Human papillomavirus-type distribution in South African women without cytological abnormalities: a peri-urban study

Van Aardt MC, FCOG (SA), MMed(O&G), MBChB, Research Fellow, Gynaecological Oncology
Dreyer G, PhD, MCOG(SA), MMed (O&G), MBChB, Head
Gynaecological Oncology Unit, Steve Biko Academic Hospital, University of Pretoria, Pretoria
Richter KL, MMedPath(MedVirol), FCPathMedVirol(SA), MBChB, Consultant
Department of Medical Virology, University of Pretoria; National Health Laboratory Service, Pretoria
Becker P, PhD, MSc, Biostatistician, Biostatistics Unit, South African Medical Research Council, Tygerberg
Correspondence to: Matthys van Aardt, e-mail: mc@vanaardt.net
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Abstract

Objectives: Knowledge of human papillomavirus (HPV) distribution in the general population is crucial for the development of new HPV vaccines and to provide a baseline from which to monitor the impact of current HPV vaccines in the future. HPV-type distribution in the Tshwane area, South Africa, might be different to that in other regions and countries.

Design: This was a retrospective descriptive study, representative of women without cervical cytological abnormalities.

Setting and subjects: Women attending primary health clinics in the region of Tshwane were screened for cervical abnormalities with conventional cytology.

Outcome measures: Women without cytological abnormalities were included, and HPV DNA typing, using HPV Linear® Array Genotyping Test (Roche Molecular Systems, Branchburg, USA) was performed on all women.

Results: Demographic data were available for 1 238 patients. The mean age was 40.9 years. The majority of the women (14.6%) were between 35 and 39 years of age. 19.4% of women were younger than 30 years of age. The prevalence of HPV types was 67.1% and high-risk HPV infections, 44.9%. The average number of HPV-type infections was 3.2 in the 845 patients with HPV infections. The most common high-risk virus was HPV 16 (10.8%), followed by HPV 51 (9.3%), and HPV 58 (7.9%). HPV 18 was observed in 5.9%, and HPV 45 in 7.5%, of participants. HPV 62 (15.6%) and HPV 84 (14.4%) were the most prevalent low-risk types.

Conclusion: HPV infections were highly prevalent in this population. The prevalence of HPV 16 and 18 was higher than that reported in other world regions. HPV 16 was the most prevalent high-risk type infection in women without cytological abnormalities. HPV infections other than HPV 16 and 18 were also prevalent, and this is important for future vaccine development.

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Introduction

Cervical cancer is the third most common malignancy worldwide diagnosed in women. It is estimated that in 2008, 530 000 new cases of cervical cancer, and 275 000 deaths from cervical cancer, occurred. Low-resource countries carry the burden as over 85% of cervical cancer occurs in these nations.[1]

Persistent infections with specific oncogenic human papillomavirus (HPV) genotypes strongly predict malignancies that relate to HPV, but HPV-type distribution in the general population is not a true reflection of high-risk types found in cancer that is attributed to HPV.[2] Precursor lesions to cervical cancer do not develop in all women with persistent high-risk HPV infections.[3] Approximately 70% of new HPV infections are cleared by women's immune systems within one year, or become undetectable.[4] However, cervical cancer is almost always caused by persistent HPV infection.[1]

The adjusted prevalence of HPV infection in women with no cytological abnormalities was estimated to be

11.7% worldwide.[5] The prevalence of HPV infections in women without cytological abnormalities differs considerably between countries and regions, as well as within regions, ranging from 1.6-41.9%. On average, HPV prevalence in regions in Europe, North America and Asia is lower than that in regions in Africa and Latin America, especially in human immunodeficiency virus (HIV)-infected groups.[1,6,7] Epidemiological data on HPV distribution in the general population is crucial in the light of new broad-spectrum HPV vaccines that are currently under development. It is also important to have this data before introducing HPV-based screening tests in a population for use in cost-benefit modelling studies, and to evaluate the impact on healthcare infrastructure. The data would also provide a baseline against which the impact of current HPV vaccines could be monitored in the future.[6,8]

