HPV and cervical cancer on Curaçao

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2018

document version
Publisher's PDF, also known as Version of record

Chapter 6
Human papillomavirus (HPV) types prevalence in cervical samples of female sex-workers on Curaçao

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Preventive Medicine Reports 2018; 11: 120-124

Outline

Abstract

Sex-workers have an increased risk for high-risk HPV(hrHPV) cervical cancer. On Curaçao, legal and illegal prostitution practice is high, and the promiscuous lifestyle is common. We aimed to gain insight in HPV-genotype prevalence in cervical scrapes of female sex workers (FSW) and related risk factors in comparison with women not working in the sex industry.

Cervical samples were taken from 76 FSW and 228 non-FSW (NFSW) age matched controls in the period between 2013-2015. HPV was detected by GP5+/6+PCR-EIA followed by genotyping via reverse line-blot.

HPV prevalence in FSWs was 25.0% and in NFSWs 29.4% (p=0.14). NFSW had more often untypable HPV-genotypes (HPV–X: 5.3% vs 0.0%; p=0.042). A trend for statistical difference was observed in HPV prevalence between FSWs from Dominican Republic (42.1%) and FSWs from Colombia (19.2%; p=0.067). Young age was the only risk factor related to HPV prevalence in FSWs. (Mean age FSW 29.2y +/-7.8 and NFSW 33y +/-6.2) Smoking and drugs consumption were significantly higher among FSW. A significant higher number of women with history of any STD was reported by NFSWs. In addition, >90% of FSW had their previous Pap smear <3years ago, while >35% NFSW never had a previous Pap smear (p<0.001). In conclusion: no significant difference in HPV prevalence is observed between FSW and NFSW. HPV prevalence in FSW was associated with a lower age. During interviews, FSW seemed more aware about prevention strategies, reported less history of STD's and were more updated with cervical cancer screening, compared to NFSWs.

Keywords: HPV prevalence, sex workers, Curaçao, the Caribbean

6.
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6.1 Introduction

Persistent infection with an oncogenic HPV type is the causative agent of cervical cancer (Walboomers et al., 1999; Bosch et al., 2002). Multiple sexual partners and young age at sexarche are factors associated with increased exposure risks (Bosch et al., 2002; Bosch et al., 2006). Studies reported that sex workers have more than twice probability of having HPV infection and also a higher prevalence of abnormal Pap smears than non-sex workers, in the general population (Valles et al., 2009; Leung et al., 2013). Globally, there are few studies published with statistical data on HPV and cancer in FSW (Soohoo et al.,2013; Gonzalez et al.,2011; Cañadas et al., 2004; Montano et al., 2011; Brown et al.,2012).

In the Caribbean region prostitution is common and the region is on the list of regions well known for sex tourism (Der Mark, 2003; Prüss-Ustün et al.,2013; Dominguez et al., 2010; Agard-Jones, 2011). Cervical cancer is the 2th most common cancer among women in Latin America and the Caribbean (ICO HPV Information Centre, 2016; Ferlay et al., 2015). For the year 2030, the incidence and mortality are projected to increase by 45% and 60%, if no action is taken (Ferlay et al.,2012; GLOBOCAN 2012). Low income- and developing-countries attribute with 85% of annual cervical cancer incidence (Ferlay et al., 2015). To be more specific, while the incidence and mortality of cervical cancer attribute to 12% of the cancer cases in Latin America and the Caribbean, statistics show 2% for both incidence and mortality in North

America (Ferlay et al., 2015). In regions with a lower gross domestic product (GDP) per capita, women are at greater risk for developing cervical cancer, and they also have a greater risk of dying from this disease (Ferlay et al., 2012; GLOBOCAN 2012).

On Curaçao, legal prostitution was established since 1949 with only one licenced brothel on the island. It has the capacity for 120 female sex workers (FSW), the number of clients per sex worker per 3 months is approximately 600 clients. Only women from abroad work at the brothel. After 3 months the work and residence licence of the FSW expires, and she moves back to her country of birth or she hops between Caribbean islands or Latin American countries. All FSW are medically examined for sexual transmitted diseases (STDs) when they start working at the brothel and receive medical check-up every two weeks thereafter. (Mr. M. Regales, Manager at the brothel, personal communication)

The Caribbean including Curaçao is known for its high HPV prevalence (ICO HPV Information Centre, 2016; Ferlay et al., 2015; Ferlay et al., 2012; GLOBOCAN 2012). When considering implementation of preventive strategies in high risk groups including FSW on Curaçao, the prevalence of HPV and HPV genotypes should also be taken into account besides social and behaviour aspects.

