study, considering that this situation could paradoxically represent a risk factor for recurrence. However, as expected, statistical analysis showed that absence of viral DNA was indeed not a risk factor. Because of this singular patient, all 7 were nevertheless included in the analysis, even though they made the case for HPV 11 weaker.

In the current study, neither age nor gender represented a risk factor for recurrence. Nevertheless, they both verge on statistical significance ($P = 0.080$ and 0.064, respectively).

Median age of the recurrence group was only 4.5 years younger than the nonrecurrence group. Thus, the population cannot be divided in 2 specific separate groups as the ages are too close. This is also supported by Nathan et al.[30] who showed that outcome of laser treated HPV-associated anal canal diseases were not affected by age.

The distribution of the 34/1 recurrent males/female makes the genders difficult to compare and the P value of 0.080 difficult to analyze.

HPV 6 and 11 were the most frequently encountered viruses. This is consistent with the results published by Dupin[31] who showed that mucosal HPV 6 and 11 are the types most frequently found in patients with genital warts or condylomata acuminata. This deduction is also supported by Kreuter et al.[32] The whole of their cohort of patients excised by surgery for intraanal condylomata carried HPV 6 or 11.

HPV 11 was associated with statistically higher recurrence rates, when present alone and also in mixed infections. Venturoli et al.[33] observed that the residual or recurrent disease in 72 women treated by surgery for HPV infection with persistence of the high-risk HPV 16 and/or 18 was higher (82.4%) than in women with persistence of at least 1 of HPV Type 31, 33, 35, 45, 52, and 58 (66.7%) and at least 1 of HPV Type 26, 39, 51, 53, 56, 59, 66, 68, 73, and 82 (14.3%). This is the only study that correlated recurrence with HPV type. However, these authors concentrated on high-risk HPVs and only on

women. They did not include low- and intermediate-risk HPVs, particularly HPV 6 and 11. Our patients' recurrences cannot be compared with Venturoli, or with the literature, as other similar cohorts have not been published.

Che et al.[34] also studied HPV and recurrence of condylomata but they did not mention exactly where the lesions recurred: whether vagina, penis, or anus. Their work concentrated on HPV DNA loads rather than HPV typing. They found HPV Type 6 or 11 in 98.4% of cases (recurrent or not) but used a Fluorescence Quantitative PCR Diagnostic Kit, which does not differentiate HPV 6 from 11. Kreuter et al.[32] studied the HPV DNA load by HIV-infected men treated with 5% imiquimod suppositories after surgical ablation, using electrocautery, of intraanal condylomata acuminata. They showed that the suppositories led to a sharp decrease in HPV DNA load and concluded that larger clinical trials are needed to find out whether relapse rates with the use of imiquimod after surgery are significantly lower than with surgery alone. Only 7 patients were included in their study and they also did not analyze the correlation between HPV type and recurrence. So, neither of these 2 studies can readily be compared with our results in terms of HPV typing and recurrence.

The present series shows that HIV seropositivity does not apparently influence ACA recurrence as suggested by Stevens et al.[35] Epidemiologic studies have shown that HIV seropositivity and other conditions are risk factors for HPV infection among women.[2,3] Nothing was said about risk factors for recurrence. Other studies need to be performed to determine if HIV seropositivity really acts as an independent risk factor on the prevalence of HPV infection and recurrent disease. The low recurrence rate demonstrated among HIV-positive patients suggests that our surgical technique was of importance: en bloc resection with a free margin of normal tissue ensured clearance, and diathermy, systematically used to obtain hemostasis, prevented any residual virus from being left on the resection site. CD4 count of the patients was not known at the time of surgical treatment, which makes their real immune competence difficult to determine. However, Sanclemente et al.[36] showed in a series of 37 patients that the efficacy of 5% imiquimod in HIV-positive male patients suffering from anogenital warts or anal intraepithelial neoplasia was not influenced by patients' CD4 count, HIV viral load, or HPV viral load, meaning that recurrence was not influenced by HIV status. This is consistent with our series.

A severity scoring system for condylomata presented at the American Society of Colon and Rectal Surgeons 2004 Meeting suggested a scale of 0 to 5 applied to 3 areas: the intraanal, perianal, and extraanal regions.[35] The 3 scores were added to create the condyloma scoring system (maximum 15) as a predictor of recurrence. The lack of information in our retrospectively analyzed cohort unfortunately did not allow use of this algorithm.

We found HPV 6 and 11 to be the most frequently identified viruses. Compared with all others, HPV 11 seemed to change the course of the disease with statistical significance ($P = 0.004$) and it confers an increased risk of recurrent ACA. Routine clinical HPV typing remains, however, questionable. In the absence of any proven benefit, the additional cost of investigation and treatment is difficult to justify when based solely on analysis of a retrospective cohort of 140 patients extending over a period of 15 years. At this point, we cannot yet recommend systematic HPV typing, its relevance not having been conclusively demonstrated. Prospective data based on recurrence, costs, and clinical implication are needed. Nevertheless, follow-up is required to identify recurrence and to treat it early, especially if HPV 11 has been indentified.

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