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Genital Human Papillomavirus Infection in Panama City Prostitutes

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Little is known of the natural history of genital human papillomavirus (HPV) infections in women from high-risk populations. Samples were collected from 183 Panama City prostitutes and assessed for HPV (filter in situ DNA hybridization) and for sexually transmitted agents. The cohort was followed for 8 mo; 51% of subjects completed four monthly return visits and 16% were sampled eight times. The proportion of women found infected with HPV increased significantly with increasing numbers of consecutive samples tested; 38 (21%) of 183 women were positive after one visit and 46 (82%) of 56 who completed six visits were infected. The pattern of viral detection over time was not random, which implied that most prostitutes were persistently infected with genital HPVs and that either scattered foci of infection or periodic reactivation of latent virus occurred. Our findings suggest that multiple sampling is necessary to accurately estimate HPV infection rates and to define whether patterns of DNA expression are present.

Human papillomaviruses (HPVs) are increasingly recognized as important and complex human pathogens. HPV has been suggested as an etiologic agent in the genesis of cervical neoplasia [1]. Genital HPVs are associated with condyloma accuminata, atypical condylomas, and cervical intraepithelial neoplasia [2–4]; HPV infection also occurs in anatomically normal genital mucosal epithelium [4–6].

Attempts to define the mechanisms by which specific types of HPV cause disease have been limited because infection must be estimated by assays for viral DNA. These assays are constrained by sampling errors and by poorly defined sensitivity. In addition, published studies associating HPV with cervical disease have not addressed issues such as viral persistence, latency, or reactivation. We attempted to assess the natural history of genital HPV infections by repeatedly sampling a cohort of prostitutes in Panama City, Republic of Panama. The women were interviewed to ascertain risk factors and had serial samples taken to define HPV infection.

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Informed consent was obtained from all participants, who were volunteers. The protocol used was reviewed and approved by the Panamanian Ministry of Health and by the Gorgas Memorial Laboratory Human Subjects Committee.

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Subjects and Methods

Study population. We enrolled 183 prostitutes between January and May 1986 as they attended mandatory weekly Ministry of Health Social Hygiene Clinics at the Chorrillo, Rio Abajo, Emiliano Ponce, and Boca la Caja District Health Centers [7]. We then followed this cohort during monthly routine clinic visits.

Detection of HPV DNA. Material for HPV DNA assays was collected at each visit by scraping the cervical os with a cotton-tipped swab. The material was suspended in 2 ml of phosphate-buffered saline, then centrifuged at 400 g for 10 min at 4°C; the pellet was then resuspended in 0.3 ml of 6× standard saline citrate (SSC) (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) containing 5 µg/ml of denatured salmon sperm DNA. The samples were then stored at −20°C until tested.

HPV DNA was detected by filter in situ hybridization [8, 9]. Briefly, cells were filtered onto three nitrocellulose filters (prehybridized with denatured salmon sperm DNA) and denatured by two 5-min

treatments with a solution of 1 *M* NaCl and 0.5 *M* NaOH followed by two 5-min treatments with 0.5 *M* Tris buffer and 3 *M* NaCl (pH 7.4). The filters were then baked at 90°C for 10 min followed by digestion with 10 ml of proteinase K (2 mg/ml in 10 m*M* Tris, pH 7.8, 0.5% sodium dodecyl sulfate, and 0.5 m*M* EDTA) at 37°C for 15 min. After deproteinization with chloroform, the filters were washed twice with 2× SSC and baked again at 90°C for 90 min.

DNA-DNA hybridization was carried out using pBR 322 and the vector containing inserts of HPV 16 and 18 or HPV 6c and 11 DNA. One set of nitrocellulose filters was probed with HPV 16 and 18, one set with 6c and 11, and one set with pBR 322. The probes were labeled with ^{32}P dCTP to a specific activity of 1×10^8 counts per minute (cpm)/µg using a commercial nick translation system (Bethesda Research Laboratories, Gaithersburg, Md). Hybridization was conducted at 42°C in a solution containing 50% formamide, 5× SSC, 5× Denhart's solution, 250 µg/ml of denatured salmon sperm DNA, and 5×10^5 cpm/ml of ^{32}P-labeled specific DNA probe.

After hybridization, filters were washed four times for 1 h each at 65°C in 2× SSC with 0.1% sodium dodecyl sulfate and then exposed to x-ray film in a Kodak intensifying screen, at −70°C for 1–3 d. All x-ray films were examined independently by three observers; films rated positive by two were considered positive. Less than 1% of specimens were positive in the pBR 322 assay; these were considered HPV-negative.