Previously we have shown that the proportion of HPV-16 and 18 associated cervical cancer is lower and consequently the attribution of the HPV genotypes 31, 45, 51, 52 and 58 is higher compared to world population (Hooi et al., 2017). Also, differences in HPV genotype prevalence in cervical cancer between the different islands exist (Hooi et al. 2018)

In this study we set out to identify HPV genotype prevalence and HPV associated risk factors in FSWs compared to age matched women not working in the sex industry.

6.2 Material and Methods

6.2.1 Study design and study population

In December 2014, seventy-six FSW from Colombia and the Dominican Republic, voluntarily participated in this study and they constitute about 60% of the sex workers working at the brothel on Curaçao. Two hundred and twenty-eight age-matched control women (referred to as non FSW, or NFSW) were obtained from women of two trials we conducted on HPV prevalence among the general female population of Curaçao. The first pilot trial was conducted in October 2014 and consists of 253 sexually active women from the general population who volunteered to participate. The second trial was conducted between March-June 2015. In this trial, 1,075 women, at random selected from the general population and equally stratified in four age groups from 25-65 years, were included. (Hooi et al. 2018) For the present study, all FSWs were age matched with controls from the general population trials (ratio 1:3, age +/- 3y), taken from the two HPV prevalence studies.

All participants provided a written informed consent and answered a questionnaire on their gynaecological medical history and sexual behaviour. Ethical approval of the study was obtained from the Institutional review board of the medical ethics committee of Fundashon Prevenshon, Curaçao (IRB board's approval number 2015/0004).

6.2.2 Data collection

The survey was carried out by a registered nurse. Personal data and information about gynaecological medical history such as history of abnormal Pap results, sexual transmitted disease (STD) and sexual behaviour such as age of sex-arche and number of lifetime sexual partners were obtained.

6.2.3 Specimen collection and handling

After completion of the anonymous questionnaire, cervical scrapes were collected with a cervical brush by a medical doctor for HPV analyses. Scrapes were conserved in phosphate buffered saline (PBS) for FSW and the Remmel® transport medium for NFSW. At the end of the day all samples were stored at -20°C.

6.2.4 HPV detection and genotyping by means of GP5+/6+ amplimer with the Enzyme immune assay (EIA kit HPV GP HR)

The MagnaPure 96 instrument was used for DNA isolation. Ten µL of extracted DNA was used as input for the broad spectrum GP5+/6+-PCR in a total volume of 50µL. Detection of hrHPV was done on 5µL GP5+/6+ amplimer with the Enzyme immune assay (EIA kit HPV GP HR; Labo Bio-medical Products, Rijswijk, The Netherlands) according to the manufacturer's instructions. This kit detects amplified DNA from HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. A 10µL aliquot of GP5+/6+ amplimer from HPV positive samples by EIA was tested with the Genotyping kit HPV GP, version 2 (Labo Bio-medical Products). This kit enables genotyping of 12 hrHPV genotypes (16/ 18/ 31/ 33/ 35/ 39/ 45/ 51/ 52/ 56/ 58/ 59), 6 possible hrHPV types (26/ 53/ 73/ 82/ 66/ 68) and 5 lrHPV types (6/ 11/ 30/ 67/ 70) (Van der Mark, 2003; Prüss-Ustün et al., 2013). Samples that were HPV positive , but were negative with the genotyping HPV GP kit were designated HPV-X.