A prospective study performed on university students in the USA showed that 30% were HPV-infected within one year after initiating sexual intercourse, and 54% within four years.[9] It appears as if high-risk HPV infections even out in American women in the general population who are older than 45 years of age, and that the percentage of women who are positive for HPV DNA drops below 5%.[10]

The five most commonly found HPV types in 99.7% of patients with cervical cancer are HPV 16, 18, 33, 45 and 31. HPV 16 and 18 are responsible for more than 70% of cervical cancer.[11,12] Other factors associated with cervical carcinogenesis include smoking, nutritional deficiencies, genetic factors, HIV co-infection and infections of the genital tract.[13]

Objectives and hypothesis

The objective of this study was to investigate the distribution of HPV types in women representative of the general population, in the Tshwane district, using normal cytological screening tests. The Tshwane district includes areas in and around South Africa's capital, Pretoria. The hypothesis of this study was that HPV-type distribution in the study population with normal cytological screening would be different to that found in the international literature.

Method

This was a retrospective descriptive study, based on data from a dataset that was created from 2008-2010. The study population was representative of women attending public healthcare facilities, and included women attending five primary health clinics in the Tshwane region. The women were invited to screen for cervical abnormalities with the use of both conventional cytology (a Papanicoloau smear) and HPV DNA genotyping. One thousand two hundred and sixty patients had no cytological abnormalities, and received HPV DNA genotyping. Complete demographic data were available for 1 238 patients.

Patients in this study utilised public health care, and the vast majority were black South African women. The samples included dry cervical swabs collected by healthcare workers and tampon samples self-collected by the patients. Patients with abnormal cytology were managed according to standard treatment protocols. Voluntary counselling and testing for HIV were offered to women during consultations at the primary clinic. Conventional cytology was performed by professionally trained nurses and qualified doctors working at the respective clinics. The collected slides were sent to the cytology laboratories at the National Health Laboratory Service (NHLS) and interpreted by qualified cytologists. The cytology laboratory, to assure the quality of the conventional cytology, utilised standard NHLS protocols. Cervical biopsies to compare the cytological findings with histology were not possible because the primary clinics did not have biopsy forceps, and it was not part of the original study design.

Immediately after the swabs and tampon specimens had been collected, they were placed in phosphate-buffered saline and a 10% methanol solution. The collected samples were sent to the Department of Medical Virology at the University of Pretoria. The swab specimens were transported and stored dry until testing. DNA was extracted by means of the DNA® Isolation Kit (Roche Molecular Systems, Branchburg, USA) on the MagNA Pure Automated Extraction System®. HPV typing was determined by the HPV Linear Array® Genotyping Test. Tests were run for 15 high-risk genotypes: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82; three probable high-risk types: HPV 26, 53 and 66; and 19 low or undetermined risk types: HPV 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108.[14]

Data were captured on Microsoft® Excel® datasheets, and analysis performed using Stata® statistical software (StataCorp, College Station, USA). Descriptive statistical methods were employed to describe this population. The data analysis consisted of descriptive statistics, mean and standard deviations for continuous data, and frequencies and percentages for categorical data. The high-risk HPV types were grouped together on the basis of their prevalence in cervical cancer. The 15 high-risk HPV types were divided into four groups: HPV 16 and/or 18, grouped together and named as "very very high risk"; HPV 35, 45 and/or 52, grouped together and named as "very high risk"; HPV 31, 33 and/or 58, grouped together as "high risk"; and the other high-risk HPV types, HPV 39, 51, 56, 59, 68, 73 and/or 82, grouped together as "high risk rest".

The different protocols for this study were approved by the Ethics Committee of the Faculty of Health Sciences of the University of Pretoria (210/2008, 189/2012).

