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# Human papillomavirus prevalence and genotypes in an opportunistically screened Irish female population

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ABSTRACT

**This study aims to evaluate human papillomavirus (HPV) prevalence and predominating genotypes in liquid-based cervical cytology samples from an Irish urban female population. In addition to use of routine cervical cytology testing, women are screened for HPV using the MY09/11 primers for the HPV *L1* gene and primers for β-globin amplification in a multiplex format. Overall, 996 women between the ages of 16 and 72 years (average age: 35) are included in the study and HPV prevalence was 19.8%. Cytology results showed that 88.9% were normal, 9% borderline or mild dyskaryosis, 1.1% moderate dyskaryosis and 0.9% severe dyskaryosis. Human papillomavirus prevalence in women under 25 was 31%, reducing to 23% in women in the 25–35 age group and to 11% in women over 35. Human papillomavirus prevalence increased with grade of cytology from 11.4% (normal) through 85.4% (borderline), 84% (mild), 100% (moderate) to 100% (severe dyskaryosis). HPV 16 (20%) and 18 (12%) were the most common high-risk types detected in the study. Other common high-risk types were (in descending order) HPV 66, 33, 53, 31 and 58. HPV 66 was associated with the detection of borderline abnormalities by cytology. This is the first population-based study of HPV prevalence in the normal healthy cervical screening population in the Republic of Ireland.**

KEY WORDS: Cervix dysplasia.
Genotype.
Human papillomavirus.
Prevalence.
Urban health.

## Introduction

Human papillomavirus (HPV) infection causes benign and malignant neoplastic lesions of the uterine cervical epithelium. Multiple HPV types are known to infect the anogenital region and these are classified as high-risk or low-risk according to their oncogenic potential.[1] High-risk types 16 and 18 are the most common HPV types found in cervical carcinomas worldwide and much research has focused on these HPV types in the elucidation of the molecular mechanisms of cervical carcinogenesis.[2] The majority of cervical HPV infections are of high-risk type; however, the geographical distribution of HPV types other than HPV 16 and 18 varies considerably worldwide.[3]

Human papillomavirus is a ubiquitous organism and it is estimated that approximately 80–85% of individuals will be infected during their lifetime.[4] Human papillomavirus infections, most of which are transient, are most common in young adults and persistent high-risk infection is the single most important risk factor in the development of cancerous and precancerous lesions.[5,6]

Precancerous cervical lesions are screened for by conventional Papanicolaou (Pap) smear examination and most developed countries have screening programmes in place. Recently, however, there has been a drive towards DNA-based testing for high-risk HPV as part of screening algorithms, as this substantially increases the sensitivity of detection of neoplasia.[7–9]

The Republic of Ireland is currently awaiting the full implementation of a national cervical screening programme, which has been piloted in one region of the country since 2000. Few data are available on HPV prevalence in the normal asymptomatic Irish female population, and thus the aim of the current study is to determine the prevalence of genital HPV infection in women presenting for opportunistic cervical screening. In the study, HPV prevalence and genotype are determined using a multiplex polymerase chain reaction (PCR) technique, followed by HPV *L1* gene sequencing.

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## Materials and methods

### *Sample collection and subjects*

Cervical PreservCyt specimens were obtained from women attending their local general practitioner (GP) in the Dublin area for cervical smear testing. Women were invited to participate in the study by their GP and 996 samples were obtained with informed consent between 2004 and 2005 (age range: 16–72 years, mean: 35 years). Following preparation of a ThinPrep slide and cytological examination, results were coded as normal, borderline or by grade of dyskaryosis

**Table 1.** Distribution of HPV prevalence according to age and cytology result ($n$=996).

| Cytology | Age of woman (years) and HPV status | | | | | | |
|---|---|---|---|---|---|---|---|
| | <25 | | 25–30 | | >35 | | |
| | HPV+ | HPV– | HPV+ | HPV– | HPV+ | HPV– | Total |
| Normal | 27 | 122 | 48 | 301 | 26 | 362 | 886 |
| Borderline* | 10 | 2 | 17 | 4 | 8 | 0 | 41 |
| Mild† | 13 | 5 | 22 | 2 | 6 | 1 | 49 |
| Moderate† | 6 | 0 | 3 | 0 | 2 | 0 | 11 |
| Severe† | 2 | 0 | 4 | 0 | 3 | 0 | 9 |
| Total | 58 | 129 | 94 | 307 | 45 | 363 | 996 |

*Borderline nuclear changes including atypical glandular and squamous cells of undetermined significance.