6.3 Statistical analysis

Overall HPV prevalence between FSW and NFWS were compared with the chi-square test, genotype specific HPV prevalence with the Fisher's exact test. Demographical and sexual behavioural data were compared between FSW and NFWS via the chi-square test (categorical data). Fisher's exact test (categorical data with small groups), the independent samples *t*-test (continuous normal data) and the Mann-Whitney U test (continuous non-normal data). Sexual risk factors between HPV positive and HPV negative FSW were compared via the chi-square test or Fisher's exact test. Categorical data were described by frequencies and percentages, continuous data by means and standard deviations (SD). For all statistical analyses, SPSS version 22 (IBM Corp., Armonk, NY) were used. The significance level was set at 0.05, p-values <0.1 were considered indicative for a statistical trend.

6.4 Results

FSW were on average 32.2 (SD 6.6) years old, and the majority were from Colombia (68%) as opposed to the Dominican Republic (25%), while country of birth was unknown for 5 women (6.6%). Mean age of NFSW was 32.5 years (SD=6.2, p=0.75). Use of oral anticonception (OAC) was more common in the FSW (74.2%) compared to NFSW (18.1%; p<0.001). (Table 6.1) NFSWs reported more often a history of sexual transmitted disease. Moreover, FSWs were more up to date with their visits for cervical cancer screening compared to the NFSWs: more than 90% of FSW had their previous Pap smear less than 3 years ago, while more than 35% of NFSW never had a previous Pap smear (p<0.001). No significant differences were found in HPV prevalence between FSWs (25.0%) and NFSWs (29.4%) (p=0.14). HPV-16 (FSW vs NFSW: 7.9% vs 3.9%, p=0.22), 31(5.3% vs 3.9%, 0.74) and -18 (3.9% vs 3.1%, p=0.71), were the three most common genotypes for

both populations. (Table 2) However, we found a significant difference in HPV-X genotypes that were untypable by reverse line blot between NFSW (5.3%) and FSW (0%; p=0.042). HPV positive FSWs were on average younger than HPV negative FSWs (29.2 (SD 7.8) versus 33.2 (SD 6.2) years, respectively (p=0.018)) (Table 6.3). Moreover, HPV positive FSWs worked shorter in prostitution than HPV negative FSW(median of 2.5 years (range: 1.0 – 4.3) vs 5.0 (range: 3.0 – 8.0), respectively; p=0.002). Lastly, there was a statistical trend for a difference in HPV prevalence in FSWs born in the Dominican Republic tended to have a higher prevalence of HPV (42.1%) compared to FSWs born in Colombia (19.2%; p=0.067).

Table 6.1
Comparison of country of birth, sexual behaviour and gynaecological medical history between FSW(N=76) and NFSW(N=228).

		FSW		NFSW		
		n	**%**	**n**	**%**	**p-value**
ethnicity						<0.001
	African	37	54.4%	204	89.5%	
	Caucasian	14	20.6%	7	3.1%	
	Asian	0	0.0%	4	1.8%	
	other	17	25.0%	13	5.7%	
	missing	*8*		*0*		
oral sex						0.30
	no	11	15.3%	47	20.8%	
	yes	61	84.7%	179	79.2%	
	missing	4		2		
anal sex						0.47
	no	54	77.1%	130	81.3%	
	yes	16	22.9%	30	18.8%	
	missing	6		68		
OAC						<0.001
	no	59	81.9%	58	25.8%	
	yes	13	18.1%	167	74.2%	
	missing	14		3		

(table 6.1 continued)

		FSW		NFSW			
		n	**%**	**n**	**%**	**p-value**	
IUD						0.17	
	no	66	91.7%	192	85.3%		
	yes	6	8.3%	33	14.7%		
	missing	4		3			
smoking						0.003	
	no	59	80.8%	210	92.9%		
	yes	14	19.2%	16	7.1%		
	missing	3		2			
alcohol						0.18	
	no	45	60.8%	14	77.8%		
	yes	29	39.2%	4	22.2%		
	missing	2		210			
drugs						0.004	*
	no	67	93.1%	225	99.6%		
	yes	5	6.9%	1	0.4%		
	missing	4		2			
history of STD						0.021	
	no	72	94.7%	191	84.5%		
	yes	4	5.3%	35	15.5%		
	missing	0		2			
previous Pap smear						<0.001	
	<3 yr.	69	93.2%	87	38.2%		
	3-5yr.	4	5.4%	25	11.0%		
	>5yr.	0	0.0%	32	14.0%		
	never	1	1.4%	84	36.8%		
	missing	2		0			

* Fisher's exact test.
Yes means current or past event.
No means has never happened before.

