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

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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 and 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 29.2 y ± 7.8 and NFSW 33 y ± 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 < 3 years 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.

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 prevention for sex tourism (Mark and Abraham-Van, 2003; Prüß-Ustün et al., 2013; Domínguez, 2010; Agard-Jones, 2011). Cervical cancer is the 2nd 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., 2015). 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., 2015).

On Curaçao, legal prostitution was established since 1949 with only

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one licensed 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 license 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; The World Bank Group, 2014). When considering implementation of preventive strategies in high risk groups including FSW in 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 HPV16 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., 2017 PLoS submitted. “Human papillomavirus (HPV) prevalence and associated risk factors in women from Curaçao”. Under review).

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.

2. Material and methods

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 in 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, 1075 women, at random selected from the general population and equally stratified in four age groups from 25 to 65 years were included. (Hooi et al. PLoS submitted.) For the present study, all FSWs were age matched with controls from the general population trials (ratio 1:3, age ± 3 y), 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 Prevension, Curaçao (IRB board's approval number 2015/0004).

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.

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 Remmels® transport medium for NFSW. At the end of the day all samples were stored at −20 °C.

2.4. HPV detection and genotyping by means of GP5+/6+ amplitimer 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 50 μL. Detection of hrHPV was done on 5 μL GP5+/6+ -amplitimer 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 10 μL aliquot of GP5+/6+ amplitimer from HPV positive samples by EIA was tested with the Genotyping kit HPV GP, version 2 (Lobo 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). (Brown et al., 2012; Mark and Abraham-Van, 2003). Samples that were HPV positive, but were negative with the genotyping HPV GP kit were designated HPV-X.

2.5. Statistical analysis

Overall HPV prevalence between FSW and NFSWs 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 NFSWs 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.

3. 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 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: > 90% of FSW had their previous Pap smear < 3 years ago, while > 35% of NFSW never had a previous Pap smear (p < 0.001).

No significant differences was 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 (0.0%; 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 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).

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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 prostitutes. These results represent only the FSWs of a legal brothel where FSWs are regularly inspected and counselled in general health prevention. However, the population which is about the half of the legal prostitutes. These results were notably higher in FSWs from Colombia compared to FSWs from the Dominican Republic (42.1%) and FSWs from Colombia (19.2%, Dominican PAHO report, 2017; Blackman et al., 2014).

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 the Dominican Republic and NFSWs from Curacao 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 the 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 (Official PAHO report, 2017; 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 (Mark and Abraham-Van, 2003; Prius-Ustún 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

### Table 1
Comparison of country of birth, sexual behaviour and gynaecological medical history between FSW(N = 76) and NFSW(N = 228).

|                      | FSW (N = 76) | NFSW (N = 228) | p-Value |
|----------------------|--------------|----------------|---------|
| Ethnicity            |              |                |         |
| African              | 37           | 204            | 0.001   |
| Caucasian            | 14           | 7              |         |
| Asian                | 0            | 4              |         |
| Other                | 17           | 13             |         |
| Missing              | 8            | 0              |         |
| Oral sex             |              |                |         |
| No                   | 11           | 47             | 0.30    |
| Yes                  | 61           | 179            |         |
| Missing              | 4            | 2              |         |
| Anal sex             |              |                |         |
| No                   | 54           | 130            | 0.47    |
| Yes                  | 61           | 179            |         |
| Missing              | 6            | 6              |         |
| OAC                  |              |                |         |
| No                   | 59           | 58             | 0.001   |
| Yes                  | 13           | 167            |         |
| Missing              | 14           | 3              |         |
| IUD                  |              |                |         |
| No                   | 66           | 192            | 0.17    |
| Yes                  | 6            | 33             |         |
| Missing              | 4            | 3              |         |
| Smoking              |              |                |         |
| No                   | 59           | 210            | 0.003   |
| Yes                  | 14           | 16             |         |
| Missing              | 3            | 2              |         |
| Alcohol              |              |                |         |
| No                   | 45           | 14             | 0.18    |
| Yes                  | 29           | 4              |         |
| Missing              | 2            | 2              |         |
| Drugs                |              |                |         |
| No                   | 67           | 225            | 0.004*  |
| Yes                  | 5            | 1              |         |
| Missing              | 4            | 2              |         |
| History of STD       |              |                | 0.021   |
| No                   | 72           | 191            |         |
| Yes                  | 4            | 35             |         |
| Missing              | 0            | 2              |         |
| Previous Pap smear   |              |                | < 0.001 |
| < 3 yrs              | 69           | 87             |         |
| 3-5 yrs              | 4            | 25             |         |
| > 5 yrs              | 0            | 32             |         |
| Never                | 1            | 84             |         |
| Missing              | 2            | 0              |         |

|                       | Yes | No | p-Value |
|-----------------------|-----|----|---------|
| Smoking               | 82  | 16 | 0.003   |
| Alcohol              | 18  | 82 | 0.18    |
| Drugs                | 22  | 78 | 0.004*  |
| History of STD       | 41  | 59 | 0.021   |
| Previous Pap smear   | 15  | 85 | < 0.001 |

Yes means current or past event. No means has never happened before.

* Fisher’s exact test.