Results

Socio-demographic characteristics

In the period from March 2009 to December 2011, 1 260 patients were recruited who showed no cervical abnormalities on a cytological test. Complete demographic data, including age, were available for 1 238 patients. The age distribution of the study population is shown in Figure 1. The mean age was 40.94 years (standard deviation 11.99).

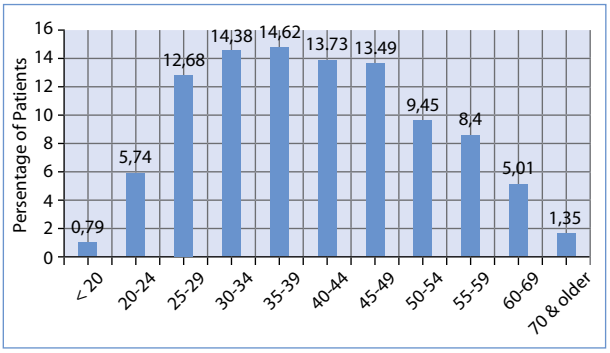


Figure 1: Age distribution (percentage) of the study population

Patients were divided according to age into five-year intervals for women aged 20-59, and into 10-year intervals for women aged 60 and older. Two hundred and forty (19.39%) women were younger than 30 years of age. Twelve (0.97%) women were younger than 20 years of age, and 19 (1.53%) women were 70 years and older.

The prevalence of human papillomavirus-type infections

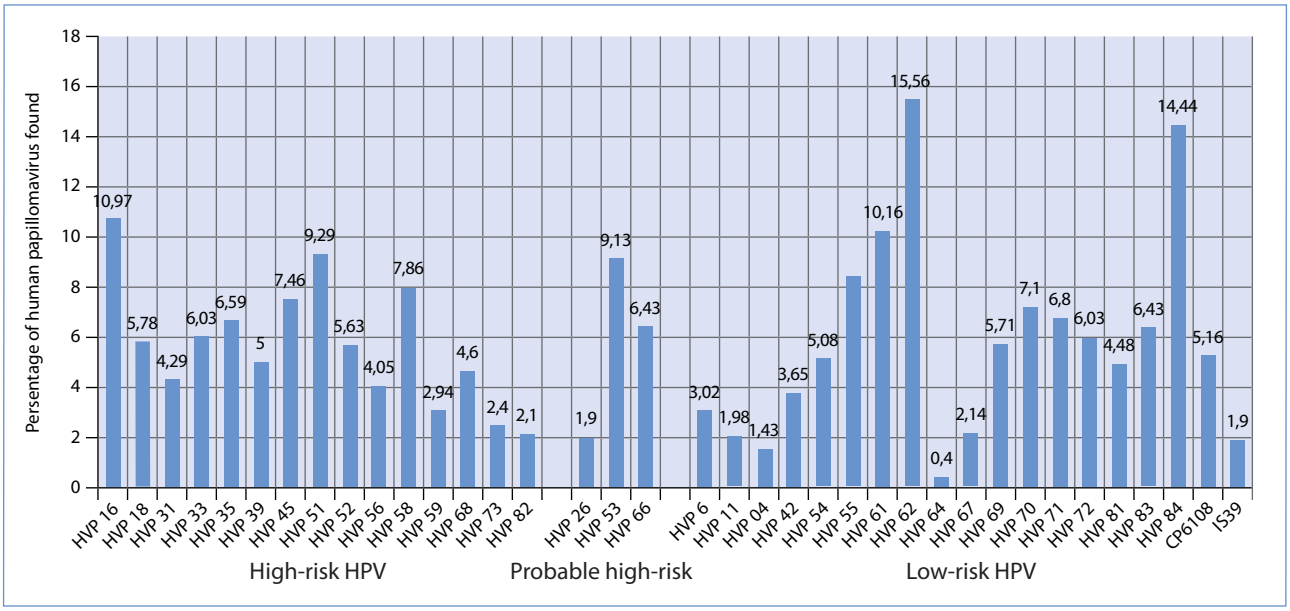
The prevalence of HPV infections was 67.06% for one or more high- and/or low-risk HPV types. One thousand and seventy high-risk, 220 probable high-risk, and 1 388 low-risk, HPV types, were found in 1 260 patients. Four hundred and fifteen (32.94%) of these patients had no viruses identified on HPV DNA typing. Of the 845 patients with HPV infections, the average number of HPV type infections was just more than three multiple HPV type infections. The distribution of specific HPV types in the general population is illustrated in Figure 2.