Table 6.2
Overall HPV prevalence and HPV type prevalence in FSW vs. controls (NFSW)

HPV type	FSW (N=76)		controls NFSW (N=228)		
	n	%	n	%	p-value
any	19	25.0%	67	29.4	0.14
6	0	0.0%	3	1.3%	0.58
16	6	7.9%	9	3.9%	0.22
18	3	3.9%	7	3.1%	0.71
30	0	0.0%	2	0.9%	1.00
31	4	5.3%	9	3.9%	0.74
33	0	0.0%	3	1.3%	0.58
35	3	3.9%	7	3.1%	0.71
39	1	1.3%	3	1.3%	1.00
45	1	1.3%	6	2.6%	0.68
51	1	1.3%	2	0.9%	1.00
52	1	1.3%	6	2.6%	0.68
53	0	0.0%	1	0.4%	1.00
56	0	0.0%	5	2.2%	0.34
58	2	2.6%	7	3.1%	1.00
59	2	2.6%	1	0.4%	0.16
66	0	0.0%	3	1.3%	0.58
67	2	2.6%	2	0.9%	0.26
68	2	2.6%	0	0.0%	0.062
70	2	2.6%	1	0.4%	0.16
73	0	0.0%	1	0.4%	1.00
X	0	0.0%	12	5.3%	**0.042**

Table 6.3
Risk factors associated with HPV prevalence in FSWs

		HPV -		HPV +			
		n	%	n	%	p-value	
age (years)						**0.018**	
	mean (SD)	33.2	(6.2)	29.2	(7.8)		
years working as FSW						**0.002**	
	median [range]	5.0 [3.0 - 8.0]		2.5 [1.0 - 4.3]			
country of birth						0.067	*
	Colombia	42	80.8%	10	19.2%		
	Dom. Rep.	11	57.9%	8	42.1%		
	missing	*4*		*1*			
ethnicity						0.30	
	African	25	67.6%	12	32.4%		
	Caucasian	12	85.7%	2	14.3%		
	other	14	82.4%	3	17.6%		
	invalid	*6*		*2*			
country of FSW						0.14	*
	diff. countries	48	80.0%	12	20.0%		
	only Curaçao	7	58.3%	5	41.7%		
	missing	*2*		*2*			
oral sex						0.13	*
	no	6	54.5%	5	45.5%		
	yes	48	78.7%	13	21.3%		
	missing/invalid	*3*		*1*			
anal sex						0.75	*
	no	40	74.1%	14	25.9%		
	yes	11	68.8%	5	31.3%		
	missing/invalid	*6*		*0*			
OAC						0.49	*
	no	46	78.0%	13	22.0%		
	yes	9	69.2%	4	30.8%		
	missing/invalid	*2*		*7*			
IUD						0.62	*
	no	51	77.3%	15	22.7%		
	yes	4	66.7%	2	33.3%		
	missing/invalid	*2*		*2*			

(table 6.3 continued)	HPV -		HPV +			
	n	**%**	**n**	**%**	**p-value**	
condom					0.99	
no	11	73.3%	4	26.7%		
work and partner	33	73.3%	12	26.7%		
only work	5	71.4%	2	28.6%		
missing/invalid	*8*		*1*			
condom (oral)					0.55	
no	8	61.5%	5	38.5%		
work and partner	33	76.7%	10	23.3%		
only work	6	75.0%	2	25.0%		
missing/invalid	*10*		*2*			
smoking					1.00	*
no	44	74.6%	15	25.4%		
yes	10	71.4%	4	28.6%		
missing/invalid	*54*		*0*			
alcohol					0.76	
no	34	75.6%	11	24.4%		
yes	21	72.4%	8	27.6%		
missing/invalid	*2*		*0*			
drugs					0.60	*
no	50	74.6%	17	25.4%		
yes	3	60.0%	2	40.0%		
missing/invalid	*4*		*0*			
history of STD					1.00	*
no	54	75.0%	18	25.0%		
yes	3	75.0%	1	25.0%		
previous Pap smear					0.84	
<3 yr.	51	73.9%	18	26.1%		
3-5yr.	3	75.0%	1	25.0%		
never	1	100.0%	0	0.0%		
missing/don't known/invalid	*2*		*0*			