†Grade of cervical dyskaryosis.

(mild, moderate or severe). Residual PreservCyt material was used for determination of HPV prevalence and genotype.

*Human papillomavirus detection*

PreservCyt specimens (4 mL) were centrifuged at 3000 x*g* and the resulting pellets washed (x2) in Tris-EDTA buffer (10 mmol/L Tris, 1 mmol/L EDTA [pH 8.0]). Extraction of DNA was performed using the QIAamp DNA mini kit (Qiagen, UK), following the manufacturer's instructions. Detection of HPV was performed using the MY09/11 consensus primers for amplification of a region of the *L1* gene and with primers for amplification of a region of the β-globin housekeeping gene, in a multiplex format, as described previously.[10]

*Human papillomavirus genotyping*

Human papillomavirus-positive samples ($n$=132) were genotyped by a direct sequencing approach using the MY09/11 product of the *L1* gene.[11] MY09/11 PCR was optimised so that the reaction mix contained 12.5 μL DNA, 10 pmol each of the forward and reverse primers, 200 μmol/L deoxynucleoside triphosphates, 1x PCR buffer (containing 10 mmol/L Tris HCl [pH 8.3], 50 mmol/L KCl), 2 mmol/L $MgCl_2$ and 1 unit of Platinum *Thermus aquaticus* (*Taq*) DNA polymerase (Invitrogen.) in a final volume of 50 μL.

The PCR was initiated by a 5 min denaturation and enzyme activation step at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 57°C and 1 min at 72°C, and completed by a 5 min extension step at 72°C. Products were visualised by electrophoresis of 5 μL of the reaction on a 1% agarose gel.

To remove excess primers, dNTPs and enzyme the MY09/11 PCR products (450 bp, 45 μL) were purified using the GenElute PCR Clean-Up kit (Sigma-Aldrich). Products were then concentrated by vacuum drying with centrifugation. Samples were sequenced using the forward MY11 degenerate primer at a concentration of 60 pmol/μL. HPV genotype was determined following BLAST (www.ncbi.nlm.nih.gov) analysis of sequences.

*Statistical analysis*

Statistical significance of the relationship of HPV status to age and abnormal cytology was determined by the Pearson $\chi^2$ test. All statistics were performed using SPSS v.11 software (SPSS Inc.).

## Results

Of the 996 samples analysed by cytology, 11.1% ($n$=110) showed some degree of cytological neoplastic abnormality. The majority of women presenting for cervical screening were aged over 25; however, the prevalence of cervical abnormalities was greatest in those aged under 25 years (Table 1).

Human papillomavirus DNA was detected in 197 (19.8%) of the 996 samples tested. (Table 2). Human papillomavirus infection was strongly associated with abnormal cytology ($P$<0.0001) and was 100% sensitive for the identification of samples with high-grade lesions. Human papillomavirus infection was associated with borderline cytology and lesions ($P$<0.0001).

**Table 2.** HPV status and cytology result ($n$=996)

| HPV Status | | Cervical cytology | | | | | |
|---|---|---|---|---|---|---|---|
| | | Normal | Abnormal* | Borderline† | Mild‡ | Moderate‡ | Severe‡ |
| Positive | ($n$=197) | 101 (51.3%) | 96 (48.7%) | 35 (17.8%) | 41 (20.8%) | 11 (5.6%) | 9 (4.6%) |
| Negative | ($n$=799) | 785 (98.2%) | 14 (1.8%) | 6 (0.8%) | 8 (1.0%) | 0 (0%) | 0 (0%) |
| Total | ($n$=996) | 886 | 110 | 41 | 49 | 11 | 9 |

*Classified as either borderline cytology or dyskaryosis.