Of the the low-risk HPV types, HPV 62 (15.56%) and HPV 84 (14.4%) were present most frequently. HPV 6 and 11 were uncommon, and were present in 38 (3.02%) and 25 (1.98%) patients, respectively.

The prevalence of high-risk human papillomavirus types

The prevalence of high-risk HPV infections was 44.92%. A single high-risk HPV type, illustrated in Figure 3, was found in 297 (23.57%) patients. Two hundred and sixty-nine (21.35%) patients had multiple high-risk HPV-type infections.

The most common high-risk virus observed was HPV 16, which was present in 136 (10.79%) of the study



HPV: human papillomavirus

Figure 2: Distribution of human papillomavirus types detected in the study population

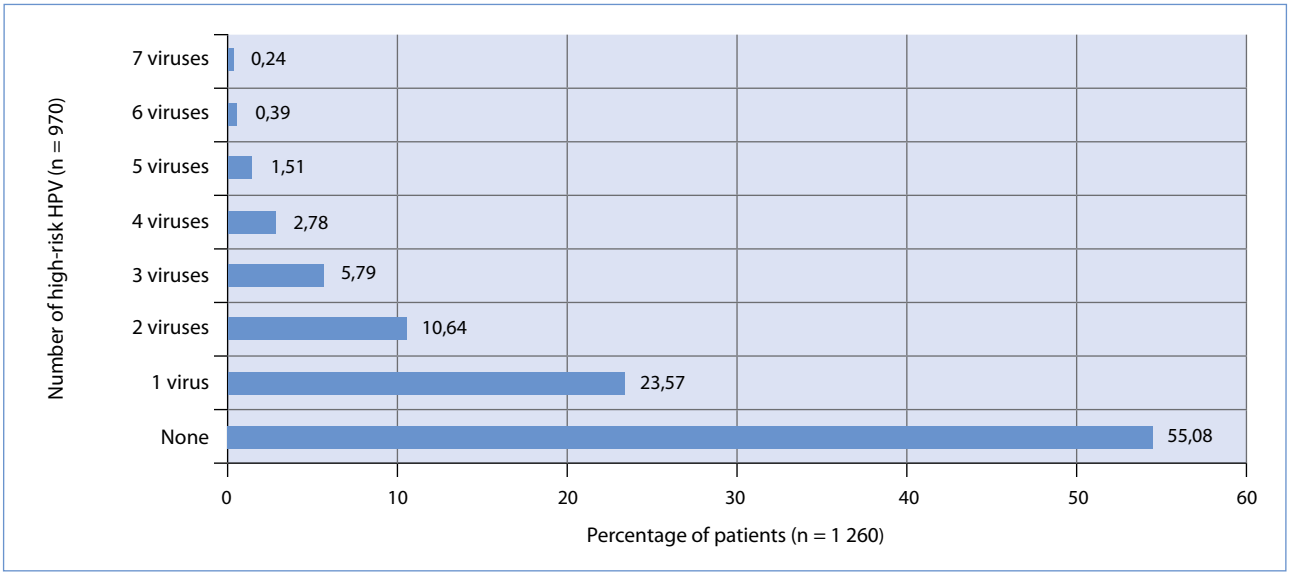
population, followed by HPV 51 and HPV 58. HPV 18 was observed in 5.87% of the study population and HPV 45 in 7.46%. Figure 4 illustrates the 10 most prevalent high-risk HPV types. One hundred and ninety-seven (15.63%) patients had HPV 16 and/or HPV 18 infections.