** tested via the Fisher's exact test.*

6.5 Discussion

In this study we showed that HPV prevalence in NFSWs was high (29.4%). HPV prevalence in FSWs (25.0%) did not differ significantly from HPV prevalence in NFSWs. The FSWs we investigated were legal sex workers labouring in a brothel and appeared more aware of STDs. They were also more up to date with their cervical cancer screening than NFSWs from the general population. In addition, we found that HPV positive FSWs were younger than HPV negative FSWs. FSWs from the Dominican Republic tended to have a higher HPV prevalence than FSWs from Colombia.

Furthermore, we will provide some possible explanations on the reason for similar HPV prevalence in FSWs and NFSWs. A low male/female ratio of 85/100 is seen in the local population (ter Bals, 2014). This might be an explanation for a promiscuous lifestyle mainly under the male population in a high-risk region and as a result of these a high HPV prevalence. However, literature indicates the family constellation on Curaçao and the role of men having different women has been described from the past (Van der Mark 2003, 21). Other social and cultural determinants, including a high rate of illegal prostitution in the general population might be another explanation.

In 2013, Soohoo et al., published a statistical meta-analysis about a global perspective of cervical HPV infection among FSWs based on 35 papers found eligible for review. Twenty-five countries in Africa, America, Eastern Mediterranean, Europe, Southeast Asia and Western Pacific were represented in this review, and in some countries multiple studies were conducted.

A median overall HPV prevalence of 42.7% (range 2.3% to 100%) was reported (Soohoo et al., 2013). Differences in cervical smear collection, in HPV assays and in statistical analysis make comparison with our analysis difficult. Only one study conducted in Oviedo, Spain, used the same HPV assay as in our study and there was no significant difference in HPV

prevalence. (Curaçao: 25.0%, N=76; Oviedo Spain: 27.8%, N=187; p=0.64) (Cañadas et al., 2004).

During the interviews, we obtained the impression that the awareness level of primary and secondary STD prevention and former STD was notably higher in FSWs from Colombia compared to FSWs from Dominican Republic and NFSWs from Curaçao natives. Although this study was not aimed to analyse the differences in awareness level, we found a significant time difference in taking the last Pap smear for cervical screening between FSW and NFSW. Moreover, we found a higher history of STDs reported by NFSW compared to FSW. These findings confirm poor awareness of cervical cancer prevention strategies under NFSW. Furthermore, we also found a trend toward significant difference in HPV prevalence between FSWs from Dominican Republic (42.1%) and FSWs from Colombia (19.2%, p=0.067). These findings are in agreement with the observation that implementation of prevention strategies, including awareness programmes in the Caribbean region is difficult (ter Bals et al., 2014, Blackman et al., 2014).

A strength of our study is that it is the first study among the Caribbean islands to report HPV prevalence of FSWs in this high risk region for HPV and cervical cancer and also well known for sex tourism and prostitution (Van der Mark, 2003; Prüss-Ustün et al., 2013). All anamnesis questions and cervical samples of the FSWs and NFSWs were collected by the same persons. Our study also has some limitations: a small sample size of FSW (N=76) in our research population which is about the half of the legal prostitutes. These results represent only the FSWs of a legal brothel where FSWs are regularly checked for diseases while the FSWs practicing illegally and without routinely medical check-ups, are not represented by this data. HPV prevalence in this group may probably be higher. Most of these women may not have a regular

medical check-up and may use less barrier contraceptives because of their social-financial status.

In conclusion, we found no significant difference in HPV prevalence between FSW and NFSW on Curaçao. FSW from Colombia have a higher awareness for STD than NFSW as the former also have less STD in the past. These data are important for health policy makers to implement cervical cancer prevention strategies.

Based on the results of our study, we advise to develop more population awareness of STDs under the general population by providing detailed information about STDs. Also, we advise the government to increase the control over illegal prostitution and to set up specialised STD centres. Moreover, we advise the ministry of health to take into account the higher attribution of non-HPV-16/18 types in cervical cancer when a vaccine for prophylactic vaccination is considered.

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