Results

We collected 741 HPV specimens from 183 prostitutes. Overall detection rates for HPV 16/18 and HPV 6/11 DNA were 25% and 12%, respectively. Of the samples, 10% were positive against both HPV 16/18 and HPV 6/11 probes; this could represent infections with more than one virus type, infection with a cross-hybridizing HPV type, or insufficient conditions of stringency. Because the filter in situ hybridization cannot distinguish between these possibilities, the remaining data analysis classified a positive hybridization reaction as any HPV DNA.

Detection of HPV infection increased significantly according to the number of consecutive specimens tested. Of the 183 women, 94 (51%) were seen on four consecutive visits and 30 (16%) completed eight follow-up visits over 8 mo. As shown in table 1, only 21% were positive after a single visit, 41% were positive at least once during their first two visits, and >80% of the sampled six times or more had HPV DNA detected at least once. This pattern suggested persistent HPV infection with periodic viral shedding.

Table 1. Human papillomavirus, infection rates in Panamanian prostitutes by number of samples taken.

No. consecutive visits	No. subjects	No. (%) positive for HPV DNA*
1	183	38 (21)
2	138	56 (41)
3	119	59 (50)
4	94	59 (63)
5	76	54 (71)
6	56	46 (82)
7	45	38 (84)
8	30	24 (80)

* HPV DNA-positive refers to DNA detected at least once up to the indicated number of visits.

Shedding from persistent viral infections should occur in a nonrandom pattern. To test this hypothesis, we analyzed the distribution of HPV DNA–positive samples as a function of clinic visits. Among women sampled on three, four, or five consecutive visits, significantly more were consistently free of HPV DNA and more were positive in three or more samples than expected by chance (table 2). Not surprisingly, the HPV DNA positivity rate was higher in samples taken subsequent to a positive sample; 74 (44%) of 167 specimens taken immediately after a positive sample were positive versus 108 positive samples immediately following 399 (27%) negative specimens ($\chi^2 = 15.3$, $P < .001$). This pattern suggests that most of the prostitutes were persistently infected with genital HPVs, characterized by either scattered foci of infection that were inconsistently swabbed or by periodic reactivation of latent virus.

We obtained genital cultures for gonorrhea, chlamydia, mycoplasma, herpes simplex virus (HSV), and cytomegalovirus (CMV) from women during their first clinic visit. We isolated gonorrhea from 8 of 164 subjects (5%), chlamydia from 20 of 137 (15%), mycoplasma from 40 of 162 (25%), and HSV and CMV from 1% and 3%, respectively. There was no association between isolation of any sexually transmitted disease agent and detection of HPV DNA.

Table 3 summarizes demographic, socioeconomic,

Table 2. Distribution of human papillomavirus-positive samples by number of samples taken.

No. samples positive	In 3 visits		In 4 visits		In 5 visits	
	Observed	Expected	Observed	Expected	Observed	Expected
0	60	52.0	35	25.7	22	13.2
1	37	49.6	27	39.4	21	27.7
2	17	15.7	20	22.6	16	23.2
3	5	1.7	11	5.8	10	9.7
4	—	—	1	0.5	6	2.0
5	—	—	—	—	1	0.2
Total	119		94		76	
χ^2 analysis	10.9 (2 df) $P < .005$		12.7 (3 df) $P < .01$		20.9 (4 df) $P < .005$	

NOTE. Expected results are estimated by binomial distribution.

and behavioral characteristics of study participants, which were similar to those documented in a previous study of this population [7]. No significant associations were observed between these variables and detection of HPV DNA. Also, there were no differences in the distribution of these characteristics by the number of follow-up visits completed.

Discussion

Most investigators have detected a high prevalence rate of HPV DNA in specimens from condylomatous, dysplastic, or invasive cervical lesions. However, viral DNA has also been detected in specimens from women without cytologic or histologic evidence of disease [5, 8, 10–19]. Clinical studies have shown that natural regression or therapeutic eradication of focal lesions is not always accompanied by elimination of the virus [10, 20].

The presence of viral DNA in anatomically normal tissue could result from sampling during the incubation period of the infection or the persistence of the virus in an occult form not associated with a proliferative response of infected epithelial cells. Our finding of HPV DNA in samples obtained from prostitutes who were followed at sufficient monthly intervals to encompass the expected incubation period supports the concept of the virus persisting in an occult form.

In addition to persistent HPV infections, there are other explanations for the pattern of genital HPV DNA detection observed in our study population. The filter in situ hybridization assay might have yielded random positive reactions that were not specific for HPV DNA. This seems unlikely since the probability of a positive reaction among women repeatedly sampled was influenced by the positivity status of prior samples, and the time-dependent pattern of HPV DNA detection among the prostitutes was not that expected by random chance.

A biased sample population, in which women with genital HPV infections were more likely to remain in the study, could increase the frequency of HPV detection with repeat testing. We do not believe this occurred.