Discussion

Age distribution

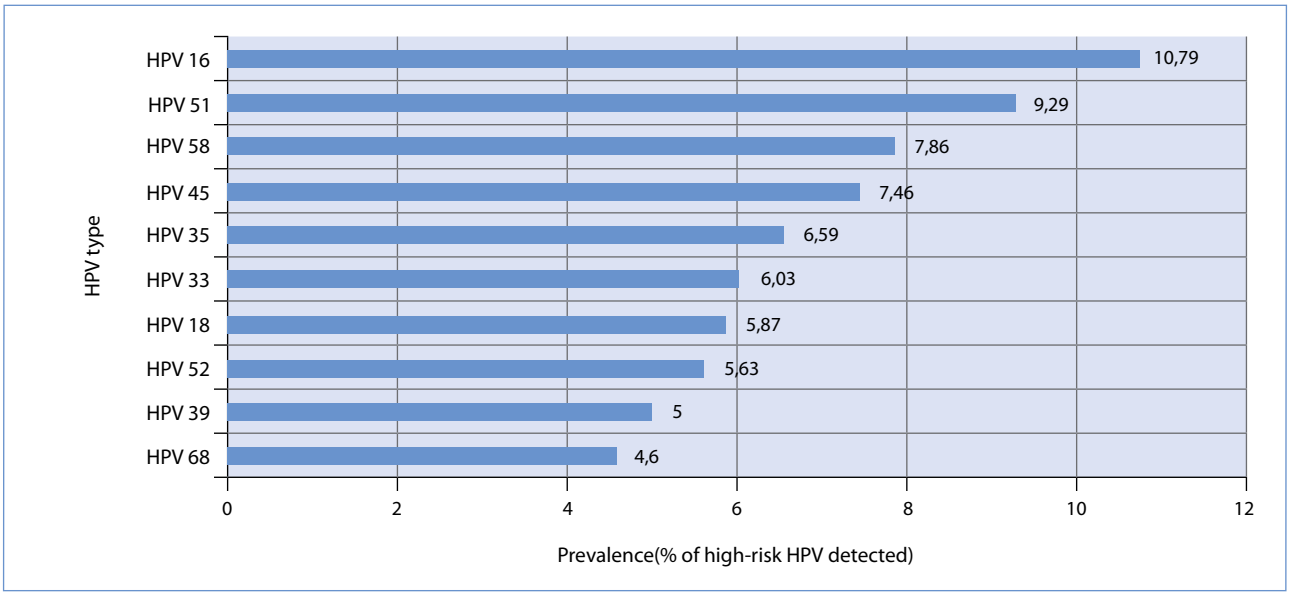
Prevalent HPV infections are less likely to persist in younger, than in older, women. Over a 36-month period, four of five immunocompetent women clear the infection spontaneously.[15] In this study, the age distribution showed a bell-shaped curve, with a peak between 35 and 39 years of age. The highest prevalence of high-risk HPV infections was observed in young women. This is similar to the findings in another South African study.[16] Fifty-six per cent of women younger than 30 years of age were infected with high-risk HPV.

Results from the current study were comparable to the findings of a Kenyan study which showed the highest



HPV: human papillomavirus

Figure 3: Total number of high-risk human papillomavirus types per patient in the study population



HPV: human papillomavirus

Figure 4: The 10 most prevalent high-risk human papillomavirus types found

prevalence of HPV infection to be in women between the ages of 25 and 29 years. Fifty-eight per cent of these patients tested positive for HPV DNA.[17] However, the findings from the Kenyan study included all women tested, regardless of cervical cytology. The peak in the prevalence of high-risk HPV infections in women between 20 and 29 years of age was also noted in a Korean study that was representative of the general population, although in this study, the infection rate was more than double the 23% prevalence found in the Korean population.[8]