†Borderline nuclear changes, including atypical glandular and squamous cells of undetermined significance.

‡Grade of dyskaryosis.

*Human papillomavirus prevalence and age*

The breakdown of HPV prevalence by age is shown in Table 1. Cumulatively, 77% (152/197) of HPV infections occurred in those aged under 35. In those aged over 35, 19% (38/197) prevalence was seen in women aged 36–49 years, and 4% (7/197) in women aged over 50 years. Prevalence decreased with age. The trend of decreasing HPV prevalence with age was highly significant ($P<0.0001$). Of the 101 HPV-positive cases with normal cytology, 75 (74%) were in women under the age of 35 years.

*Human papillomavirus genotype prevalence*

Genotyping of 132 HPV-positive samples was performed by sequencing the MY09/11 HPV *L1* gene PCR product. Overall, 20 different HPV genotypes were identified. The most common genotypes (in descending order) were 16, 18, 66, 33, (53 and 6), 61, (70, 31 and 58), 83, (81, 62, and 68), (59 and 54), and (73, 52, 11, 84 and 88) (Fig. 1). The most common high-risk types were 16 and 18, and the most common low-risk types were 6 and 61.

High-risk HPV genotypes predominated (74%) and overall there was a higher frequency of high-risk types than low-risk types for each cytology grade. High-risk HPV types 16, 18, 66, 33, 53, 31 or 58 were found in 49/66 (74%) of abnormal cytology samples and 10/12 (83%) of samples with high-grade lesions.

Analysis of HPV genotype and cytology revealed that HPV 16 was the most common type both in mild dyskaryosis (29%) and in samples with moderate or severe dyskaryosis (33%). In those cases with borderline cytology, HPV 66 predominated. In samples with normal cytology, HPV 16 and 18 were the most common HPV types.

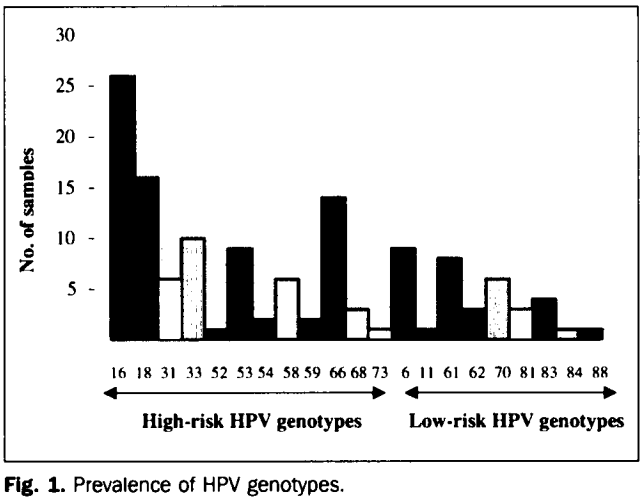


**Fig. 1.** Prevalence of HPV genotypes.

## Discussion

The aim of the present study is to provide an analysis of the cytological and HPV status of Irish women undergoing opportunistic cervical screening in an urban setting. In this study, 11% of women presenting for opportunistic screening had an abnormal smear result, consisting of 4% borderline nuclear change, 5% mild, 1% moderate and 1% severe dyskaryosis. A similar rate of 10% abnormal cytology has been reported previously in the United Kingdom, with approximately 3% borderline, 4% low-grade dyskaryosis and 3% high-grade dyskaryosis.[12]

Cervical abnormalities in Irish women, particularly those with high-grade lesions, have been reported previously to be more common in women under 25 years of age and their prevalence within this group is thought to be increasing.[13] Currently, the American Cancer Society recommends cervical screening for women within three to five years of commencement of sexual activity and no later than at 21 years of age,[14] while results from a longitudinal study conducted by the Icelandic Cancer Society between 1979 and 2002 on the development of high-grade lesions indicate that screening should start before the age of 25, with a maximum three-year screening interval.[15]

In the Republic of Ireland, cervical screening is currently recommended for women over the age of 25 years and the proposed Irish Cervical Screening Programme plans to offer free cervical screening to women aged 25–60 (www.icsp.ie). Findings of the present study suggest that introducing Irish women to the cervical screening system before the age of 25 may be beneficial and may identify those at risk of developing later abnormalities.