### Table 2
Overall HPV prevalence and HPV type prevalence in FSW vs. controls (NFSW).

| HPV type      | FSW (N = 76) | NFSW (N = 228) | p-Value |
|---------------|--------------|----------------|---------|
| Any           | 19           | 67             | 0.14    |
| 6             | 0            | 3              | 0.58    |
| 16            | 6            | 9              | 0.22    |
| 18            | 3            | 7              | 0.71    |
| 30            | 0            | 2              | 1.00    |
| 31            | 4            | 9              | 0.74    |
| 33            | 0            | 3              | 0.54    |
| 35            | 3            | 7              | 0.71    |
| 39            | 1            | 3              | 1.00    |
| 45            | 1            | 6              | 0.68    |
| 51            | 1            | 2              | 1.00    |
| 52            | 1            | 6              | 0.68    |
| 53            | 0            | 1              | 1.00    |
| 56            | 0            | 5              | 0.34    |
| 58            | 2            | 7              | 1.00    |
| 59            | 2            | 1              | 0.46    |
| 66            | 0            | 3              | 0.58    |
| 67            | 2            | 2              | 0.26    |
| 68            | 2            | 0              | 0.062   |
| 70            | 2            | 1              | 0.16    |
| 73            | 0            | 1              | 1.00    |
| X             | 0            | 12             | 0.042   |

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 the Dominican Republic and NFSWs from Curacao 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 the 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 (Official PAHO report, 2017; Blackman et al., 2014).
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 in 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-HPV16/18 types in cervical cancer when a vaccine for prophylactic vaccination is considered.

**Contributorship statement**

Design of the study: Hooi, Pinedo, Meijer.

Data collection: Hooi, Quint, de Koning.

HPV detection and genotyping: Hooi.

Statistics: Lissenberg-Witte, Meijer, Hooi.

Writing: Hooi, Lissenberg-Witte, Meijer, Kenter.

All authors critically commented on all versions of the manuscript.

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**Conflicts interests**

D.J. Hooi, B.I. Lissenberg-Witte, M. de Koning, H.M. Pinedo, G. Kenter, has no COI. CJLM Meijer served occasionally on the scientific advisory board of Qiagen, and SPMSD/Merck. and by occasion as consultant for Qiagen. He is minority stock holder of Diassay b.v. W Quint has obtained projects from GSK and Qiagen and is stockholder of DDL Diagnostic Laboratory has meant a lot to us. This analysis was suggested after a critical observation by Mrs. R. Pinedo, on a possible correlation between the island's high number of prostitution and high HPV prevalence. It was not possible to conduct this trial without the grants obtained from the Kramer and Lem foundation to whom we are very grateful. The work was supported by Fundashon Prevension grant number 2015-0001.

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| Table 3 Risk factors associated with HPV prevalence in FSWs. |
|-----------------|-----------------|-----------------|-----------------|
|                | HPV −           | HPV +           | p-Value         |
|                | n   | %   | n   | %   |          |
| Age (years)    | 33.2 | 6.2 | 29.2 | 7.8 | 0.018   |
| Mean (SD)      | 5.0 | [3.0–8.0] | 2.5 | [1.0–4.3] | 0.002   |
| Country of birth | 42 | 80.8 | 10 | 19.2 | 0.067   |
| Colombian      | 11 | 57.9 | 8 | 42.1 |          |
| Ethnicity      | 4 | 1 | | | 0.30    |
| African        | 25 | 67.6 | 12 | 32.4 |          |
| Caucasian      | 12 | 85.7 | 2 | 14.3 |          |
| Other          | 14 | 82.4 | 3 | 17.6 |          |
| Country of FSW | 6 | 2 | 0.02 |          |
| Diff. countries | 48 | 80.0 | 12 | 20.0 | 0.14    |
| Only Curacao   | 7 | 58.3 | 5 | 41.7 |          |
| Missing        | 2 | 2 | 0.13 |          |
| Oral sex       | 6 | 54.5 | 5 | 45.5 |          |
| Yes            | 48 | 78.7 | 13 | 21.3 |          |
| Missing/invalid | 3 | 1 | 0.75 |          |
| Anal sex       | 40 | 74.1 | 14 | 25.9 |          |
| Yes            | 11 | 68.8 | 5 | 31.3 |          |
| Missing/invalid | 6 | 0 | 0.49 |          |
| OAC            | 46 | 78.0 | 13 | 22.0 |          |
| Yes            | 9 | 69.2 | 4 | 30.8 |          |
| Missing/invalid | 2 | 7 | 0.62 |          |
| IUD            | 51 | 77.3 | 15 | 22.7 |          |
| Yes            | 4 | 66.7 | 2 | 33.3 |          |
| Condom         | 3 | 2 | 0.99 |          |
| No             | 11 | 73.3 | 4 | 26.7 |          |
| Yes            | 5 | 71.4 | 2 | 28.6 |          |
| Missing/invalid | 8 | 1 | 0.55 |          |
| HIV (oral)     | 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 | 1.00 |          |
| Smoking        | 44 | 74.6 | 15 | 25.4 |          |
| Yes            | 10 | 71.4 | 4 | 28.6 |          |
| Missing/invalid | 54 | 0 | 0.76 |          |
| Alcohol        | 34 | 75.6 | 11 | 24.4 |          |
| Yes            | 21 | 72.4 | 8 | 27.6 |          |
| Missing/invalid | 2 | 0 | 0.60 |          |
| Drugs          | 50 | 74.6 | 17 | 25.4 |          |
| Yes            | 3 | 60.0 | 2 | 40.0 |          |
| Missing/invalid | 4 | 0 | 1.00 |          |
| History of STD | 54 | 75.0 | 18 | 25.0 |          |
| Yes            | 3 | 75.0 | 1 | 25.0 |          |
| Previous Pap smear | 51 | 73.9 | 18 | 26.1 | 0.84   |
| < 3 yr.        | 3 | 75.0 | 1 | 25.0 |          |
| Never          | 1 | 100.0 | 0 | 0.0 |          |
| Missing/unknown | 1 | 0 | 2 | 0 |          |

* * Tested via the Fisher’s exact test.
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