In contrast to other African and international studies which showed a decline in HPV prevalence in the general population with increasing age, there was a peak in the prevalence of high-risk HPV infections in the women in this population between 60 and 69 years of age without cytological abnormalities.[7,18,19] However, a large meta-analysis on women with normal cervical cytology found a similar second peak in older women from southern Africa, southern Europe and southern Asia.[6]

The reasons for the peak in this age group were not clear. They might relate to sexual behaviour and/or unscreened and untreated patients secondary to poor cervical cancer screening implementation, and the highly sensitive method used to test for HPV DNA.[7] Reactivation of latent infections might also be associated with a decline in immune function associated with ageing.[15] Gravitt et al suggest that an increased risk of latent HPV infection at 50 years of age might be responsible for the higher prevalence of HPV infection in older women.[20]

The prevalence of any human papillomavirus infection and high-risk human papillomavirus infection types

The prevalence of HPV infection was high. 67.06% of patients were infected with one or more HPV type, and 44.92% of patients were infected with one or more oncogenic HPV type. In comparison to other South African data, the prevalence was slightly lower in this cohort of women with normal cytology than that of 74.6% and 54.3% for all HPV and high-risk HPV infections, respectively, as reported by Richter et al, for women with and without cytological abnormalities.[7]

The prevalence of high-risk HPV infections in women with cytological results within normal limits was 11.9% from Cape Town data, but this study was limited to HIV-noninfected women.[16] A global review of the age-specific prevalence of HPV infections reported the prevalence of HPV infections to be between 7% and 60% for Africa, irrespective of cytological findings.[18] Bruni et al reported the HPV prevalence in women with normal cytology in sub-Saharan Africa to be 24%.[6] The crude prevalence of HPV infections in South Africa, regardless of cervical cytological findings, was reported as 42.2% by Vinodhini et al. These authors also illustrated a marked difference between developed and less developed countries.[1]

The burden of HPV infections in extended Middle East and North Africa were reported as ranging between 0% and 25% in women with normal cytology.[5] The most recently available prevalence of HPV infections in South Africa, obtained from the World Health Organization/*Institut Català d'Oncologica (ICO)* HPV information centre was 21% in women without cytological abnormalities.[21]

The prevalence of high-risk HPV infections in this study was lower than that reported by Richter et al,[7] but higher than that reported in other studies carried out in sub-Saharan Africa.[16] This is expected because this study only included women with normal cytology. No differentiation was made between HIV-infected and HIV-noninfected women in this study, which might explain the higher prevalence in this study. The prevalence of any HPV infection in women with and without cervical disease from the USA was reported to be 42.5%, and of any high-risk type, 29%.[2] A community-based cohort study in Korea reported the prevalence of high-risk HPV infections to be 12.6% for study participants.[8]

The prevalence of high-risk HPV infections in older women in the current study population was almost eight times higher in women aged 60-70 years than that in women who were older than 57 years in the USA.[22]

Utilising HPV testing as a screening method in the USA has become an acceptable alternative to cytology. Current recommendations are that screening is extended to three years if both HPV DNA and cytology are negative. The treatment for women with high-risk HPV infections and cytological abnormalities is clear. Currently, there are no universally accepted guidelines for women testing positive for high-risk HPV without cytological abnormalities. These women have a higher risk of future high-grade cervical intraepithelial lesions (II and III).[23]

Specific low- and high-risk human papillomavirus types

The most common HPV types were HPV 62, followed by HPV 84 and HPV 16. These findings correlate with data from the USA where HPV 62 and HPV 84 were the most frequent low-risk HPV infections.[2] Previous published data from southern Africa reported HPV 83 and HPV 53 to be the most prevalent types.[21]

In a more recent publication from Cape Town, HPV 35, 16 and 58 were the most commonly seen infections in women with normal cytology.[16] HPV 56 was the most prevalent in Korea, followed by HPV 18 and HPV 52 in women with and without cervical disease, and HPV 6, 11, 16 and 18 in women with no cytological abnormalities in extended Middle East and North Africa.[4,8]