Human papillomavirus prevalence in the present study was 19.8%. In the UK a similar prevalence rate of 20% was detected,[12] while in Italy, in a similar urban population, a prevalence of 16% was detected, also using consensus HPV detection methods.[16]

Great variation in prevalence figures for total HPV infection has been reported worldwide. A recent IARC study, which surveyed 10 population-based studies on approximately 15,000 samples from four continents, showed that the prevalence of HPV infection varied greatly (2%–30%).[2]

Several studies report high HPV prevalence in young women, with a decrease in prevalence with age.[16–18] In the present study, HPV prevalence decreased with age, from 31% in those aged under 25 years to 23% in the 25–35 age group to 11% in those aged over 35.

It has been shown previously that HPV testing has a high sensitivity for the detection of high-grade lesions,[19] and most studies demonstrate the high negative predictive value of HPV testing in primary screening.[20–22] In a similar population-based HPV prevalence study using consensus primers, HPV was found in 12.7% of the negative cytology cases, 85% of borderline cases, 96% of mild abnormalities and 94% of high-grade disease.[12] The present study reports similar prevalences rates in these cytological categories.

This is the first Irish study to generate population-based HPV genotype prevalence data. To date, three other HPV genotyping studies have been performed in Ireland. One involved genotypic mapping of cervical adenocarcinomas,[23] while the others correlated HPV status with cellular biomarkers predictive of squamous and glandular pre-invasive lesions.[24,25]

While the direct sequencing approach used in the present study cannot identify multiple HPV infections, the dominant HPV infection present at the cervix can be identified. In the present study, high-risk HPV types predominated in all grades of cytology, and confirms the findings of other studies.[26,27] Human papillomavirus types 16 and 18 were the most common high-risk types detected in the present study, with prevalences of 20% and 12%, respectively, while, HPV 6 (7%) was the most common low-risk type. Other recent European population-based HPV

typing studies also report a predominance of HPV 16 infection, but prevalence of HPV types other than HPV 16 varied.[28,29]

Interestingly, the third most common HPV type detected in this Irish cohort was HPV 66, which has not been reported previously in Irish HPV genotyping studies. However, this may be a consequence of using typing methods that do not identify a wide range of HPV types. Other studies in which HPV 66 has been detected have involved the use of the line-probe assay (LiPa, Roche Biochemicals) or direct sequencing of consensus HPV PCR products.[30,31] In these studies, HPV 66 was detected at moderate frequencies of 2% and 4%.[28,29]

In a study by Melchers *et al.*, conducted in The Netherlands, which used the LiPa assay and sequencing to identify HPV types in various grades of cytology, HPV 66 was the second most common high-risk type identified.[30] In the study, HPV 66 was associated with abnormal cytology and was found in women with borderline cytology, low-grade lesions and high-grade lesions, but was most common in the borderline group. In the present study, 10/14 cases of HPV 66 identified occurred in women under the age of 30 years, and all but three cases of HPV 66 were associated with abnormal cytology.

More recently, HPV 66 was the fourth most common HPV type identified at a frequency of 6% in a university population in Rome,[31] and it was the fourth most common HPV type detected in a high-risk Spanish population.[11]

This highlights the need for population-based HPV typing studies before decisions are made on HPV screening using commercial systems, which may not cover the most common high-risk types in a given population. Other studies have highlighted the need for a full evaluation of HPV type distribution in a given population, particularly with regard to the development of vaccines against high-risk types other than HPV 16.[32,33] It is hoped to determine the effects of multiple HPV types in the Irish population using the LiPa assay in a future study.

The present study suggests that if HPV testing is to be used as part of a screening algorithm then preliminary HPV genotyping studies that facilitate multiple type detection should be performed to determine the impact of high-risk HPV types other than 16 and 18 in a given population. □

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