Weekly attendance at Ministry of Health Social Hygiene Clinics is mandatory for all women who work in houses of prostitution, bars, cantinas, massage parlors, and similar establishments. Compliance is enforced by inspections and stiff fines for women who lack current clinic documentation and for the establishment in which she is working. During clinic visits women are examined for gross signs of gonorrhea and Gram's stained cervical smear is examined for evidence of gonorrheal infection. Infected women are treated and not allowed to work until treatment is completed. The clinics do not routinely screen for dysplasia or genital warts and do not provide treatment for such conditions.

The Panama City prostitute population is quite mobile. Eighty per cent were non-Panamanian nationals temporarily residing in the city. The "drop out" rate we observed is similar to the general turnover within clinics. Finally, there was no association between specific demographic, socioeconomic, or behavioral characteristics and either detection of HPV DNA or duration of follow-up in the study.

The periodic detection of HPV DNA in the geni-

Table 3. Demographic, socioeconomic, and behavioral characteristics of prostitutes in Panama studied for human papillomavirus.

	No. (%)
Country of birth:	
Colombia	107 (58.8)
Panama	37 (20.3)
Dominican Republic	14 (7.7)
Costa Rica	10 (5.5)
Brazil	7 (3.8)
Other*	7 (3.8)
Race:	
Mestizo	93 (51.1)
White	65 (35.7)
Black	18 (9.9)
Asian	6 (3.3)
Number customers/week:†	
1–5	62 (49.2)
6–10	13 (10.3)
11–20	4 (3.2)
21–50	38 (30.2)
>50	9 (7.1)
Amount charged:‡	
≤$5	3 (2.3)
$6–$10	30 (23.1)
$11–$20	14 (10.8)
$21–$30	21 (16.1)
$31–$50	19 (14.6)
>$50	43 (33.1)

	Range	(Average ± SD)
Age (years)	16–57	(28 ± 7.1)
Years living in Panama§	.08–20	(1.6 ± 3.7)
Years as prostitute	.08–30	(3.9 ± 4.8)
Age at first intercourse (years)	12–27	(18.2 ± 2.9)
Age began prostitution (years)	15–53	(24.1 ± 5.5)

* Other countries of birth: Chile, 2; USA, Peru, Honduras, El Salvador, and Haiti, 1 each.

† Answers were provided by 126 subjects; 14 denied prostitution and 32 did not respond to the question.

‡ Answers were provied by 130 subjects; 24 denied prostitution and 28 did not respond to the questions.

§ Includes 145 foreign-born women.

tal tract of these high-risk women could result from repeated reinfection by male sexual partners. In such instances, positive results would reflect virus deposition from the male genital tract or focal replication in the cervical epithelium. The sensitivity of the hybridization assay is probably not sufficient to detect virus introduced via semen; however, we cannot exclude repeated occult reinfections.

The distinction between reinfection and persistent virus infections in terms of natural history has important implications. Repeat sampling of our prostitute population revealed that ~80% of women seen repeatedly for 8 mo were positive for HPV DNA on one or more occasions. If this was due to reinfection then the presence or absence of HPV DNA in genital secretions of low-risk women should reasonably predict their HPV infection status.

However, if the high detection rates on repeat sampling of the prostitutes represents persistent infection or reactivation of latent virus, our findings imply that a single sampling of cervical cells will be inadequate to accurately assess genital HPV infection. This latter interpretation is supported by the findings of Schneider and coworkers [21]. They noted that a greater proportion of pregnant than nonpregnant women were positive for cervical HPV DNA; also HPV-positive pregnant women had larger quantities of viral DNA than positive nonpregnant women. These findings suggest that factors associated with pregnancy influence expression of persistent HPV infections.

A potential limitation of our observation is the reliance on the filter in situ hybridization assay to detect HPV DNA. The simplicity of this method permits its use in large epidemiologic surveys [15, 18]. A comparison of filter in situ with other hybridization methods, using samples from patients with histologically confirmed lesions, suggests that it is adequately sensitive and specific for this purpose [9]. However, information on comparative studies of specimens from women without detectable lesions is not available.

HPV DNA detection rates in exfoliated cervical cells from women without cervical abnormalities have generally been similar regardless of the assay used. For example, the filter in situ hybridization method has yielded positivity rates of 2% [11], 2%–13% [15], and 7%–13% [18]. Southern blot analysis of similarly obtained specimens has yielded rates of 11% [22] and 12% and 28%, respectively, among nonpregnant and pregnant women [21]. Although these reports suggest comparable sensitivity of the assay methods, the specificity of the filter in situ method is suspect since there is no inherent characteristic of the reaction patterns that identifies the target of the probe as HPV DNA.

Our study design controlled for reactions with plasmid DNA but spurious reactions with other substances cannot be excluded. Clearly, additional investigations into the shedding patterns of genital papillomavirus among groups of women at varying risks of sexually transmitted infections are needed.