When a comparison was made of women with normal cytological findings from different regions globally, HPV 16 was the most common type, except in western Africa, where HPV 31 was the most prevalent. HPV 6 and 11 were uncommon in this population, but slightly more prevalent than the 0.8% reported prevalence for Africa.[6]

HPV 16 was the most common high-risk HPV type, but in contrast to worldwide reported data, it was followed by HPV 51 and 58, instead of HPV 18.[6] HPV 18 was ranked seventh of all the oncogenic HPV types.

Based on findings from Denny et al,[24] the most frequently identified HPV types in women with invasive cervical cancer in sub-Saharan Africa were HPV 16, 18, 45, 35 and 52. This finding highlights that HPV 45 significantly contributed to cervical cancer.[24] Of the five most frequent HPV types in this study, three high-risk HPV types were present, namely HPV 16, 45 and 35. The prevalence of HPV 45 was 7.46%, much more common than the reported prevalence of 0.5% in other women with normal cytology, as reported by Bruni et al.[6]

Human papillomavirus 16 and/or 18

The prevalence of HPV 16 and/or HPV 18 was 15.63%, considerably higher than reported in other world regions in women with and without cervical disease.[1,6] McDonald et al found HPV 35 and 16 infections to be the most common types in women without cytological abnormalities. HPV 35 was as prevalent as, or more prevalent than, HPV 16.[16] The prevalence (reported by McDonald et al) of high-risk types HPV 16 and/or 18 in women testing high-risk HPV positive without cytological abnormalities was 25.39%, compared to 34.64% in the current study.[16]

Approximately one in five women aged 25 years and younger are already exposed to HPV 16 and/or 18, so prophylactic vaccines should be administered before sexual debut in order to prevent HPV 16 and/or 18 infections.[7] The prevalence of HPV 16 and/or 18 was 15.66% in women younger than 25 years of age in the study population. In comparison to other types, Bruni et al showed that HPV 16 was the most prevalent type and had the highest relative contribution.[6]

In Africa and other areas where HPV is particularly common, the higher prevalence of other HPV types could explain the inverse correlation between the overall HPV prevalence and the contribution of HPV 16.[6] The prevalence of HPV 16 and/or 18 was more than four times that reported by the HPV information centre for South Africa in women with normal cytology, almost twice (15.63% vs. 8.7%) that in Korean women, and almost triple that in females in the USA (15.63% vs. 6.6%) with and without cytological abnormalities.[2,8,21] Smith et al reported the prevalence of HPV 16 and/or 18, stratified by age, to be less than 8%.[18]

Infection with multiple human papillomavirus types

Infections with numerous HPV types are frequently found in women who are sexually active, and especially in HIV-infected women.[25,26] There has been a rise from 4% to 15.7% in multiple HPV infections found in cervical cancer in the past 20 years.[25] However, from the results of one study, it did not seem as if multiple HPV infections had an influence on the natural history of one another.[27]

Almost as many women had a single high-risk HPV type infection as those with multiple high-risk HPV infections (23.57% vs. 21.35%). It was rare to find multiple HPV infections simultaneously in the earliest studies on HPV, probably because of diagnostic test limitations. Today, it is clear that a woman can harbour multiple HPV infections with different oncogenic types.[25,26]

Conclusion

HPV infections were highly prevalent in this study of women without cervical cytological abnormalities. The most prevalent HPV types were HPV 62, 84 and 16. The prevalence of HPV 16 and/or 18 was higher than that reported in other world regions, and occurred at a young age. The high prevalence of high-risk HPV types in women with normal cytology is important to consider before implementing HPV-based screening tests in this population, and needs to be addressed with regard to a cost-benefit analysis and the potential impact that it might have on health care in South Africa. These findings are also important to guide future vaccine development and to support the need for early vaccination in this population.

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